DOWNLOAD PDF ELISA AND OTHER SOLID PHASE IMMUNOASSAYS

Chapter 1 : ELISA and Other Solid Phase Immunoassays : D. M. Kemeny :

This is a quick-reference manual on practical aspects of immunoassay. Providing a theoretical and practical basis for modern applications of solid-phase immunoassays, this text brings together experts who have used ELISA and other assays in a variety of fields.

Principle[edit] Immunoassays rely on the ability of an antibody to recognize and bind a specific macromolecule in what might be a complex mixture of macromolecules. In immunology the particular macromolecule bound by an antibody is referred to as an antigen and the area on an antigen to which the antibody binds is called an epitope. In some cases, an immunoassay may use an antigen to detect for the presence of antibodies, which recognize that antigen, in a solution. In other words, in some immunoassays, the analyte may be an antibody rather than an antigen. In addition to the binding of an antibody to its antigen, the other key feature of all immunoassays is a means to produce a measurable signal in response to the binding. Most, though not all, immunoassays involve chemically linking antibodies or antigens with some kind of detectable label. A large number of labels exist in modern immunoassays, and they allow for detection through different means. Many labels are detectable because they either emit radiation, produce a color change in a solution, fluoresce under light, or can be induced to emit light. History[edit] Rosalyn Sussman Yalow and Solomon Berson are credited with the development of the first immunoassays in the s. Yalow accepted the Nobel Prize for her work in immunoassays in , becoming the second American woman to have won the award. This type of immunoassay is now used in around million clinical tests every year worldwide, enabling clinicians to measure a wide range of proteins, pathogens and other molecules in blood samples. Labels are typically chemically linked or conjugated to the desired antibody or antigen. Enzymes[edit] Possibly one of the most popular labels to use in immunoassays is enzymes. These enzymes allow for detection often because they produce an observable color change in the presence of certain reagents. In some cases these enzymes are exposed to reagents which cause them to produce light or Chemiluminescence. Radioactive isotopes[edit] Radioactive isotopes can be incorporated into immunoassay reagents to produce a radioimmunoassay RIA. Radioactivity emitted by bound antibody-antigen complexes can be easily detected using conventional methods. RIAs were some of the earliest immunoassays developed, but have fallen out of favor largely due to the difficulty and potential dangers presented by working with radioactivity. Surface plasmon resonance is an example of technique that can detect binding between an unlabeled antibody and antigens. Immunoassays can be run in a number of different formats. Generally, an immunoassay will fall into one of several categories depending on how it is run. The amount of labelled, unbound analyte is then measured. In theory, the more analyte in the sample, the more labelled analyte gets displaced and then measured; hence, the amount of labelled, unbound analyte is proportional to the amount of analyte in the sample. Two-site, noncompetitive immunoassays usually consist of an analyte "sandwiched" between two antibodies. ELISAs are often run in this format Competitive, heterogeneous immunoassays[edit] As in a competitive, homogeneous immunoassay, unlabelled analyte in a sample competes with labelled analyte to bind an antibody. In the heterogeneous assays, the labelled, unbound analyte is separated or washed away, and the remaining labelled, bound analyte is measured. One-site, noncompetitive immunoassays[edit] The unknown analyte in the sample binds with labelled antibodies. The unbound, labelled antibodies are washed away, and the bound, labelled antibodies are measured. The intensity of the signal is directly proportional to the amount of unknown analyte. Two-site, noncompetitive immunoassays[edit] The analyte in the unknown sample is bound to the antibody site, then the labelled antibody is bound to the analyte. The amount of labelled antibody on the site is then measured. It will be directly proportional to the concentration of the analyte because the labelled antibody will not bind if the analyte is not present in the unknown sample. This type of immunoassay is also known as a sandwich assay as the analyte is "sandwiched" between two antibodies. Clinical tests[edit] A wide range of medical tests are immunoassays, called immunodiagnostics in this context. Many home pregnancy tests are immunoassays, which detect the pregnancy marker human chorionic gonadotropin. Illuminated by a modulated light at a plasmon resonance wavelength, the nanoparticles generate strong acoustic signal, which

can be measured using a microphone.

Chapter 2 : All Posts - ELISA AT1R - Solid Phase Immunoassays

ELISA and Other Solid Phase Immunoassays Theoretical and Practical Aspects D. M. Kemeny, Department of Medicine, and S. J. Challacombe, Department of Oral Medicine and Pathology, United Medical and Dental Schools of Guy's and St Thomas's Hospitals, London, UK This guide to the modern applications of solid phase immunoassays is written by.

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Chapter 3 : ELISA - Wikipedia

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This section does not cite any sources. Please help improve this section by adding citations to reliable sources. Unsourced material may be challenged and removed. March Learn how and when to remove this template message As an analytic biochemistry assay, ELISA involves detection of an "analyte" i. As a heterogenous assay, ELISA separates some component of the analytical reaction mixture by adsorbing certain components onto a solid phase which is physically immobilized. In ELISA, a liquid sample is added onto a stationary solid phase with special binding properties and is followed by multiple liquid reagents that are sequentially added, incubated and washed followed by some optical change e. The quantitative "reading" usually based on detection of intensity of transmitted light by spectrophotometry , which involves quantitation of transmission of some specific wavelength of light through the liquid as well as the transparent bottom of the well in the multiple-well plate format. The sensitivity of detection depends on amplification of the signal during the analytic reactions. Since enzyme reactions are very well known amplification processes, the signal is generated by enzymes which are linked to the detection reagents in fixed proportions to allow accurate quantification â€" thus the name "enzyme linked". The analyte is also called the ligand because it will specifically bind or ligate to a detection reagent, thus ELISA falls under the bigger category of ligand binding assays. The ligand-specific binding reagent is "immobilized", i. Conventionally, like other forms of immunoassays , the specificity of antigen - antibody type reaction is used because it is easy to raise an antibody specifically against an antigen in bulk as a reagent. Alternatively, if the analyte itself is an antibody, its target antigen can be used as the binding reagent. History[edit] Before the development of the ELISA, the only option for conducting an immunoassay was radioimmunoassay , a technique using radioactively labeled antigens or antibodies. In radioimmunoassay, the radioactivity provides the signal, which indicates whether a specific antigen or antibody is present in the sample. Radioimmunoassay was first described in a scientific paper by Rosalyn Sussman Yalow and Solomon Berson published in A suitable alternative to radioimmunoassay would substitute a nonradioactive signal in place of the radioactive signal. When enzymes such as horseradish peroxidase react with appropriate substrates such as ABTS or TMB , a change in color occurs, which is used as a signal. However, the signal has to be associated with the presence of antibody or antigen, which is why the enzyme has to be linked to an appropriate antibody. This linking process was independently developed by Stratis Avrameas and G. A technique to accomplish this was published by Wide and Jerker Porath in These new reporters can have various advantages, including higher sensitivities and multiplexing. However, given that the general principles in these assays are largely similar, they are often grouped in the same category as ELISAs. In , an ultrasensitive, enzyme-based ELISA test using nanoparticles as a chromogenic reporter was able to give a naked-eye colour signal, from the detection of mere attograms of analyte. A blue color appears for positive results and red color for negative. Note that this detection only can confirm the presence or the absence of analyte not the actual concentration. ELISA tests are broken into several types of tests based on how the analytes and antibodies are bonded and used. A buffered solution of the antigen to be tested for is added to each well of a microtiter plate , where it is given time to adhere to the plastic through charge interactions. A solution of nonreacting protein, such as bovine serum albumin or casein , is added to well usually well plates in order to cover any plastic surface in the well which remains uncoated by the antigen. The primary antibody with an attached conjugated enzyme is added, which binds specifically to the test antigen coating the well. A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength. The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound, the enzyme molecules will produce many signal molecules. Within common-sense limitations, the enzyme can go on producing color indefinitely, but the more antibody is bound, the faster the color will develop. A major

disadvantage of the direct ELISA is the method of antigen immobilization is not specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result yes or no for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation error inherent in a test is often used to distinguish positive from negative samples. In quantitative ELISA, the optical density OD of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule. For example, if a test sample returns an OD of 1. When the "primary" antibody is of interest, e. A surface is prepared to which a known quantity of capture antibody is bound. Any nonspecific binding sites on the surface are blocked. The antigen-containing sample is applied to the plate, and captured by antibody. The plate is washed to remove unbound antigen. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen. The plate is washed to remove the unbound antibody-enzyme conjugates. A chemical is added to be converted by the enzyme into a color or fluorescent or electrochemical signal. The absorbance or fluorescence or electrochemical signal e. The image to the right includes the use of a secondary antibody conjugated to an enzyme, though, in the technical sense, this is not necessary if the primary antibody is conjugated to an enzyme which would be direct ELISA. However, the use of a secondary-antibody conjugate avoids the expensive process of creating enzyme-linked antibodies for every antigen one might want to detect. By using an enzyme-linked antibody that binds the Fc region of other antibodies, this same enzyme-linked antibody can be used in a variety of situations. Without the first layer of "capture" antibody, any proteins in the sample including serum proteins may competitively adsorb to the plate surface, lowering the quantity of antigen immobilized. Use of the purified specific antibody to attach the antigen to the plastic eliminates a need to purify the antigen from complicated mixtures before the measurement, simplifying the assay, and increasing the specificity and the sensitivity of the assay. A sandwich ELISA used for research often needs validation because of the risk of false positive results. Unlabeled antibody is incubated in the presence of its antigen sample. The plate is washed, so unbound antibodies are removed. The more antigen in the sample, the more Ag-Ab complexes are formed and so there are less unbound antibodies available to bind to the antigen in the well, hence "competition". The secondary antibody , specific to the primary antibody, is added. This second antibody is coupled to the enzyme. A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal. The reaction is stopped to prevent eventual saturation of the signal. The labeled antigen competes for primary antibody binding sites with the sample antigen unlabeled. The less antigen in the sample, the more labeled antigen is retained in the well and the stronger the signal. Commonly, the antigen is not first positioned in the well. Two specific antibodies are used, one conjugated with enzyme and the other present in serum if serum is positive for the antibody. Cumulative competition occurs between the two antibodies for the same antigen, causing a stronger signal to be seen. If antibodies are present, the antigen-antibody reaction occurs. No antigen is left for the enzyme-labelled specific HIV antibodies. These antibodies remain free upon addition and are washed off during washing. Substrate is added, but there is no enzyme to act on it, so a positive result shows no color change. This test leaves the antigens suspended in the test fluid. The sample is then passed through the Scavenger container. This can be a test tube or a specifically designed flow through channel. These can be identical or sufficiently similar to the primary antigens that the free antibodies will bind. The Scavenger container must have sufficient surface area and sufficient time to allow the Scavenger Antigens to bind to all the excess Antibodies introduced into the sample. The sample, that now contains the tagged and bound antibodies, is passed through a detector. This device can be a flow cytometer or other device that illuminates the tags and registers the response. This test allows multiple antigens to be tagged and counted at the same time. This allows specific strains of bacteria to be identified by two or more different color tags. If both tags are present on a cell, then the cell is that specific strain. If only one is present, it is not. This test is done, generally, one test at at time and cannot be done with the microtiter plate. The equipment needed is usually less complicated and can be used in the field. It has also found applications in the food industry in detecting

potential food allergens , such as milk , peanuts , walnuts , almonds , and eggs [19] and as serological blood test for coeliac disease. The plate is then washed to remove all other components of the serum. A specially prepared "secondary antibody" â€” an antibody that binds to other antibodies â€” is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme. Thus, the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence. ELISA results are reported as a number; the most controversial aspect of this test is determining the "cut-off" point between a positive and a negative result. A cut-off point may be determined by comparing it with a known standard. Unknowns that generate a stronger signal than the known sample are "positive.

Chapter 4 : Immunoassay - Wikipedia

Increased sensitivity and ease of performance have made solid-phase immunoassays increasingly popular. Freedom from the hazards of radioisotopes and the expensive equipment needed to measure them have helped to make the enzyme-linked immunosorbent assay (ELISA) one of the fastest growing immunological tests used today.

Many immunoassays are performed on blood serum or plasma specimens. Physicians draw blood at the office, hospital clinic, or other locations, and send samples to laboratories for testing. Urine has a long history as a diagnostic sample. Urine-based tests are available for tests including pregancy, ovulation, and menopause tests, as well as for sexually transmitted diseases and drugs of abuse testing. In addition, non-lateral flow tests such as POC urine analyzers and biochemical-based POC urinalysis tests have been available for many years. Saliva-based diagnostics have long enjoyed industry attention. Nucelic acids are present in saliva, and saliva samples are DNA sources for genotyping analysis. Centralized laboratories use saliva specimens for other analytes including hormones. Saliva contains proteins including antibodies , nucleic acids, and hormones. Saliva glands produce many analytes actually found in saliva samples, into which blood can also pass. Mechanisms including passive diffusion or active transport of molecules can cause this. Analytes found in blood can also be found in saliva, generating industry interest in saliva as a diagnostic specimen. As a diagnostic specimen, saliva is noninvasive and does not require highly trained specialists to perform. Specimens are also easy to both store and transport. Saliva is an attractive test sample for diagnostics companies that are considering utilizing it for new test applications. Considerations will include test accuracy, cost effectiveness, and the outcome of research to identify and validate new biomarkers in saliva. The overall market for immunoassays is highly competitive and growing slowly, but has specific areas of opportunity that will be maximized. Significant growth opportunities in the immunoassay market may be possible through further innovations. This includes both new platforms and novel biomarkers. The attractive opportunities these innovations may make possible have attracted many diagnostics companies. Immunoassays are basic, long-standing tests existing in laboratories worldwide. Globally, prospects for market growth are good in certain geographical regions developing countries. Facots such as economics and increased health insurance availability is fueling market growth in developing countries, making them promising market segments. Many companies are targeting these markets, including major worldwide diagnostic companies as well as smaller ones. Future testing growth in these markets will occur in existing technology ELISA and lateral flow tests. Immunoassay innovations are expanding the market in new and emerging disease areas, noted in Immunoassay Markets. Kalorama details these innovations in immunoassays in this report, and highlights three in particular: GI and transplant management in medical coniditon assays, and saliva-based testing as an interestind and innovative vehicle. Leading names in immunoassays will not surprise, and leading companies often lead in IVD as well. Other immunoassay leaders include: More than other companies compete for immunoassay market share, while smaller players with ELISa tests serve local markets. Strong niche competitors such as Fujirebio, DiaSorin, Tosoh, Wako, Thermo Fisher Scientific, and internatial companies market their products worldwide. Other smaller companies have experienced remarkable growth, but their total revenues only contribute small portions to the total market. Abbott generated most of its IVD sales outside the U. Alere, now merged with Abbott, participates in nearly all immunoassay POC test segments but has a dominating position in rapid cardiac markers, drugs of abuse, infectious disease, pregnancy and fertility testing. Beckman Coulter continues to add novel and proprietary tests from different sources including tests for: Infectious Disease Immunoassay Market Opportunity: High Growth, High Impact: Infectious diseases require fast and effective detection for proper outcomes, and immunoassays are as important as molecular-based technologies in testing for these diseases. Common infectious disease immunoassays performed in central laboratories are typically categorized into groups such as hepatitis, retroviral testing, sexually transmitted diseases, TORCH, and other infectious diseases. Infectious disease immunoassays are used in point-of-care setting or when rapid testing is needed. Immunoassays are also used to screen blood and blood products prevent transfusion-transmitted infections. In terms of the market , infectious disease

immunoassays beat the overall IVD market in terms of revenue growth. The healthcare market researcher said that infectious disease immunoassay market is large because of the many different infectious agents affecting the population. Point of Care Immunoassays Market Opportunity: The market for immunoassay testing can be defined in three markets: Central, blood screening, and point-of-care. The central lab-based immunoassays market is by far the largest segment, with a Centralized laboratories include reference laboratories such as Laboratory Corporation of America or LabCorp, Quest Diagnostics, and others , hospital laboratories, laboratories operated by health maintenance organizations HMOs , and other centralized laboratories. In developed countries, all units of blood and blood products are already screened for several infectious agents. The point of care testing markets consists of two segments: However, there are many locations where POC testing can occur within these segments, especially the professional POC market. Professional POC testing is performed in a number of different settings, such as hospitals, physicians office laboratories POLs and clinics, retail clinics, and many other locations. This curated page attempts to outline the scope of the immunoassay market. Because the market is worth billions of dollars and has competitors both large and small, this page does not represent a complete market perspective. It is common for organizations to use substantially more content from Knowledge Centers than from individual purchases. Please contact your representative regarding this solution. Kalorama Information For more than 30 years, Kalorama Information has been a leading publisher of market research in medical markets, including the biotechnology, diagnostics, medical device, and pharmaceutical industries. Our comprehensive, timely, quality research and innovative approach to analysis and presentation of market intelligence have made Kalorama Information a premier source of market information for top industry decision makers.

Chapter 5 : Kemeny, D. M. [WorldCat Identities]

Volume , number 2 FEBS LETTERS February to solid phases, the role of antibody affinity, quan- titation and the use of enzyme-mediated end-.