Immunoassay technology and format of HIV assay development
愛滋病病毒的免疫測定技術及格局的發展

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Introduction

Since 1985, when testing of all blood donations for the presence of antibody to HIV was implemented, considerable progress has been made in improving assay sensitivity, especially during the early stages of infection. Early detection and diagnosis of infection continues to be a challenge in the detection of HIV antigen (Ag) and antibodies (Ab) during the early viremic window period. Since the launch of the first HIV Ab detection assay in 1985, Abbott Diagnostics has put a large amount of effort in developing HIV/AIDS disease management solutions especially in the development of immunoassay testing technology. Below is a short summary of the assay development.

Assay Technology

Starting in the late 1970s and throughout the 1980s-90s, major advances in automation and sensitivity of immunoassays, and EIA in particular, were achieved. Fluorescence Polarization Immunoassay (FPIA) and Microparticle Enzyme Immunoassay (MEIA) technologies represented the predominant technologies for some years. More recently, Chemiluminescent Magnetic Immunoassay (CMIA) technology has been routinely applied, and a new generation of chemiluminescent label has been employed using Abbott patented acridinium derivative with N-sulphonyl Carboxamide groups (Figure 1). The reaction steps for ChemiFlex (the trademarked name for Abbott's version of CMIA) utilize noncompetitive sandwich assay technology to measure analytes. The latest HIV Ag and HIV Ab assay has been developed on both MEIA and CMIA platform. (Figure 2)

The methods of MEIA and CMIA both use microparticles to anchor antibodies, but there are other similarities and differences as well (Table 1). The label, separation step and measurement technology differ between these two methods.

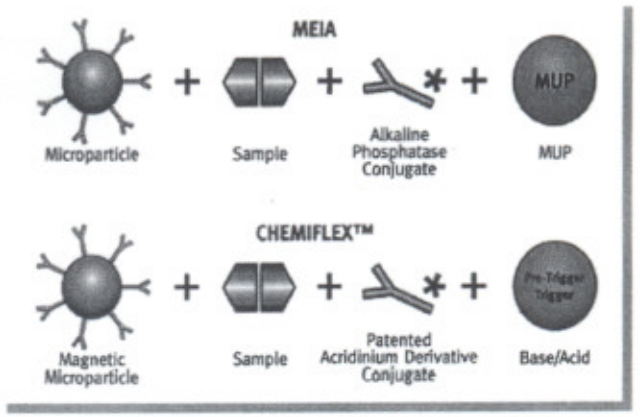

Figure 1. Abbott Architect chemiluminescence label for CMIA

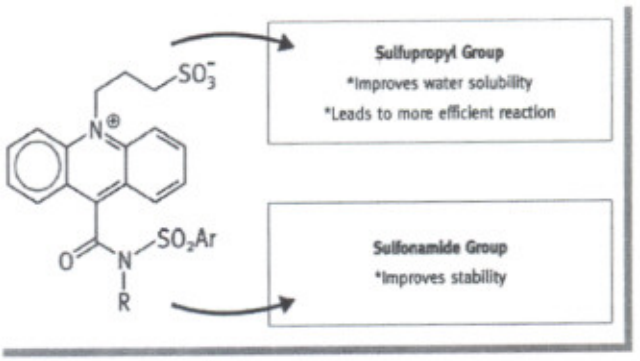

Figure 2. Comparison of MEIA and CHEMIFLEX methods

Table 1. Comparison of The Steps in MEIA and CMIA Methods

Technology	Solid Phase	Separation Step	Label	Detection Technology
MEIA	Latex Microparticle	Glass Fiber Matrix	Alkaline Phosphatase Enzyme	Fluorescence Detector
CMIA	Magnetic Microparticle	Magnet	Chemiluminescent Compound	Chemiluminescence Photomultiplier Tube

A Brief History of Using Chemiluminescent Label

Chemiluminescence technology was first published in the 1930s, and in around 1960s the family of compounds was further expanded. Chemiluminescence immunoassay was first seen in 1980s.

Compounds like Luminol/isoluminol, dioxetanes, acridinium phenyl esters and ruthenium salts have been adapted in different commercial platforms (Table 2).

Compound used as a substrate for the detection of conventional enzyme-labeled conjugates are luminol for Horse Radish Peroxidase and dioxetanes for Alkaline Phosphatase, still relying on an enzyme-mediated base. Though all compounds have a similar end-point, there are differences in the preparation, purification and stability of the conjugates, the sensitivity to interference substances, and the kinetics of the light-emitting reaction. The older chemiluminohores may still have limitations such as insolubility in aqueous buffers which render an inconsistent background levels, and instability which directly affects the frequency of calibration and assay performance. The recent Abbott Architect new class of acridinium chemiluminophores which are based on the acridinium (N-sulphonyl) carboxamides are designed to overcome such shortcoming seen in current utilized label. Though there are intrinsic characteristics in the choice of

Table 2. Chemiluminescence in immunoassays

Automated Immunoassay Platform	Type of Chemiluminescence Used
Bayer ACS180 , Centaur	Acridinium Ester
Abbott Architect	Acridinium Carboxamide
DPC Immulite	Dioxetane
Roche Elecsys	Electrochemiluminescence
Ortho Vitros Eci	Luminol based

label, there are other factors of the assay design which can influence the performance and ultimately the accuracy of an immunoassay more than the chemiluminescent label technology. These factors are the selection of capture antibody, conjugate, assay protocol, solid phase in suspension, buffer types, washing procedures, and pipetting precision. In HIV testing, the choice of antibody and antigen on the solid phase, the target epitopes, and the recognition of all HIV subtypes are important factors for the reliability and quality of a testing system.

Types of Tests and Format

In HIV testing, there are differences between direct and indirect tests. Direct tests directly detect viral components. They are used primarily for diagnostic and monitoring purposes, such as clinical progression and response to therapy in seropositive individuals. These tests measure the p24 core antigen, the viral RNA genome in plasma, and the viral DNA in infected cells. Indirect tests detect the presence of specific antibodies to the virus. Viral proteins are fixed to a solid phase and used to capture Ab to HIV. Captured Ab are detected by using a conjugate, which consists of a color-producing enzyme attached to an immunologic component. The nature of this component can influence test performance.

Three gernerations of indirect assays for the detection of antibody to HIV-1 and HIV-2 are depicted below. The emerge of the development of simultaneously detecting HIV Ag and Ab assay considered to be the 4th generation of testing format represents a new era of testing is coming in a diagnostic settings.

First Generation *(Detect only HIV-1 Ab)*
It is an antigen-antibody sandwich reaction, which uses purified HIV viral lysate coated on a solid support, and goat anti-human antibody conjugated to Horseradish Peroxidase (HRPO) for detection.

Table 3. A Summary of the Development of Abbott HIV Assays.

Assay (Year)	Solid Phase	Conjugate	Antibody Detection	Antigen Detection
1st generation (1985)	Virus Lysate	Polyclonal anti-IgG	HIV-1	No
2nd generation (1989)	Recombinant antigen	Polyclonal anti-IgG	HIV-1/HIV-2	No
3rd generation (1995)	Recombinant antigen + synthetic peptide	Recombinant antigen and peptide	HIV-1/HIV-2 & HIV group O	No
4th generation (2002)	Recombinant antigen + synthetic peptide + anti-p24	Recombinant antigen and peptide + anti-p24	HIV-1/HIV-2 & HIV group O	Yes (p24)

The first generation assay can only detect human IgG anti-HIV-1 antibodies.

Second Generation *(Detect only HIV-1/2 Ab)*
It is also an antigen-antibody sandwich format. It uses recombinant HIV-1/2 viral proteins on the solid phase and the same goat anti-human antibody conjugate as the one used in the first generation assay. The second generation assay can detect human IgG anti-HIV-1/2 antibodies.

In first and second generation format, the conjugate is made with anti-human antibodies, which are not specifically directed to anti-HIV antibodies. As a result, false positive reactions may occur via non-specific interaction with the conjugate. Also, antibody conjugates can bind only to the heavy chain (Fc) of HIV antibody, which makes it very difficult to detect pentameric IgM antibodies.

Third Generation *(Detect HIV-1/2 Ab with increased sensitivity)*
It is also an antigen-antibody sandwich format. It uses recombinant viral proteins on the solid phase for the capture of antibodies and for the detection of bound antibodies, as conjugated to HRPO. This format can detect both human IgG and IgM anti-HIV-1/2 antibodies.

In the third generation format, recombinant proteins are used both as conjugates and as antigens on the solid phase. The main advantage of this format is that the conjugate binds to one of the other sides (Fab) of the antibody, which is still free after binding the antigen on the solid phase. Because of this, the antigen conjugate can easily bind to any type of antibody, including IgM. Thus, the sensitivity to HIV antibodies can be significantly increased, especially at the early stage of seroconversion.

Fourth Generation *(Detect HIV-1/2 Ab and HIV Ag simultaneously)*

AxSYM HIV Combo is used as an example. It uses a blend of microparticles as solid phase coated with HIV recombinant antigens, and for the capture of antibodies and microparticles coated with HIV-1 p24-specific monoclonal antibodies for the capture of HIV Ag in a test sample. The assay is designed with 3 washing steps, and using biotin-antibiotin signal enhancing measurement (Table 3).

Antigen (p24) testing in a combined format has been reported to reduce the seroconversion window by as few as several days to around 2 weeks compared with third generation antibody detection assays.[1, 2, 6, 8] It is critical that in a combined format, the sensitivity and specificity is not compromised. Sensitivity of the assay is demonstrated to be around 25 pg of HIV Ag/mL for the detection of HIV-1 group M, subtypes A to G and CRF A/E, and HIV-1 group O isolates.[6]

Summary

Transmission of HIV through blood transfusion and diagnosis of infection in hospitals, public health services, and in a diagnostics setting will continue to be our concern. Considering progress has been made in improving assay sensitivity, especially during the early stages of infection. Early prevention or treatment will be available only if we can identify infection earlier. Studies show that moving to antigen-antibody combination detection is more sensitive than antibody assays alone.[7] With a better assay, early detection is made possible to curb the rise of HIV infection and a new era of testing using 4th generation HIV Ag and Ab assay is at the doorstep now.

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