Updates in Immunoassays: Virology

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LEARNING OBJECTIVES:

1. Describe various immunoassay methods available for viral detection.
2. Compare and contrast automated immunoassay analyzers available for diagnostic use.
3. Discuss current and alternate algorithms for HIV testing.
4. Review FDA-cleared assays for HIV, HBV and HCV.
5. List lateral flow assays available for viral detection.

ABBREVIATIONS: Ag/Ab = antigen/antibody; CAP - College of American Pathologists; CLIA - chemiluminescent immunoassay; CMIA - chemiluminescent microparticle immunoassay; CMV - cytomegalovirus; EBV - Epstein-Barr virus; EIA - enzyme immunoassay; ELFA - enzyme-linked fluorescent assay; ELISA - enzyme-linked immunosorbent assay; FDA - Food and Drug Administration; GS - Genetic Systems; HBV - hepatitis B virus; HCV - hepatitis C virus; HIV - human immunodeficiency virus; HSV - *Herpes simplex* virus; IFA - immunofluorescent assay; MEIA - microparticle enzyme immunoassay; RSV - respiratory syncytial virus; VZV - *Varicella zoster* virus.

INDEX TERMS: Chemiluminescent immunoassay; enzyme-linked fluorescent assay; human immunodeficiency virus; immunoassay; lateral flow assay; microparticle enzyme immunoassay.

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The rapid identification of viral infectious agents has always been a challenge to clinical microbiologists. The long standing gold standard for diagnosing viruses is the cell culture,[1] however most cultures take up to 7 days for an accurate identification.[2] This turnaround time is unacceptable in healthcare where a rapid diagnosis and appropriate treatment is essential for positive patient outcomes and reduced hospital stays. With emerging technology and the variety of molecular platforms available, identifying viruses can be achieved in a matter of hours.[3,4] Although nucleic-acid amplification tests are highly sensitive and specific, "the performance of molecular assays can vary significantly due to nucleic acid extraction methods, primer and probe design, amplification and detection technologies, instrumentation, and technical expertise."[4] In recent years some of the self-contained molecular systems such as the GeneXpert System and SmartCycler System by Cepheid have eliminated these issues.[5] Although many laboratories have taken on molecular assays for identifying viruses, immunoassays are still widely used in the identification process due to the rapid, sensitive, and accurate results they produce, not to mention their cost effectiveness and ease of use.

Rapid direct antigen testing and immunofluorescent assays are widely used in the clinical laboratory; however, controversy exists regarding the sensitivity and specificity of some of the methodologies used for viral detection. In a recent review published in CAP Today, several invited speakers at the Association for Molecular Pathology's 2011 conference commented on the use of molecular platforms in identifying viruses and compared the sensitivity and specificity with rapid direct antigen testing.[6] The consensus was that caution must be taken when using rapid and fluorescent assays in viral detection due to the lower sensitivity, specificity, and positive predictive value as compared to molecular assays.[6] Clinical laboratories nevertheless still utilize rapid detection and fluorescent assays even though the literature suggests otherwise.[3,4,6]

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This article provides an overview of some of the automated and semi-automated platforms and assays available for viral detection. The principles and methodologies such as enzyme immunoassays (EIA), enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassays (CLIA), enzyme-linked fluorescent assays (ELFA), and microparticle enzyme immunoassays (MEIA) are discussed along with a list of some of the viral assays each instrument performs.

EIA and ELISA

These two terms tend to be used interchangeably however, ELISA should be used when either antigen or antibody is absorbed to a solid support like a microtiter plate, latex beads, or nitrocellulose membranes.[3,7] The ELISA is one of the most commonly used EIAs in the clinical laboratory. Many automated and semi-automated ELISA platforms are available and measure drug levels, hormone levels, tumor markers, infectious agents whether bacterial, viral, or parasitic, producing highly sensitive and specific results.[3,7,8]

In the ELISA, if *antigen* is absorbed to the solid support, the patient's antibody, if present, binds to the antigen and is detected after addition of a second anti-immunoglobulin enzyme-labeled antibody.[3,7] The amount of enzyme present is directly proportional to the amount of antibody found in the patient's serum.[7] If the *antibody* however is absorbed to the solid support the assay is refer to as either a "sandwich" or "capture" assay.[3,7] In this technique, antibody is absorbed to the solid support and the patient's serum (antigen) is added and allowed to react. An enzyme-labeled antibody is then added which "captures" the antigen at different epitopes causing the antigen to be "sandwiched" between the two antibodies.[3] Modifications have been made to this procedure by incorporating additional immunoreactants or by using monoclonal antibodies to increase sensitivity.[3,7,8]

Automated EIA and ELISA Instrumentation

There are several instruments on the market that are completely automated and provide "walk-away" workstations. Dynex Technologies[9] markets the DSX™ Four-Plate Automated ELISA Processing System which is user friendly, cost effective and can process up to four 96 well plates and 12 immunoassays simultaneously.[10] Dynex also distributes the DS2™ Two-Plate Automated ELISA Processing System which offers the same features but processes up to two 96 well plates and 12 different assays simultaneously, ideal for small volume testing.[11] Bio-Rad markets the EVOLIS™ System which is a self-contained automated, walk-away system using EIA technology. This instrument can accommodate mid- to high-volume testing (30,000 to 100,000 tests per year) and can process up to four plates with four separate assays per plate at one time.[12] Although there are several FDA cleared tests that are validated to run on the EVOLIS™ System, only one test has obtained clearance for use on the instrument itself—the GS HIV Combo Ag/Ab EIA manufactured by Bio-Rad.[13,14]

Diamedix Corp. markets the Mago® Plus and Mago® 4S systems, which utilize automated ELISA methodology.[15,16] The Mago® Plus can process up to four 96 well plates in one run and up to 9 different assays simultaneously.[15] The Mago® 4S System is a new generation automated immunoassay system that offers both ELISA and immunofluorescent assay (IFA) testing simultaneously. This system can process up to four ELISA plates and 16 IFA slides separately or in combination which increases workflow and laboratory productivity.[16,17] ELISA and IFA test kits for viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), *Herpes simplex* virus (HSV), rubella, mumps, measles, *Varicella zoster* virus (VZV) and respiratory syncytial virus (RSV) are available from Diamedix and are validated for both Mago® Plus and Mago® 4S systems.[18,19]

Human Immunodeficiency Virus Testing

One of the most widely tested viruses using ELISA and EIA technology in the clinical laboratory is the human immunodeficiency virus (HIV). The current algorithm for HIV immunoassay testing is screening with an EIA or ELISA and confirmation of positive specimens with either the western blot or an immunofluorescent assay.[20,21] The western blot is considered the gold standard and, although highly specific, lacks the sensitivity of the screening tests; therefore both tests are used in combination for a definitive result.[20,21] Because the western blot procedure is labor intensive, expensive, and difficult to interpret, several alternate algorithms have been proposed to eliminate its shortcomings. The proposed algorithms include an initial screening with an EIA/ELISA and confirmation of positive specimens

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with either a different EIA/ELISA, a nucleic acid amplification test, or a rapid test.[21,22,23] These alternate algorithms were presented at the 2010 HIV Diagnostics Conference[24] and the detailed report can be found at the following website http://www.aphl.org/aphlprograms/infectious/hiv/Documents/StatusReportFINAL.pdf.[22]

There are several FDA-cleared rapid HIV tests that have been available in the US since December, 2011. They include the *INSTI™ HIV-1 Antibody Test Kit* manufactured by bioLytical Laboratories, Inc.; *Reveal Rapid HIV-1 Antibody Test* by MedMira Laboratories, Inc.; *Uni-Gold Recombigen HIV* by Trinity Biotech plc.; *Multispot HIV-1/HIV-2 Rapid Test* by Bio-Rad Laboratories; *SURE CHECK HIV 1/2 Assay* and *HIV 1/2 STAT-PAK Assay* by Chembio Diagnostic Systems, Inc; and the *OraQuick ADVANCE Rapid HIV-1/2 Antibody Test* by OraSure Technologies.[25] Although controversy exists over the use of rapid tests[6] and additional tests are required for confirmation, these assays are a valuable tool when rapid results are needed, especially in an outreach or community setting.[26]

FDA-Cleared Viral Assays Using EIA Methodology

In addition to HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) can be identified using EIA technology. There are several FDA-cleared assays that utilize this methodology. Table 1 lists the trade name, infectious agent, and manufacturer.[25]

Chemiluminescent Immunoassays (CLIA)

For CLIAs, antigen and antibody complexes are identified by measuring the amount of light emitted in the reaction process read and interpreted on matched instruments.[3,7] Acridium esters and luminol are commonly used as they emit light when they move to a higher energy level.[3,7]

DiaSorin manufacturers the LIAISON® analyzer, which utilizes a chemiluminescent detection system and a magnetic microparticle solid phase.[27] It is a fully automated "walk-away" instrument that can run up to 15 different assays simultaneously with a maximum throughput of 180 tests per hour.[27] Kits are available which can detect measles, mumps, rubella, VZV, EBV, CMV, HSV-1 and HSV-2 and hepatitis A virus.[28]

In May 2011, the FDA cleared the Immulite 2000 XPi Immunoassay System manufactured by Siemens Healthcare Diagnostics for use in the U.S.[29] According to the press release, this instrument "offers the largest automated immunoassay test menus available today and features several innovative hardware and software solutions to enhance productivity and efficiency for medium- to high-volume clinical laboratories."[29] Test kits are available which identify CMV, HSV-1 and HSV-2, rubella, hepatitis and EBV.[30] In addition, Siemens Healthcare Diagnostics markets the ADVIA Centaur® XP Immunoassay System which utilizes direct chemiluminescent technology with a maximum throughput of 240 tests per hour.[31] In the area of viral detection, test kits are available that identify Hepatitis, HIV, CMV and Rubella.[32] There are several FDA cleared assays using chemiluminescent and chemiluminescent microparticle immunoassay (CMIA) technology for identification of HIV, HBV and HCV. These assays can be found along with the trade name, infectious agent, format and manufacturer in Table 2.[25]

Table 1. FDA-Cleared Viral Assays using EIA Technology[25]

Trademark	Infectious Agent	Manufacturer
GS HBsAg EIA 3.0	HBV	Bio-Rad Laboratories
ORTHO Antibody to HBsAg ELISA Test System	HBV	Ortho Clinical Diagnostics
Hepatitis C Virus Encoded Antigen (HCV Encoded Antigen/Enzyme ImmunoAssay (EIA) Version 3.0/ Recombinant and Synthetic)	HCV	Ortho Clinical Diagnostics
GS rLAV EIA	HIV-1	Bio-Rad Laboratories
Avioq HIV-1 Microelisa System	HIV-1	Avioq Inc.
HIVAB HIV-1 EIA	HIV-1	Abbott Laboratories
GS HIV-2 EIA	HIV-2	Bio-Rad Laboratories
Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA	HIV-1, HIV-2	Abbott Laboratories
GS HIV-1/HIV-2 Plus O EIA	HIV-1, HIV-2	Bio-Rad Laboratories
Ortho VITROS HIV-1/HIV-2	HIV-1, HIV-2	Ortho Clinical Diagnostics
Bio-Rad GS HIV Ag/Ab Combo EIA	HIV-1, HIV-2	Bio-Rad Laboratories

Abbreviations: AB or Ab: antibody, Ag: antigen, GS: Genetic Systems, HBsAg: hepatitis B surface antigen, rDNA: recombinant deoxyribonucleic acid, rLAV: recombinant HIV-1 strain labeled LAV

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Enzyme-linked Fluorescent Assays (ELFA)

The principle of the ELFA was discussed in the accompanying article entitled *"Updates in Immunoassays—Bacteriology"* and utilizes ELISA technology with a final fluorescent detection process.[33] The VIDAS® and miniVIDAS® manufactured by bioMérieux utilize this technology and provide various kits to detect viral infections agents. Assays are available to identify measles, mumps, rubella, CMV, VZV, and rotavirus.[34]

Table 2. FDA Cleared Viral Assays using CLIA or CMIA Technology[25]

Trademark	Infectious Agent/ Format	Manufacturer
ABBOTT PRISM HBsAg; ABBOTT PRISM HBsAg Confirmatory	HBV/CLIA	Abbott Laboratories Abbott Park, IL
ABBOTT PRISM HBcore	HBV/CLIA	Abbott Laboratories
ABBOTT PRISM HCV	HCV/CLIA	Abbott Laboratories
ABBOTT PRISM HIV O Plus	HIV/CLIA	Abbott Laboratories
ADVIA Centaur HIV 1/O/2Enhanced Ready Pack Reagents	HIV-1/HIV-2/ CMIA	Siemens Healthcare Diagnostics, Inc.
ARCHITECT HIV Ag/Ab Combo	HIV-1/HIV-2/ CMIA	Abbott Laboratories

Abbreviations: Ag: antigen, Ag/Ab: antigen/antibody, CLIA: chemiluminescent immunoassay, CMIA: chemiluminescent microparticle immunoassay, HBsAg: hepatitis B surface antigen, HBcore: hepatitis B core, HCV: hepatitis C, HIV: Human immunodeficiency virus, rDNA: Recombinant deoxyribonucleic acid, rLAV: Recombinant HIV-1 strain labeled LAV

Microparticle Enzyme Immunoassays (MEIA)

This technique, which is a variant of the ELISA method, utilizes *microparticles*; tiny beads where antibodies are bound.[3,35] If the antigen is present, it will form an antigen-antibody complex with the antibody-coated microparticles. The addition of an enzyme labeled antibody reacts on the substrate to produce a fluorescent product which is then measured in an automated analyzer.[3,35] Abbott Diagnostics markets the AxSYM Immunochemical Analyzer which utilizes MEIA technology.

The AxSYM, which can process up to 120 tests per hour and can run a wide range of tests concurrently, offers several tests that detect viral agents. These include CMV, rubella, hepatitis A, B, and C and HIV.[36,37] It should be noted that the lamp used in this instrument contains mercury so proper disposal techniques must be followed according to local, state and federal regulations.[36]

Lateral Flow Assays

The principle of lateral flow assays or immunochromatographic assays was discussed in the accompanying article, *Updates in Immunoassays—Bacteriology*. Several companies utilize this technology and market rapid, user-friendly kits in the area of virology. Table 3 lists some of the current assays available.[38]

Table 3. Lateral Flow Immunoassays for Viral Detection.[38]

Trademark	Manufacturer
FirstVue™ HIV, HBsAg	AT First Diagnostic LLC
ICON® Mono	Beckman Coulter Inc.
HIV 1/2 STAT-PAK®	Chembio Diagnostics, Inc.
OSOM ® Influenza, Mono	Genzyme Diagnostics
Clearview RSV, Mono, BinaxNOW® RSV, Influenza A & B	Alere, Inc.
ImmunoCard STAT! ® Rotavirus	Meridian Bioscience, Inc.
Xpect™ RSV, Xpect™ Rotavirus, Xpect™ Flu A&B	Oxoid Ltd.
QuickVue® RSV, Influenza A+B	Quidel Corporation

Abbreviations: HIV: human immunodeficiency virus, HBsAg: hepatitis B surface antigen, RSV: respiratory syncytial virus

Summary

Virus identification is a challenge to the clinical microbiologist since growing viruses in traditional cell culture is labor intensive, time consuming, and subject to contamination. The advent of rapid and automated immunoassays has eliminated this problem by generating positive results in minutes to hours. For example, testing for infectious mononucleosis can yield a positive result in 3–8 minutes as seen with the Beckman Coulter, Inc. ICON® Mono test[39] or in 5–15 minutes with the MONO Mononucleosis Rapid Test Device marketed by ACON Laboratories, Inc.[40] Fully automated immunoassay analyzers[41] provide fast, accurate, sensitive results that aid in a prompt and accurate diagnosis for the patient. Turnaround times are shortened, allowing for timely medical intervention and treatment. The priority in any hospital or medical facility is to treat the patient as quickly and appropriately as possible. By using immunoassays,

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clinical laboratory professionals are able to report out correct results in a timely manner, ensuring overall positive patient outcomes and improved quality of healthcare.

The author does not endorse any particular company or product and has no financial gain or interest in the products presented.

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