Updates in Immunoassays: Introduction

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LEARNING OBJECTIVES:

1. Discuss the evolution and development of immunoassays.
2. Explain the principle of various immunoassays used in diagnosis.
3. List emerging methodologies in the area of immunological assays.
4. Describe the importance of immunoassay testing in the clinical laboratory.

ABBREVIATIONS: ANA - anti-nuclear antibody; CBC - complete blood count; CDC - Centers for Disease Control and Prevention; CLIA - chemiluminescent immunoassay; CMV - cytomegalovirus; CRP - C-reactive protein; DFA - direct fluorescent antibody; EIA - enzyme immunoassay; ELFA - enzyme-linked fluorescent assay; ELISA - enzyme-linked immunosorbent assay; FIA - fluorescent immunoassay; HCG - human chorionic gonadotropin; HIV - human immunodeficiency virus; IFA - indirect fluorescent assay; IgG - immunoglobulin G; IgM – immunoglobulin M; RSV - respiratory syncytial virus.

INDEX TERMS: Immunoassays, enzyme, fluorescent, chemiluminescent

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The immune system is one of the most intricate and complex systems of the human body. The cascade of events that occurs at both the cellular and molecular level from the moment foreign matter enters the body is astounding. Once antibodies are synthesized by plasma cells in response to the invading entity, medical laboratory professionals have the ability to measure and identify the type of antibody present by a variety of immunological assays. Detection methods range from rapid latex agglutination assays to enzyme, fluorescent and chemiluminescent assays that can measure both IgM and IgG levels qualitatively and quantitatively.

Immunoassays have evolved considerably when compared to some of the earlier methods of identifying antigen/antibody complex formation. The Ouchterlony double immunodiffusion assay developed in the 1940s by Swedish immunologist Örjan Ouchterlony[1] is one of the older methods where both antigen and antibody diffuse through a semi-solid gel independently of each other resulting in a precipitation line if the specific antigen/antibody complex is formed (identity pattern).[2] This assay can take up to 48 hours to completely diffuse and render a positive result. To improve turn-around times, assays were designed using electrical currents during the diffusion process to speed up the reaction time. One method developed by Grabar and Williams in 1953 was the rocket immunoelectrophoresis technique.[3] In this assay, antigen is added to an appropriate medium, then subjected to an electrical current where protein fractions are separated according to size. Antiserum is then placed in a trough cut into the gel and allowed to diffuse and react with the antigen. A precipitin arc appears if the specific antigen/antibody complex is formed.[2] However, this technique can be difficult to interpret and can require up to 24 hours for a definitive result.

In the late 1950's, Yalow and Berson developed the first radioimmunassay that utilized radioactive isotopes.[2,4] These radioactive labels emit gamma rays that can be measured by a gamma counter. The advantage of using this type of technique is the overall high sensitivity. The disadvantage is the exposure to radioactive materials,

cumbersome disposal of hazardous, radioactive material, and adherence to federal regulations.[2]

Utilizing labels in immunoassays not only resulted in high sensitivity and specificity but also yielded rapid results. Enzyme labels eventually replaced radioactive labels and in 1971, Engvall and Perlmann published their work using alkaline phosphatase as the label in quantifying IgG in rabbit serum.[5] The assay they developed and reported on was the enzyme linked immunosorbent assay (ELISA). Concurrently, van Weemen and Schuurs published their work on enzyme immunoassays (EIA) using horseradish peroxidase as the label by quantitatively measuring human chorionic gonadotropin hormone (HCG) in urine.[6] As a result, ELISA and EIA methods are largely used in the clinical laboratory as both screening and confirmatory tests. In addition, western blot immunoassays that are commercially prepared are used in the confirmation of positive ELISA screening tests in the case of specific infectious diseases *e.g.* Lyme disease and human immunodeficiency virus (HIV).

In 1975, the advent of monoclonal antibodies by Milstein and Köhler revolutionized the field of immunology and serology.[2,7] This technique using hybridoma technology and a myeloma cell line improved the development of immunoassays by using monoclonal antibodies specific to a particular epitope on the antigen thus increasing sensitivity and specificity of the reaction.[2,8] Monoclonal antibodies are widely used for *in vitro* diagnostic testing and in biotherapy treatments. In 1984 the Nobel Prize in Physiology and Medicine was awarded to Milstein and Köhler for their development of monoclonal antibodies and their outstanding contribution to the field of immunology.[9]

Fluorescent immunoassays (FIA) whether direct (DFA) or indirect (IFA) are widely used in the clinical and public health laboratories and are useful in identifying numerous infectious agents e.g., *Treponema pallidum*, *Legionella pneumophilia*, *Bordetella pertusis*, *Giardia lamblia*, *Toxoplasma gondii,* respiratory syncytial virus (RSV), rabies, and cytomegalovirus (CMV).[2,8,10,11]

Enzyme linked fluorescent assays (ELFA) combine the methodology of ELISA testing with utilization of a fluorescent label in the final detection process.[12] Due to the use of combined labels, results yield a high level of sensitivity and specificity with rapid turnaround times.

Chemiluminescent immunoassays (CLIA) identify antigen/antibody complexes by measuring the amount of light emitted as a result of a chemical reaction. Molecules such as acridinium esters and luminol are used in the reaction process due to their ability to emit light once excited.[2,8,10,11] This methodology requires specific instrumentation depending on the chemicals used to read and interpret the results.

Some of the newer and emerging methodologies in the area of immunological assays include the use of immunoblot and recombinant immunoblot assays, lateral flow immunoassays (also referred to as immunochromatographic assays), quantum dots, and magnetic labeling technology.[8] This focus series will discuss various methodologies and provide updates in immunoassays as they relate to the detection of infectious disease. The principle of each method mentioned will be discussed in more detail as it is introduced in the article. Updates in immunoassays in bacteriology, virology, and parasitology will be discussed along with some of the guidelines set forth by the Centers for Disease Control and Prevention (CDC) regarding syphilis screening and confirmatory testing as well as QuantiFERON testing for tuberculosis. Although not infectious agents, immunoassays used for anti-nuclear antibodies (ANA) will be discussed along with the use of C-reactive proteins (CRP) and procalcitonin as biomarkers in bacterial sepsis.

In summary, the ultimate goal of the medical laboratory scientist is to report accurate final results in a timely manner. Whether the result is a stat glucose or cardiac enzymes, a complete blood count (CBC), the results of a crossmatch for transfusion purposes or the rapid detection of an infectious agent, accurate and precise results ensure the patient will receive the appropriate treatment improving patient outcomes and overall quality of life. Since patient outcomes correlate with the delivery of prompt and accurate test results, laboratorians are embracing new cutting edge technology such as molecular diagnostics and immunologic assays for quick turnaround times. Although the field of molecular diagnostics in the clinical laboratory has escalated in the past two decades, simple, rapid immunoassays that are highly sensitive and specific, cost effective, and easy to use still remain

the method of choice for quick and accurate results.

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