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Performance evaluation of three automated human immunodeficiency virus antigen–antibody combination immunoassays

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Abstract

Three fourth-generation antigen/antibody combination assays (Elecsys, AxSYM, Architect HIV) and two third-generation (AxSYM, Centaur) HIV antibody immunoassays were evaluated. The evaluation panel of 479 samples included: nine tissue culture derived HIV-1 strains at four different p24 antigen concentrations ($n = 36$), a p24 antigen sensitivity panel ($n = 10$), 149 HIV-1 or HIV-2 confirmed antibody positive samples, ten anti-HIV-1 positive low titer samples, three seroconversion panels ($n = 21$), and 253 HIV negative samples. The Architect had the best sensitivity for detection of HIV-1 antigen across eight HIV-1 subtypes, followed by the AxSYM while the Elecsys could not detect the highest antigen concentration evaluated (25 pg/mL) for eight of nine virus isolates. All assays showed 100% sensitivity for detection of HIV-1, group M, group O, and HIV-2 antibody positive samples. The Architect Ag/Ab Combo assay detected the first positive bleed of the three seroconversion panels and detected infection 4–26 days earlier than the third generation assays. Based on evaluation of 253 negative samples, assay specificity varied from 98.0% to 99.6%. The Architect HIV Ag/Ab Combo exhibited the best performance for specificity and detection of p24 antigen leading to closure of seroconversion window and demonstrating its utility for early diagnosis of HIV infection.

Keywords: Fourth generation immunoassay; Human immunodeficiency virus (HIV); p24 antigen; Seroconversion

1. Introduction

Early diagnosis of HIV infection is very important to prevent spread of HIV and to improve the opportunities for therapy. The window period for antibody detection usually lasts for approximately 30 days after infection. During this period clinical symptoms are mild, and replication of virus is evident based on presence of viral RNA and p24 antigen in blood (Vasudevachari et al., 2002; Huang et al., 2005). Over the past two decades, serologic methods for detection of HIV infection have evolved considerably. The early, first generation tests used HIV viral lysate proteins to capture antibodies and detection was based on use of antibodies, to human immunoglobulin IgG, conjugated to an enzyme such as horse radish peroxidase. The second generation assays for detection of HIV antibodies used HIV recombinant antigens for capture while the third generation assays used double antigen sandwich method. The third generation antibody assays were more sensitive as this format facilitated detection of IgM antibodies thus reducing the seroconversion window (Ly et al., 2001; Vasudevachari et al., 2002). The fourth generation HIV tests are designed to detect both HIV p24 antigen and antibody in single immunoassay (Weber et al., 1998; Sickinger et al., 2004). Several commercially available fourth generation assays are currently used worldwide and are rapidly replacing

Abbreviations: CRP, C-reactive protein; HBV, hepatitis B virus; HIV, human immunodeficiency virus; NASBA, nucleic acid sequence-based amplification; NT, test was not performed; RLU, relative light units; RT-PCR, reverse transcription-polymerase chain reaction; S/CO, signal to cut-off

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third generation assays (Courouce, 1999; Brust et al., 2000; Ly et al., 2001; Weber et al., 2002a, 2002b; Canna et al., 2003). The ability of the fourth generation assays to detect p24 antigen and reduce seroconversion window is the most important factor that has led to replacement of third generation assays. However, as reported in the literature, p24 antigen and seroconversion sensitivity of the fourth generation assays varies significantly. Several fourth generation assays have sensitivity equivalent to antibody assay or in some instances, have compromised antibody detection (Weber et al., 2002a, 2002b; Weber, 2003; Ly et al., 2004). In this study, we have evaluated sensitivity and specificity of three fourth-generation assays and two third-generation HIV assays currently available in Korea.

2. Materials and methods

2.1. HIV assays

All assays were performed following the manufacturers' package insert.

2.1.1. Architect HIV Ag/Ab Combo

Architect HIV Ag/Ab Combo (Abbott Laboratories GmBH, Delkenheim, Germany) is a chemiluminescent magnetic microparticle-based immunoassay used to determine presence of HIV-1 p24 antigen and antibody to HIV-1 group M, HIV-1 group O, and HIV-2 by an automated, random access instrument. The Architect HIV Ag/Ab Combo uses recombinant antigens and synthetic peptides derived from HIV viral transmembrane proteins (TMP) of HIV-1 group M, HIV-1 group O, and HIV-2 for antibody detection and anti-p24 monoclonal antibodies for antigen detection. Briefly, serum or plasma sample is incubated with recombinant antigen and monoclonal antibody-coated microparticles. After a wash step, acridinium-labeled HIV antigens and HIV p24 antibody conjugates are added that bind to the captured antibody or antigen in the test sample. After a wash step, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLU). The concentration of HIV p24 antigen and antibody in sample are directly proportional to chemiluminescent signal in the reaction. Specimens with signal to cut-off (S/CO) values greater than or equal to 1.00 are considered reactive.

2.1.2. AxSYM HIV Ag/Ab Combo

AxSYM HIV Ag/Ab Combo (Abbott Laboratories GmBH, Delkenheim, Germany) is a microparticle enzyme immunoassay used to determine presence of HIV p24 antigen and antibody. This assay utilizes a blend of microparticles coated with HIV recombinant antigens, representing the TMP for HIV-1 group M, HIV-1 group O, and HIV-2, for the capture of antibodies and microparticles coated with HIV-1 p24 monoclonal antibodies for the capture of HIV antigen. The AxSYM assay uses biotinylated conjugates that are detected by rabbit anti-biotin antibody conjugated to alkaline phosphatase. The assay principle is similar to the Architect combination assay and was described in details previously (Ly et al., 2001, 2004; Sickinger et al., 2004).

2.1.3. Elecsys 2010 HIV Combi

Elecsys 2010 HIV Combi (Roche, Switzerland) is electrochemiluminescence immunoassay. It detects HIV-1/HIV-2 antibodies using recombinant antigens derived from the polymerase and envelope regions of HIV-1 and HIV-2 and for the detection of HIV-1 p24 antigen, monoclonal antibodies are used. The serum or plasma of each sample is incubated with biotinylated monoclonal anti-p24 antibody, HIV recombinant antigens, and HIV peptides, and with monoclonal anti-p24 antibodies, HIV-specific recombinant antigens, HIV-specific peptides labeled with ruthenium complex, and then incubated with the streptavidin-coated microparticles. After incubation, the reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured. The electro-chemiluminescence signal is proportional to antigen and/or antibody present in the test sample and is detected by Elecsys automated instrument.

2.1.4. AxSYM HIV 1/2/O

AxSYM HIV 1/2/O (Abbott Laboratories, GmBH, Delkenheim, Germany) is a three step microparticle enzyme immunoassay that detects HIV antibodies to HIV-1 group M, HIV-1 group O, and HIV-2. Antibodies are captured by recombinant HIV antigen-coated microparticles. Captured antibodies are bound to biotin labeled recombinant antigen and peptide, and detected by an anti-biotin alkaline phosphate conjugate.

2.1.5. Centaur HIV 1/2/O

Centaur HIV 1/2/O (Bayer, Germany) is a microparticle chemiluminometric immunoassay that detects antibodies to HIV-1 group M, HIV-1 group O, and HIV-2. The assay is a two wash antigen sandwich immunoassay described in details previously (van Helden et al., 2004). The solid phase contains a preformed complex of streptavidin-coated microparticles and biotinlylated HIV-1 and HIV-2 recombinant antigens and peptides. These antigens capture antibodies to HIV-1 and HIV-2 in the patient sample. HIV-1 and HIV-2 recombinant antigens and peptides labeled with acridinium ester detect anti-HIV and HIV-2 antibodies captured by the solid phase.

2.1.6. Elecsys HIV antigen

Elecsys HIV Ag assay (Roche, Switzerland) is electrochemiluminescence immunoassay for qualitative determination of HIV-1 p24 antigen. The serum or plasma of each sample is incubated with a biotinylated monoclonal anti-p24 antibody and a monoclonal HIV p24 antibody labeled with ruthenium and allowed to form a sandwich complex. After addition of streptavidin-coated microparticles and incubation, the reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured on the surface of the electrode. The Elecsys software determines results automatically by comparing the electro-chemiluminescence signal obtained from sample with the cut-off values previously obtained by HIV antigen calibration.