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Comparison of 3 Automated Immunoassays for Detection of Anti-Hepatitis A Virus Immunoglobulin M in a Tertiary Care Hospital

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Three automated immunoassay kits for anti-Hepatitis A Virus (HAV) IgM – Architect, (Abbott Laboratories, USA), Elecsys (Roche Diagnostics, Germany), and ADVIA Centaur (Siemens Healthcare Diagnostics Inc., USA) – were compared. We included 178 consecutive samples, for which an anti-HAV IgM test was requested at Seoul National University Hospital from September 2009 to January 2010. Reviewing of medical records, reverse transcription (RT)-PCR for HAV RNA, or total anti-HAV assay were performed on 16 (9.0%) samples with discrepant results. The percent agreements (kappas) of the Architect and ADVIA Centaur, Architect and Elecsys, and ADVIA Centaur and Elecsys kits were 96.6% (0.91), 96.6% (0.92), and 97.8% (0.94), respectively. Eight out of 16 discrepant samples showed gray-zone values in Architect but were nonreactive in the others. Slightly earlier seroconversion was suspected in Elecsys. The 3 assays showed comparable performances with excellent agreements in a tertiary care hospital setting.

Key Words: Anti-hepatitis A virus immunoglobulin M, Immunoassay, Agreement

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An anti-hepatitis A virus (HAV) IgM test is crucial to diagnose current HAV infection. Commercialized anti-HAV IgM chemiluminescence immunoassay has been widely used recently because of its significantly improved specificity and technical simplicity [1], although reports on performance are scarce [2, 3]. Performance of 3 anti-HAV IgM assays – Architect HAV Antibody (HAVAb)-IgM (Abbott Laboratories, Abbott Park, IL, USA), Elecsys Anti-HAV IgM (Roche Diagnostics, Mannheim, Germany), and ADVIA Centaur HAV IgM (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) – was compared under routine conditions in the clinical laboratory of Seoul National University Hospital.

The study included 178 consecutive samples for immediate anti-HAV IgM testing using Architect HAVAb-IgM between September 2009 and January 2010. We collected the remaining sera as aliquots in 1.5 mL tubes immediately after the Architect HAVAB-IgM test and stored them at -80°C until analysis. Elecsys and ADVIA Centaur HAV IgM were performed on the same day according to the manufacturers' instructions. For Architect, signal-to-cutoff (S/CO) values of 0.80-1.20 were considered gray-zone values. For ADVIA Centaur, an S/CO $\geq$0.80 and $<$1.20 was considered equivocal.

Medical records were reviewed, or reverse transcription (RT)-PCR for HAV and ADVIA Centaur total HAV were performed for 16 sera showing discrepant results. RNA was extracted using a Chemagic Viral DNA/RNA preparation kit (Chemagen, Baesweiler, Germany), and RT-PCR was performed using the AccuPower HAV Real-Time RT-PCR kit (Bioneer Corp., Daejeon, Korea). This

study was approved by the Seoul National University Hospital Institutional Review Board (E-1110-046-381).

The agreements (kappas) between assays were calculated [4]. Correlations in S/CO values between assays were evaluated by a Spearman's test, excluding those results exceeding the measurable range using SPSS for Windows (version 12.0; SPSS Inc., Chicago, IL, USA).

Among 178 samples, 45 (25.3%) were reactive and 117 (65.7%) were nonreactive for all 3 kits. When the gray-zone results of Architect and ADVIA Centaur were interpreted as nonreactive, the percent agreements (kappas) between Architect and ADVIA Centaur, Architect and Elecsys, and ADVIA Centaur and Elecsys were 96.6% (0.91), 96.6% (0.92), and 97.8% (0.94), respectively. Among the 16 (9.0%) discrepant sera, 8 (case 1-8, Table 1) showed gray-zone values with Architect, but they were nonreactive with ADVIA Centaur and Elecsys. The negative anti-HAV IgM follow-up tests indicated that cases 1 and 2 were less likely to have HAV infection. For cases 3-8, HAV infection could not be ruled out from additional test results (HAV RT-PCR, negative; total anti-HAV, reactive). Case 9 (Architect, reactive; others, nonreactive) and Case 10 (ADVIA Centaur, reactive; others, nonreactive) were also less likely to have HAV infection considering the negative HAV RT-PCR, although very high levels of AST and ALT were seen.

Cases 11 and 12, confirmed as HAV+ (positive RT-PCR), were nonreactive with ADVIA Centaur but reactive with Elecsys. Cases 13 and 14, confirmed as HAV+ from reactive results with higher S/CO values of follow-up anti-HAV IgM tests in all 3 assays, showed gray-zone results with Architect and were reactive with Elecsys. Case 13 was nonreactive with ADVIA Centaur.

Cases 15 and 16, with infection history (7 and 8 months ago, respectively), (reactive anti-HAV IgM and clinical course consistent with HAV infection) were reactive with ADVIA Centaur and Elecsys and nonreactive and in the gray-zone with Architect, respectively.

Although, these assays were not quantitative, their S/CO values were moderately correlated with each other. Spearman's correlation coefficient (r) between Architect and the ADVIA Centaur HAV IgM was 0.757 ($P<0.001$); Architect and Elecsys, 0.732 ($P<0.001$); and Elecsys and ADVIA Centaur, 0.776 ($P<0.001$) (Fig. 1).

Here, 3 kits showed excellent overall agreement (kappas: 0.91-

Table 1. Clinical characteristics of cases with discrepant results among Architect, ADVIA Centaur, and Elecsys Anti-HAV IgM assays (N=16)

Case No.	HAV IgM Architect (S/CO)*	HAV IgM ADVIA Centaur (S/CO)*	HAV IgM Elecsys (S/CO)*	F/U HAV IgM (days since first bleed)	Anti-HAV IgG	Total anti-HAV	RT-PCR	AST/ALT (IU/L)	T.bil/D.bil (mg/dL)	Clinically suspected diagnosis
1	G (0.9)	N (0.05)	N (0.25)	N (8)	NT	R	N	1,015/190	2.3/1.5	Common bile duct stone
2	G (1.0)	N (<0.02)	N (0.24)	N (3)	N	R	N	532/342	1.1/0.4	Gallbladder stone
3	G (0.8)	N (0.03)	N (0.26)	NT	R	R	N	27/42	1.0/0.2	Toxic hepatitis
4	G (1.0)	N (0.08)	N (0.21)	NT	NT	N	N	45/74	0.7/NT	Leptospirosis
5	G (1.0)	N (0.07)	N (0.23)	NT	NT	R	N	64/306	1.1/NT	Diabetes mellitus, hepatitis
6	G (0.9)	N (<0.02)	N (0.22)	NT	NT	N	N	147/204	1.6/0.4	Amyopathic dermatomyositis
7	G (1.1)	N (0.07)	N (0.30)	NT	NT	R	N	316/84	2.0/1.1	Metastatic breast cancer
8	G (0.9)	N (0.21)	N (0.26)	NT	NT	R	N	129/325	22.8/16.3	Toxic hepatitis
9	R (1.5)	N (0.06)	N (0.23)	NT	NT	N	N	1,031/3,467	0.7/NT	Toxic hepatitis
10	N (0.4)	R (4.21)	N (0.20)	N (4)	N	N	N	15,864/8,340	8.0/NT	Alcoholic hepatitis
11	G (0.9)	N (0.66)	R (1.17)	NT	N	R	P	3,385/2,627	1.3/NT	HAV hepatitis
12	R (1.4)	N (0.77)	R (1.04)	R (1)	N	R	P	2,150/703	2.0/NT	HAV hepatitis
13	G (0.9)	N (0.22)	R (3.75)	R (4)	NT	R	NT	2,134/3,053	2.4/NT	HAV hepatitis
14	G (1.1)	R (2.85)	R (4.23)	R (3)	NT	R	NT	382/1,407	3.5/NT	HAV hepatitis
15	N (0.5)	R (1.50)	R (1.09)	NT	NT	R	NT	19/18	0.8/NT	Resolving HAV hepatitis
16	G (0.9)	R (1.66)	R (1.14)	NT	R	R	NT	587/557	14.5/8.5	Resolving HAV hepatitis

*For Architect HAVAb-IgM, specimens with signal-to-cutoff (S/CO) values 0.80-1.20 were considered gray-zone. For ADVIA Centaur HAV IgM, S/CO values ≥0.80 and <1.20 were considered equivocal.
Abbreviations: T.bil, total bilirubin; D.bil, direct bilirubin; F/U, follow up; HAV, hepatitis A virus; N, nonreactive or negative; G, gray zone; R, reactive; P, positive; NT, not tested; S/CO, signal-to-cutoff; RT-PCR, reverse transcription-PCR.

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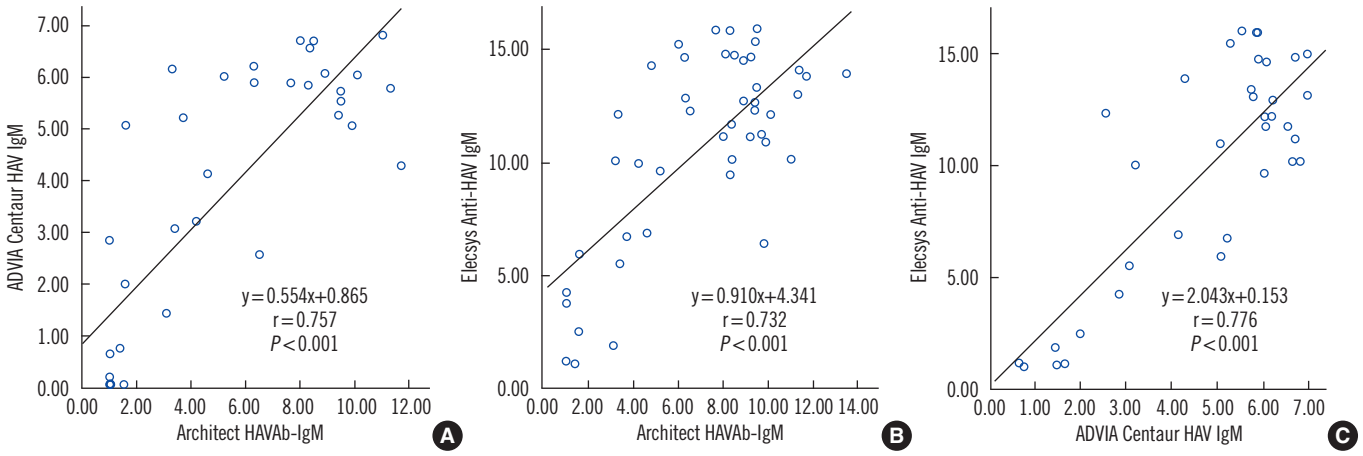

Fig. 1. Correlations of signal-to-cutoff (S/CO) values among Architect HAVAb-IgM, ADVIA Centaur HAV IgM, and Elecsys Anti-HAV IgM assays. (A) Scatter plot of S/CO values of Architect HAVAb-IgM and ADVIA Centaur HAV IgM assays, (B) Architect HAVAb-IgM and Elecsys Anti-HAV IgM assays, and (C) ADVIA Centaur HAV IgM and Elecsys Anti-HAV IgM assays.
Abbreviation: HAV, hepatitis A virus.

0.94) when the gray-zone values of Architect were considered nonreactive (ADVIA Centaur showed no equivocal results). Architect showed gray-zone results in 12 samples: HAV infections, 4; less-likely infections, 2; uncertain for infection, 6. The agreement was slightly lower (kappa values: Architect and ADVIA Centaur, 0.81; Architect and Elecsys, 0.87; data not shown) when the gray-zone values of Architect were considered reactive.

ELISAs can exhibit false-reactive results in various conditions, including autoimmune diseases or renal failure [5]. Rheumatoid factor or heterophilic antibodies can also interfere with immunoassay results [6, 7]. Nonspecific binding of serum IgM to the microparticle bead induces false reactivity in the Liaison system adopting chemiluminescence immunoassay, in the absence of rheumatoid factor or paraprotein; the use of chemical-blocking reagents eliminated this problem [8]. The Architect system adopts a different assay principle (direct coating of HAV antigens on a microparticle bead) from that of the other assays (using streptavidin-coated microparticles and biotinylated mouse anti-human IgM antibodies). Further investigations are needed to determine if gray-zone results, more frequently observed with Architect, could be partially explained by the nonspecific adsorption of proteins to the microparticle bead.

In cases 11-14, in the early phase of HAV infection, the ADVIA Centaur and Architect showed slightly later seroconversions compared to the Elecsys. Two cases with history of HAV infection (~7-8 months ago) were reactive with ADVIA Centaur and Elecsys with low S/CO values (1.09-1.66), whereas Architect showed nonreactive in one sample and gray-zone in another, suggesting a slight difference in the sensitivity for the detection of decreasing anti-HAV IgM in patients who had recovered from previous HAV infection.

Although all 3 kits are not quantitative tests, the S/CO values showed moderate correlations among them. For samples from patients with resolving HAV infection, S/CO values were low (1.09-1.66), suggesting very low anti-HAV IgM levels. Further development of quantitative tests for anti-HAV IgM may be helpful in patients showing atypical disease courses during HAV infection or HAV reactivation after transplantation [9].

In conclusion, 3 automated immunoassay kits showed comparable performances, with excellent overall agreement among them when performed on samples submitted to a tertiary care hospital and can be successfully applied in clinical laboratory practice.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Dufour DR, Talastas M, Fernandez MD, Hwang JH, Kim JW, Kim NY, et al. Chemiluminescence assay improves specificity of hepatitis C antibody detection. Clin Chem 2003;49:940-4.
2. Hess G, Faatz E, Melchior W, Bayer H. Analysis of immunoassays to detect antibodies to hepatitis A virus (anti-HAV) and anti-HAV immunoglobulin M. J Virol Methods 1995;51:221-8.
3. Wiedmann M, Boehm S, Schumacher W, Swysen C, Zauke M. Evaluation of three commercial assays for the detection of hepatitis a virus.

Eur J Clin Microbiol Infect Dis 2003;22:129-30.
4. Clinical and Laboratory Standards Institute. Assessment of laboratory tests when proficiency testing is not available; Approved guideline. 2nd ed. CLSI document, GP29-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
5. Mylonakis E, Paliou M, Lally M, Flanigan TP, Rich JD. Laboratory testing for infection with the human immunodeficiency virus: established and novel approaches. Am J Med 2000;109:568-76.
6. Cavalier E, Carlisi A, Chapelle JP, Delanaye P. False positive PTH results: an easy strategy to test and detect analytical interferences in routine practice. Clin Chim Acta 2008;387:150-2.
7. Kellar KL, Kalwar RR, Dubois KA, Crouse D, Chafin WD, Kane BE. Multiplexed fluorescent bead-based immunoassays for quantitation of human cytokines in serum and culture supernatants. Cytometry 2001;45:27-36.
8. Berth M and Bosmans E. Prevention of assay interference in infectious-disease serology tests done on the liaison platform. Clin Vaccine Immunol 2008;15:891-2.
9. Eisenbach C, Longerich T, Fickenscher H, Schalasta G, Stremmel W, Encke J. Recurrence of clinically significant hepatitis A following liver transplantation for fulminant hepatitis A. J Clin Virol 2006;35:109-12.

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