Clinical Biochemistry 48 (2015) 1330–1336

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Development and evaluation of analytical performance of a fully automated chemiluminescent immunoassay for protein induced by vitamin K absence or antagonist II

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ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 9 July 2015
Accepted 13 July 2015
Available online 22 July 2015

Keywords:
ARCHITECT
Chemiluminescent microparticle immunoassay
DCP
Hepatocellular carcinoma
PIVKA-II
Tumor marker

ABSTRACT

Objectives: Protein induced by vitamin K absence or antagonist II (PIVKA-II), an abnormal form of prothrombin, has been used as an aid in the diagnosis of hepatocellular cancer (HCC) as a tumor marker. We developed a fully automated quantitative immunoassay for PIVKA-II on the ARCHITECT® *i* systems. The aim of this study was to evaluate the analytical performance of this assay.

Design and Method: Assay imprecision, sensitivity, dilution linearity, high dose hook effect, sample type equivalency, assay interferences of potential interfering materials and correlation with Picolumi PIVKA-II (Eidia, Tokyo, Japan) were evaluated.

Results: The percentage coefficient of variation (%CV) of total imprecision ranged from 2.8% to 5.4% with 10 levels of samples. The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were less than 0.63 mAU/mL, 1.62 mAU/mL, and 8.25 mAU/mL, respectively. Linearity up to 30,000 mAU/mL, no high dose hook effect, no difference among sample types and no interference of common drugs and endogenous substances were observed. Correlation study with the Picolumi PIVKA-II gave a correlation coefficient of 0.93 and a regression slope of 1.07.

Conclusions: The results demonstrate that the fully automated prototype ARCHITECT PIVKA-II assay is an accurate, highly sensitive and precise assay for the measurement of PIVKA-II levels in human sera and plasmas.

1. Introduction

Liver cancer is the fifth most common cancer in men and the seventh in women but is the third leading cause of cancer death, because of its high fatality rate (mortality is 93% of incidence) [1,2]. About 85% of the burden is in developing counties with more than 50% in China alone. The regions with high incidence of liver cancer are West and Central Africa, and East and Southeast Asia. In contrast, incidence rates are generally low in developed countries except Japan and Southern Europe [1,2]. Hepatocellular carcinoma (HCC) is the major histologic type among primary liver cancers occurring worldwide, accounting for 70% to 85% of total burden [3].

Chronic infection by either hepatitis B virus (HBV) or hepatitis C virus (HCV) is a major risk factor for HCC. Approximately 80% of HCC is estimated to be attributable to HBV or HCV worldwide [4]. Carcinogenic risk of HBV carriers is 223-fold that of non-carriers [5]. Infection by HCV is the most common predisposing factor in some developed countries, including Japan [6].

Recently in some historical high risk regions, liver cancer rates decreased, possibly due to a reduction in HBV infection by improvement of hygiene and sanitation conditions. In contrast, incident rates are increasing in some developed areas, including the United State and Central Europe, possibility due to the obesity epidemic and the rise in HCV infection through continued transmission by injection drug users [7]. During the past three decades from 1980 to 2010, the overall age-adjusted incidence rate of liver cancer in the United State has tripled from 2.63 to 8.07 per 100,000 persons [8].

One of the reasons for the high fatality rate of HCC is the difficulty of detection for HCC in early stage since it is an asymptomatic disease, therefore many patients are not treated in the early stages. Early detection of HCC by surveillance in high risk groups is an appropriate way to improve the survival rate in patients with HCC [9–14].

Abbreviations: PIVKA-II, Protein induced by vitamin K absence or antagonist II; HCC, hepatocellular cancer; LoB, limit of blank; LoD, limit of detection; LoQ, limit of quantitation; %CV, percentage coefficient of variation; HBV, hepatitis B virus; HCV, hepatitis C virus; DCP, des-gamma-carboxy prothrombin; AFP, alpha-fetoprotein; AFP-L3, AFP fraction with affinity to the Lens culinaris agglutinin; GLA, gamma-carboxyglutamic acid.

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http://dx.doi.org/10.1016/j.clinbiochem.2015.07.023
0009-9120/

Protein induced by vitamin K absence or antagonist II (PIVKA-II), also known as des-gamma-carboxy prothrombin (DCP), is an abnormal form of the coagulation protein, prothrombin. PIVKA-II is a functionally defective prothrombin resulting from decline of the carboxylation of 10 glutamic acid residues at the N-terminus [15].

The level of PIVKA-II is elevated in patients with HCC. PIVKA-II level is not correlated with the levels of alpha-fetoprotein (AFP) or AFP fraction with affinity to the Lens culinaris agglutinin (AFP-L3), which are other representative biomarkers for HCC. Therefore PIVKA-II can be used along with AFP or AFP-L3 as a complementary biomarker for HCC. Thus, the combination of two markers enhances the sensitivity of diagnosis of HCC, but minimizes the decrease in specificity [15–18]. The Japan Society of Hepatology recommends measurement of two or more tumor markers for the diagnosis of small HCC [19].

Many studies showed serum PIVKA-II levels are related to tumor size, vascular invasion, intrahepatic metastasis and frequency of recurrence after treatment, so PIVKA-II also can be used as a prognostic predictor in patients with HCC [20–24]. PIVKA-II may assume a crucial role to decide the most effective therapy according to tumor characterization.

PIVKA-II has been used as an aid for diagnosis of HCC and monitoring of high risk patients (HCV infections, hepatitis/cirrhosis, HBV infections) for development of HCC for more than 20 years in Japan [19].

We report here our development and evaluation of the analytical performance of a fully automated immunoassay for PIVKA-II on the ARCHITECT *i* systems (Abbott Japan, Tokyo, Japan).

2. Materials and methods

2.1. Assay principle

The ARCHITECT PIVKA-II assay is a two-step immunoassay, using chemiluminescent paramagnetic microparticle technology for quantitative determination of PIVKA-II. The analyte, PIVKA-II, is captured by paramagnetic microparticles coated with a recombinant murine monoclonal anti-PIVKA-II antibody 3C10 (Abbott Laboratories, IL, USA), which recognizes an epitope in PIVKA-II within the gamma-carboxyglutamic acid (GLA) domain (amino acid 13–27) at the N-terminus. The analyte-microparticle complex is detected with an acridinium labeled murine anti-prothrombin monoclonal antibody MCA 1–8 (Abbott Laboratories) conjugate, which recognizes an epitope at the N-terminus in prothrombin (Fig. 1).

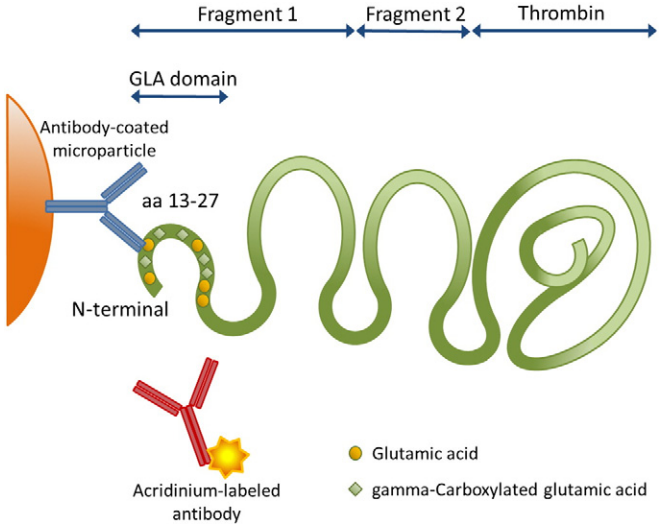

Fig. 1. Assay principal of the prototype Architect PIVKA-II assay. Capture antibody recognize an epitope in the gamma-carboxyglutamic acid (GLA) domain (amino acid (aa) 13–27), tracer antibody recognize an epitope in the prothrombin part.

In the first step, 30 μl of sample, 50 μl of assay buffer and 50 μl of anti-PIVKA-II antibody coated microparticles are combined and incubated for 18 min. After washing, 50 μl of acridinium labeled anti-prothrombin antibody conjugate is added and incubated for 4 min. Following an additional wash step, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). The RLUs detected by the ARCHITCT optical system is related to the amount of PIVKA-II Ag in the sample. The assay is fully automated and an assay result can be obtained within 30 min after initial sample aspiration. The measuring range of this assay was designed from 10 to 30,000 mAU/mL. Calibrators were prepared from PIVKA-II antigen diluted in buffer solution containing bovine serum albumin. The calibrator levels are 0, 40, 100, 300, 5000 and 30,000 mAU/mL.

2.2. PIVKA-II antigen

PIVKA-II antigen was prepared from human prothrombin (Enzyme Research, IN, USA) by a thermal decarboxylation method described by Bajaj et al. [25]. PIVKA-II value of the antigen was determined by comparison to the Picolumi PIVKA-II Assay (Eidia, Tokyo, Japan).

2.3. Specimens

AFP positive specimens were obtained from ProMedDx, LLC (MA, USA). Normal specimens from apparently healthy individuals were obtained from ProMeDx, LLC, C-C Biotech (CA, USA) and Denka Seiken Co., Ltd. (Tokyo, Japan). All human specimens used for this study were collected under institutional review board approved protocols.

2.4. Evaluation methods

Assay imprecision was evaluated according to the Clinical and Laboratory Standards Institute (CLSI) guideline EP5-A3 [26]. Three levels of buffer based PIVKA-II quality controls, four levels of serum based panels and three levels of plasma based panels were assayed in replicates of three at two separate times per day for over 20 days using three lots of reagents and four instruments including three different versions (two $i1000_{SR}$, one $i2000_{SR}$ and one *i*2000). A high plasma panel spiked with PIVKA-II antigen at ~75,000 mAU/mL level was tested using the 1:10 autodilution protocol.

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined according to the CLSI guideline EP17-A2 [27]. Four zero-concentration samples and eight low concentration PIVKA-II samples (0.625, 1.25, 2.5, 5, 7.5, 9, 10, 15 mAU/mL) were assayed in replicates of two during five different days using three reagent lots and five instruments (two $i1000_{SR}$ and three *i*2000). LoB was set as the 95th percentile of the zero-concentration samples' values. For LoD calculation, normality of the distribution of the lowest concentration PIVKA-II sample which 95% replicate showed over LoB was confirmed using the Shapiro–Wilk test. After the confirmation, LoD was calculated using the equation: $LoD = LoB + (C_\beta * SD_S)$, where $C_\beta = 1.645 / [1 - 1 / (4 * f)]$, f = degrees of freedom of SD_S, SD_S = standard deviation (SD) of the low concentration samples. LoQ was set as the lowest concentration that showed a total error of 30% (10% bias + 2 × 10% CV). LoB, LoD and LoQ were calculated from merged data of all reagent lots on each instrument.

Reagent on-board stability studies were performed with three lots of reagents using nine instruments. Three levels of buffer based PIVKA-II quality controls, four levels of serum based panels and three levels of plasma based panels were assayed in replicates of five at 20 test points using reagents stored on-board the instrument during 30 days. A calibration curve was established on the initial day and concentration of each sample was determined using the stored calibration curve. Concentrations of the samples were evaluated for trending over time.

Dilution linearity was assessed according to the CLSI guideline EP06-A [28] with two serum and one plasma specimens spiked with PIVKA-II

Table 1
Total assay imprecision (%CV).

n = 120

Sample	Instrument type	*i*1000$_{SR}$			*i*1000$_{SR}$			*i*2000			*i*2000$_{SR}$		
	Reagent lot number	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
Quality control-1	Mean (mAU/mL)	49.39	51.22	48.52	48.58	47.48	49.04	52.20	47.91	48.67	50.92	49.12	50.01
	SD (mAU/mL)	2.07	1.62	1.83	2.08	1.68	2.06	1.73	1.70	1.83	1.63	1.50	1.47
	CV	4.2%	3.2%	3.8%	4.3%	3.5%	4.2%	3.3%	3.5%	3.8%	3.2%	3.1%	2.9%
Quality control-2	Mean (mAU/mL)	484.59	512.08	470.81	478.84	484.71	475.68	490.87	471.31	477.95	488.49	487.93	496.88
	SD (mAU/mL)	18.56	16.56	14.70	16.51	17.88	19.14	14.94	15.00	13.50	13.90	13.90	14.75
	CV	3.8%	3.2%	3.1%	3.4%	3.7%	4.0%	3.0%	3.2%	2.8%	2.8%	2.8%	3.0%
Quality control-3	Mean (mAU/mL)	9779.61	9810.52	9392.19	9807.30	10,020.86	9505.93	9988.98	9284.40	9441.06	9877.19	9591.38	10,273.99
	SD (mAU/mL)	388.25	358.94	312.28	368.43	413.75	385.90	486.79	374.25	271.04	530.93	441.68	398.87
	CV	4.0%	3.7%	3.3%	3.8%	4.1%	4.1%	4.9%	4.0%	2.9%	5.4%	4.6%	3.9%
Serum panel-1	Mean (mAU/mL)	20.43	22.10	21.38	20.63	20.70	22.29	21.67	20.28	21.18	22.15	21.49	22.51
	SD (mAU/mL)	0.92	0.97	0.90	1.00	1.06	1.03	1.04	0.88	0.85	1.01	0.91	0.98
	CV	4.5%	4.4%	4.2%	4.8%	5.1%	4.6%	4.8%	4.3%	4.0%	4.6%	4.2%	4.4%
Serum panel-2	Mean (mAU/mL)	43.76	46.85	45.52	44.27	44.49	46.75	45.97	43.59	45.08	46.89	45.46	47.17
	SD (mAU/mL)	1.66	1.53	1.38	1.79	1.85	1.68	1.33	1.75	1.61	1.85	1.87	1.66
	CV	3.8%	3.3%	3.0%	4.0%	4.1%	3.6%	2.9%	4.0%	3.6%	3.9%	4.1%	3.5%
Serum panel-3	Mean (mAU/mL)	243.29	253.92	247.70	247.88	253.26	256.67	245.00	236.37	244.29	252.81	250.30	257.94
	SD (mAU/mL)	7.98	7.75	8.91	9.45	8.04	8.26	6.95	7.67	7.30	7.56	8.13	8.38
	CV	3.3%	3.1%	3.6%	3.8%	3.2%	3.2%	2.8%	3.2%	3.0%	3.0%	3.2%	3.2%
Serum panel-4	Mean (mAU/mL)	1934.50	1980.87	1909.98	1984.26	2048.24	1982.26	1922.53	1875.93	1911.39	1940.36	1974.14	2041.62
	SD (mAU/mL)	57.55	64.10	55.73	71.37	69.50	59.05	61.05	59.63	61.92	66.57	69.08	68.79
	CV	3.0%	3.2%	2.9%	3.6%	3.4%	3.0%	3.2%	3.2%	3.2%	3.4%	3.5%	3.4%
Plasma panel-1	Mean (mAU/mL)	26.33	28.51	28.49	26.52	26.60	29.63	27.42	25.84	27.92	27.95	26.92	29.08
	SD (mAU/mL)	1.03	1.36	1.04	1.11	1.32	1.14	1.22	1.06	1.20	1.16	1.09	1.17
	CV	3.9%	4.8%	3.6%	4.2%	5.0%	3.9%	4.4%	4.1%	4.3%	4.2%	4.0%	4.0%
Plasma panel-2	Mean (mAU/mL)	251.77	264.19	256.71	256.86	260.46	265.01	254.59	246.51	257.36	261.02	259.16	268.43
	SD (mAU/mL)	7.17	8.16	10.30	7.62	9.27	8.22	8.27	7.25	7.93	8.07	9.43	8.02
	CV	2.8%	3.1%	4.0%	3.0%	3.6%	3.1%	3.2%	2.9%	3.1%	3.1%	3.6%	3.0%
Plasma panel-3	Mean (mAU/mL)	77,267.12	78,785.77	74,362.72	78,800.74	81,700.78	76,808.19	75,779.80	71,850.25	72,301.50	75,424.25	76,125.15	78,788.15
	SD (mAU/mL)	2302.29	2864.63	2426.01	2505.25	2351.07	2837.68	3225.02	2580.41	2507.28	3713.16	3626.03	2888.44
	CV	3.0%	3.6%	3.3%	3.2%	2.9%	3.7%	4.3%	3.6%	3.5%	4.9%	4.8%	3.7%

CV: coefficient of variation, SD: standard deviation.

Table 2
LoB, LoD and LoQ using 5 instruments.

Instrument type/number	LoB (mAU/mL)	LoD (mAU/mL)	LoQ (mAU/mL)
*i*2000-1	0.58	1.61	6.92
*i*2000-2	0.34	1.17	4.91
*i*2000-3	0.37	0.85	2.38
$i1000_{SR}$-1	0.00	0.73	8.25
$i1000_{SR}$-2	0.63	1.37	4.81

LoB: limit of blank, LoD: limit of detection, LoQ: limit of quantitation.

antigen. The specimens were diluted from 100% to 0.01% with the ARCHITECT PIVKA-II calibrator diluent across the measuring range. Regression analyses of the observed diluted concentrations were compared to expected values based on the corresponding concentration of the undiluted specimen.

High dose hook effect was assessed by testing of high level PIVKA-II samples beyond the measuring range of the assay. The high level PIVKA-II samples were prepared from pooled serum spiked with PIVKA-II antigen to 60,000, 100,000, 200,000, 300,000, 400,000, and 600,000 mAU/mL. All samples were assayed in replicates of five using four instruments (three *i*2000 and one $i1000_{SR}$). Mean RLU of each sample was compared with mean RLU of Calibrator-F, the highest level calibrator.

Sample type equivalency was evaluated with specimens drawn from 16 normal individuals using seven types of blood collection tubes (plain serum tube (Terumo, Tokyo, Japan), SST tube (Becton, Dickinson and Company (BD), NJ, USA), EDTA K2 tube (Terumo), EDTA Na2 tube (Terumo), lithium heparin tube (Terumo), lithium heparin PST tube (BD) and sodium heparin tube (Terumo)). 20 mAU/mL or 200 mAU/mL of PIVKA-II antigen was spiked into each specimen and assayed in replicates of three. The mean of the PIVKA-II value of each sample was compared to mean value of the corresponding plain serum tube sample.

Assay interferences of potential interfering materials including endogenous substances, nutritional supplements and therapeutic agents were evaluated based on the CLSI guideline EP7-A2 [29]. Each material was diluted with appropriate diluents and spiked into the five low level serum samples and five high level serum samples prepared by spiking with a high titer specimen. The diluent used for the preparation of the spiked materials was spiked into the low level and high level samples in the same manner for use as controls. All samples were assayed in replicates of twelve. The mean of the PIVKA-II value of each test sample was compared to mean value of the corresponding control sample.

Correlation of the prototype ARCHITECT PIVKA-II with Picolumi PIVKA-II across the measuring range was evaluated using 81 AFP positive sera and 151 normal sera. Statistical analyses for the correlation were performed using Analyse-it version 2.30 (Analyse-it Software, Ltd., Leeds, UK).

3. Results

The total imprecision determined with each sample type and instrument type is shown in Table 1. The percentage coefficient of variation (%CV) was between 2.8 and 5.4 over the investigated concentration range from 20.28 to 78,800.74 mAU/mL.

The LoB, LoD and LoQ ranged from 0.00 to 0.63, from 0.73 to 1.61 and from 2.38 to 8.25 mAU/mL, respectively (Table 2).

The reagent on-board stability results showed no significant change of sample concentrations. The 95% confidence interval of concentration shift% from initial day to 30 days was 2.0% to 3.2%.

The linearity of this assay over the range of 3.85 to 39,991.70 mAU/mL using three samples is shown in Fig. 2-A. The Spearman's correlation coefficient between expected values and observed values of each sample was 1.00. The recovery of diluted samples ranged from 89% to 105%.

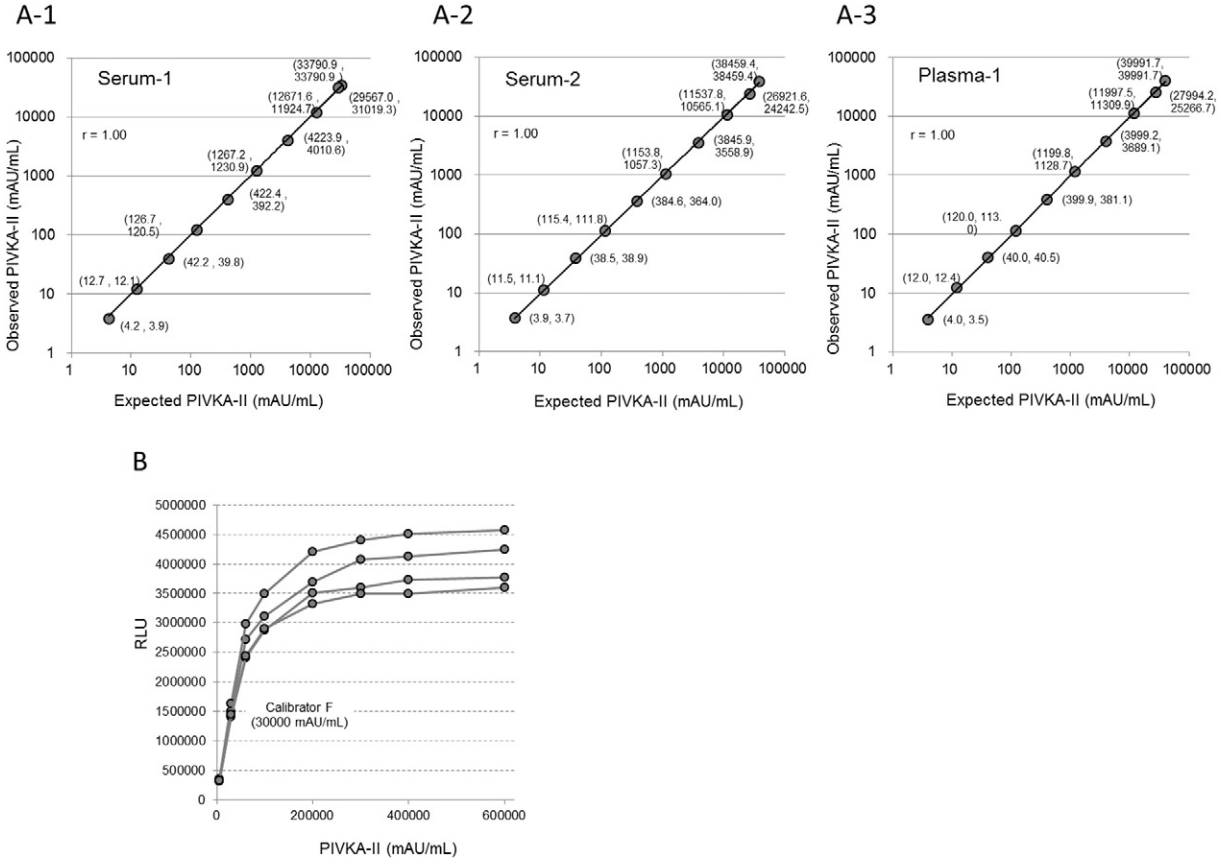

Fig. 2. Evaluation of linearity and high dose hook effect. A) Relationship between expected PIVKA-II concentrations calculated from dilution ratio and neat samples' concentration and observed concentrations in three samples (A-1 and A-2: serum samples, A-3: plasma sample). B) Relationship between PIVKA-II concentrations and relative light units (RLUs) on 4 instruments. Higher concentration gave higher RLU up to 600,000 mAU/mL.

Table 3
Evaluation of sample type equivalence and potential interfering materials including endogenous substances, nutritional supplements and therapeutic agents.

a) Sample type equivalence

n = 48 (16 donors × 3 replicates) per condition

Specimen type	Spiked PIVKA-II concentration	% difference from plain tube serum	
		Mean	95% confidence interval
SST serum	20 mAU/mL	2.1%	0.1% to 4.1%
	200 mAU/mL	3.7%	1.6% to 5.8%
EDTA K2 plasma	20 mAU/mL	1.5%	−0.6% to 3.6%
	200 mAU/mL	1.1%	−1.0% to 3.2%
EDTA Na2 plasma	20 mAU/mL	0.4%	−1.3% to 2.1%
	200 mAU/mL	0.9%	−0.8% to 2.6%
Li Heparin plasma	20 mAU/mL	−0.4%	−2.1% to 1.3%
	200 mAU/mL	1.8%	0.0% to 3.6%
Li Heparin (PST) plasma	20 mAU/mL	0.8%	−1.0% to 2.6%
	200 mAU/mL	2.6%	1.0% to 4.2%
Na Heparin plasma	20 mAU/mL	1.0%	−0.7% to 2.7%
	200 mAU/mL	3.1%	1.4% to 4.8%

b) Potential interfering materials

n = 60 (5 donors × 12 replicates) per condition

Potentially interferents	Concentration	PIVKA-II low level (20 mAU/mL)		PIVKA-II high level (200 mAU/mL)	
		% Difference from control		% Difference from control	
		Mean	95% confidence interval	Mean	95% confidence interval
Bilirubin (conjugated)	20 mg/dL	−2.0%	−3.5% to −0.5%	−1.6%	−2.3% to −0.9%
Bilirubin (unconjugated)	20 mg/dL	1.2%	−1.9% to 4.3%	0.8%	−1.4% to 3.0%
Hemoglobin	500 mg/dL	7.2%	5.8% to 8.6%	5.2%	3.8% to 6.6%
Prothrombin	15 mg/dL	2.2%	−1.2% to 5.6%	0.0%	−1.5% to 1.5%
Total protein	12 g/dL	−4.0%	−6.9% to −1.1%	−6.4%	−7.5% to −5.3%
Triglycerides	3000 mg/dL	2.4%	−0.2% to 5.0%	2.6%	−0.5% to 5.7%
Acetaminophen	600 μg/mL	1.6%	−0.1% to 3.3%	−1.2%	−2.2% to −0.2%
Acetylsalicylic acid	500 μg/mL	1.0%	−0.5% to 2.5%	−1.8%	−6.5% to 2.9%
Ascorbate	500 μg/mL	−0.2%	−3.8% to 3.4%	0.2%	−2.0% to 2.4%
Cisplatin	7.24 μg/mL	−1.0%	−4.9% to 2.9%	−0.2%	−1.8% to 1.4%
Fluorouracil	100 μg/mL	−2.2%	−6.3% to 1.9%	−0.4%	−3.4% to 2.6%
Galactose	2 mg/mL	−2.0%	−4.9% to 0.9%	0.6%	−0.8% to 2.0%
Glucose	10 mg/mL	−2.2%	−4.6% to 0.2%	−0.1%	−1.6% to 1.4%
Ibuprofen	400 μg/mL	−2.0%	−6.9% to 2.9%	−0.4%	−3.0% to 2.2%
Interferon α	3000 IU/mL	1.8%	−0.2% to 3.8%	0.6%	−0.5% to 1.7%
Interferon β	3000 IU/mL	−1.8%	−4.6% to 1.0%	0.4%	−1.3% to 2.1%
Interferon γ	3000 IU/mL	0.8%	−1.2% to 2.8%	0.0%	−0.9% to 0.9%
Vitamin B1	140 μg/mL	−2.3%	−5.1% to 0.5%	−0.6%	−2.7% to 1.5%
Vitamin B6	500 μg/mL	−2.2%	−4.9% to 0.5%	−0.2%	−1.8% to 1.4%
Vitamin B12	250 μg/mL	−0.2%	−3.9% to 3.5%	−0.6%	−2.0% to 0.8%

a) Evaluation of sample type.
b) Evaluation of potential interfering materials.

No high dose hook effect was observed in the testing conditions, up to 600,000 mAU/mL (Fig. 2-B). Higher concentration samples gave higher RLU and no samples showed lower RLU than Calibrator-F RLU (30,000 mAU/mL, upper limit of the measuring range).

The value differences between plain serum and various types of specimens are shown in Table 3-a. The mean percentage differences of each specimen type ranged from −0.4% to 3.7%.

The effects of potential interfering materials are shown in Table 3-b. The mean percentage differences between test samples and control samples ranged from −6.4% to 7.2%.

The correlation between the Picolumi PIVKA-II and the prototype ARCHITECT PIVKA-II is shown in Fig. 3. The regression slope calculated using the Passing-Bablok regression method was 1.07. The Spearman's correlation coefficient was 0.93.

4. Discussion

In the present study, the analytical performance of ARCHITECT PIVKA-II assay was evaluated. The assay demonstrated good precision and high sensitivity. There is viability in LoQ (2.38–8.25 mAU/mL), but the highest LoQ was less than 10 mAU/mL against the reported cut-off of 40 mAU/mL [30]. The good linearity over the measuring range, no hook effect up to 600,000 mAU/mL and no interference of common therapeutic agents, nutritional supplements and endogenous substances were confirmed. We demonstrated that the ARCHITECT PIVKA-II Assay can be used with various types of serum and plasma samples. The correlation data showed the prototype ARCHITECT PIVKA-II gives equivalent PIVKA-II values to those of the Picolumi PIVKA-II, the regulatory approved PIVKA-II assay in Japan.

In Japan, PIVKA-II has been commonly employed as a HCC specific tumor marker. The Japan Society of Hepatology recommended the measurement of tumor markers combined with ultrasonography at intervals of 2–6 months for regular screening in high risk patients with type C chronic liver disease, type B chronic liver disease and cirrhosis [19]. Since PIVKA-II, AFP and AFP-L3 are complementary biomarkers, measurement of two or more tumor markers is useful for a precise diagnosis of HCC [15–18]. The National Health Insurance in Japan covers measurements of AFP, PIVKA-II and AFP-L3. In contrast, outside Japan only ultrasonography and AFP are commonly used for monitoring of the high risk patients and there are no guidelines regarding PIVKA-II usage for diagnosis of HCC so far.

Although prompt determination of the PIVKA-II level is important for an appropriate treatment decision, it has not been achieved so far

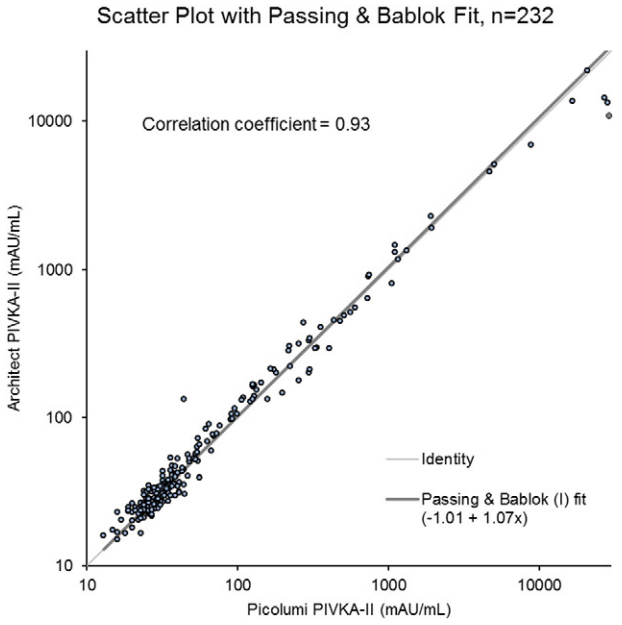

Fig. 3. Correlation between the Japan-approved PIVKA-II assay kit, Picolumi PIVKA-II, and the Architect PIVKA-II assay.

even in Japan. Because the instrument used for the most predominant assay kit for PIVKA-II in Japan is not prevalent in clinics and hospitals. Therefore most samples are shipped to and assayed in commercial clinical laboratories which take several days to report the assay results after the initial sample collection. Because the second major assay kit, Lumipulse PIVKA-II, in Japan can't use plasma samples, collected blood samples must be held for more than 30 min. to allow the serum to clot, it is difficult to obtain the assay result before medical treatment for ambulatory in a routine medical care process within a day.

Although human prothrombin is digested to several subfragments by serine proteases activated in the coagulation cascade [31], the assay can detect PIVKA-II in both serum and plasma without the influence of degradation of analyte and the interference by the subfragments because both antibodies used for the ARCHITECT PIVKA-II assay recognize epitopes at the N-terminus in PIVKA-II.

Current assay kits for PIVKA-II on market have some weaknesses. Picolumi kit needs manual dilution steps for sample preparation and Lumipulse and μTAS Wako DCP can't be used for plasma samples. The ARCHITECT PIVKA-II assay is a convenient fully automated assay which has a good analytical performance with high throughput (200 tests/h) without the pretreatment of specimens. The ARCHITECT PIVKA-II assay was designed to be used with various types of plasma as well as serum samples. Since the incubation time for coagulation is not required for plasma samples, the assay results can be obtained earlier than serum samples after the sample collection. These features allow to test and obtain the test result before medical treatment in a day when ambulatory patients visit.

This assay provides a convenient automated method for measurement of PIVKA-II in both serum and plasma in clinics, hospitals and clinical laboratories. It can support the global use of PIVKA-II for management of HCC and the early detection of HCC in high risk groups.

Conflict of interest

KF, HK and TY are employees of Abbott Japan which markets the ARCHITECT PIVKA-II Assay. KO, YI and HS are employees of Denka Seiken which manufactures the ARCHITECT PIVKA-II Assay under contract with Abbott Laboratories. There are no other conflicts of interest.

Acknowledgment

The authors are grateful the assay development teams at Abbott Japan, Denka Seiken and Abbott Laboratories for their efforts in the assay development and support for evaluation of the ARCHITECT PIVKA-II assay.

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