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ORIGINAL ARTICLE

Analytical Evaluation and Reference Interval Investigation for Chinese Han Population in Wuhan of CA72-4 Performed on an Architect i2000sr System

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SUMMARY

Background: **Carbohydrate antigen 72-4 (CA72-4) is a mucin-like, tumor-associated glycoprotein. Its elevation in serum has been found to be associated with various carcinomas. The purpose of this study was to evaluate the analytical performance of the Architect chemiluminescent immunoassay CA72-4 on the Architect i2000sr platform and to determine the reference interval (RI) of CA72-4 for the Chinese Han population in the city of Wuhan.**
Methods: **We evaluated the precision, analytical sensitivity, linearity and interference according to the guidelines of the Clinical Laboratory Standardization Institute. Additionally, the Abbott Architect i2000sr CA72-4 assay was compared to the Roche Cobas E602 CA72-4 assay. A total of 448 healthy individuals were selected to determine the RIs of CA72-4.**
Results: **The assay had an imprecision of 1.38% - 2.63% within runs, 1.41% - 2.73% between runs and a total imprecision of 2.54% - 4.10%. The limit of blank, limit of detection and limit of quantitation concentrations were 0.19 U/mL, 0.21 U/mL, and 0.21 U/mL. Linearity was confirmed across the entire measurable range. Additionally, the carryover was less than 0.065%. Interferences with hemoglobin, conjugated bilirubin and lipemia were acceptable with changes of less than 10%. A correlation coefficient of 0.9450 was determined through the method comparison experiment (95% CI 0.9318 - 0.9557). The RI for serum CA72-4 in the Han population was established as an upper limit, 11.13 U/mL (90% CI 9.39 - 12.99) by using the Abbott Architect i2000sr system.**
Conclusions: **This study establishes a RI for the Chinese Han population in Wuhan using the Abbott Architect i2000sr CA72-4 assay and demonstrates an analytical performance for clinical use.**
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Supplementary Data

Imprecision

Imprecision of CA72-4 was verified based on guidance from Clinical and Laboratory Standards Institute (CLSI) document EP05-A2, on Architect i2000sr. Briefly, three serum pools indicating three different concentration levels were prepared with fresh serum, split into aliquots, and kept at -20°C. For each serum pool, samples were measured in duplicate, two runs per day, over a minimum of 20 days, generating 80 replicates by one operator using one reagent lot. Supplemental Table 1 documents the imprecision testing carried out in our laboratory.

Analytical sensitivity

For evaluation of the analytical sensitivity of Architect i2000sr CA72-4, the limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were evaluated confirmed according to CLSI document EP17-A2. For the LoB evaluation, four blank samples were tested three times per day for five days. The LoB was calculated by using the formula:

LoB (U/L) = median value (U/L) + SD (U/L) × 1.645 = 0.19 U/mL

Results are shown in Supplemental Table 2. Similarly, LoD and LoQ were established by seven very low samples. The LoD value was calculated according to the formula:

LoD = LoB + 1.645 SD = 0.21 U/mL

Results are shown in Supplemental Table 3. To calculate LoQ, the relationship between the error of the measurement (expressed as % CV values) and CA72-4 concentrations was interpolated by means of a nonlinear regression curve (Supplemental Figure 1) LoQ value was 0.21 U/mL when CV was 20%.

Linearity

Linearity of CA72-4 was confirmed according to CLSI document EP6-A. Series dilutions of different concentrations which were prepared from two samples, one with very low concentration and another with high concentration, were mixed together with the specific ratios and measured in triplicate. Polynomial regression analysis was then performed and linear or non-linear coefficient was assumed to be statistically significant if p was < 0.05. Linearity of Architect CA72-4 was demonstrated up to 280.74 U/mL. Results are shown in Supplemental Table 4 and 5 and Supplemental Figure 2.

Carryover

One sample with a high concentration (H: 220 U/mL) and another patient sample with low concentration (L: 7 U/mL) were used for the carryover test. The high sample was run four times followed by four runs of the low sample. During the carryover test, no other specimens or tests were assayed on the platform. The carryover was calculated by using the formula:

$$\text{carryover} = \frac{L1-(L3+L4)/2}{(H3+H2)/2-(L3+L4)/2} = 0.065\%$$

Results are shown in Supplemental Table 6.

Interference

The potential interferences of hemoglobin, bilirubin and lipemia were evaluated by using the Sysmex Interference Check A Plus kit (Sysmex, Kobe, Japan) based on the package insert from the kit and in accordance with CLSI document EP7-A2. For each interferent, there are two sets of vials in the kit; one is interferent and the other is interferent blank (control). Each vial was reconstituted with 2.0 mL of purified water. Then, 1 mL of the reconstituted interferent was spiked to 9 mL of the base plasma sample to prepare sample A, and the same manner was used to prepare sample B with control. The dilution series was then prepared by mixing plasma sample A and sample B. One reagent batch was used for interference testing. The differences observed between the mean spiked and control sample values were examined and assessed according to acceptance. Interference was calculated using the formula below: interference = (mean spiked value - mean control value) / mean control value × 100%, and interference on more than 10% is clinically acceptable.

To determine potential interferences in the specific detection of CA72-4, one serum pool with CA 72.4 at approximately 15 U/mL was prepared. Results are shown in Supplemental Table 7 a-c.

Table S1. Imprecision estimated for CA72-4 performed on Architect i2000sr.

Serum pools	Mean (U/mL)	Within-run		Between-run		Between-day		Total	
		S.D.	CV (%)	S.D.	CV (%)	S.D.	CV (%)	S.D.	CV (%)
Level 1	2.46	0.07	2.63	0.07	2.73	0.04	1.56	0.10	4.10
Level 2	36.69	0.62	1.68	0.52	1.41	0.47	1.28	0.93	2.54
Level 3	126.39	1.74	1.38	2.17	1.71	1.96	1.55	3.40	2.69

Table S2. LoB estimated for CA72-4 performed on Architect i2000sr.

Day 1	Day 2	Day 3	Day 4	Day 5
Sample 1				
0.11	0.09	0.15	0.09	0.13
0.21	0.17	0.19	0.16	0.18
0.19	0.10	0.22	0.11	0.11
Sample 2				
0.09	0.12	0.05	0.04	0.08
0.11	0.01	0.07	0.03	0.08
0.16	0.06	0.13	0.10	0.05
Sample 3				
0.00	0.08	0.14	0.12	0.18
0.11	0.11	0.12	0.10	0.09
0.23	0.09	0.11	0.07	0.11
Sample 4				
0.10	0.08	0.10	0.09	0.05
0.12	0.05	0.04	0.13	0.07
0.07	0.09	0.05	0.05	0.10

LoB (U/L) = median value (U/L) + SD (U/L) × 1.645.

Table S3. LoD estimated for CA72-4 performed on Architect i2000sr.

	Mean (U/mL)	n	SD (U/mL)	CV
Sample 1	0.12	15	0.052	44.34%
Sample 2	0.22	15	0.031	13.73%
Sample 3	0.32	15	0.041	12.67%
Sample 4	0.49	15	0.043	8.84%
Sample 5	0.71	15	0.069	9.70%
Sample 6	0.8	15	0.048	5.94%
Sample 7	0.86	15	0.042	4.91%

LoD = LoB + 1.645 SD.

Table S4. Measurement range verified for CA 72-4 by using Calibrators.

Numbers	Ratio	Observed 1 (U/mL)	Observed 2 (U/mL)	Observed 3 (U/mL)	Mean (U/mL)	Expected (U/mL)
1	L (Cal A)	0	0	0.02	0.01	0
2	0.9L + 0.1H	29.65	29.96	29.63	29.75	30
3	0.8L + 0.2H	58.61	58.57	58.63	58.60	60
4	0.7L + 0.3H	88.79	90.29	90.98	90.02	90
5	0.6L + 0.4H	117.33	119.63	119.55	118.84	120
6	0.5L + 0.5H	145.18	149.23	150.71	148.37	150
7	0.4L + 0.6H	178.57	172.38	175.87	175.61	180
8	0.3L + 0.7H	202.87	205.22	210.15	206.08	210
9	0.2L + 0.8H	240.34	241.13	241.5	240.99	240
10	0.1L + 0.9H	270.5	270.33	265.69	268.84	270
11	H (Cal F)	290.46	294.2	> 300	-	300

Table S5. Measurement range verified for CA 72-4 by using serum pools.

Numbers	Ratio	Observed 1 (U/mL)	Observed 2 (U/mL)	Observed 3 (U/mL)	Mean (U/mL)	Expected (U/mL)
1	L	0.09	0.16	0.00	0.08	0.08
2	0.9L + 0.1H	27.35	26.8	27.43	27.19	28.11
3	0.8L + 0.2H	53.66	51.28	53.46	52.80	56.15
4	0.7L + 0.3H	76.93	82.7	79.8	79.81	84.18
5	0.6L + 0.4H	109.62	108.14	111	109.59	112.21
6	0.5L + 0.5H	143.95	135.67	133.68	137.77	140.25
7	0.4L + 0.6H	166.94	167.17	168.79	167.63	168.28
8	0.3L + 0.7H	189.17	192.28	191.78	191.08	196.31
9	0.2L + 0.8H	227.43	226.78	218.34	224.18	224.34
10	0.1L + 0.9H	244.18	246.9	249.98	247.02	252.38
11	H	273.64	282.79	284.81	280.41	280.41

Table S6. Carryover test for CA72-4 performed on Architect i2000sr.

	H1	H2	H3	H4	L1	L2	L3	L4
CA72-4 (U/mL)	223.24	223.77	218.23	218.69	7.19	7.26	7.16	6.94

Table S7a. Interference of conjugated bilirubin to Architect CA72-4 assay.

Conjugated Bilirubin (CB, mg/dL)		CA72-4 (U/mL)		
		Mean	S.D.	Interference
Control	0	15.50	0.16	
CB1	4	15.23	0.27	-1.70%
CB2	8	15.42	0.23	-0.49%
CB3	12	15.10	0.06	-2.54%
CB4	16	14.81	0.23	-4.41%
CB5	20	15.01	0.29	-3.16%

Table S7b. Interference of hemoglobin to Architect CA72-4 assay.

Hemoglobin (HB, mg/dL)		CA72-4 (U/mL)		
		Mean	S.D.	Interference
Control	0	14.54	0.22	
HB1	100	15.52	0.39	0.15%
HB2	200	15.56	0.03	0.39%
HB3	300	15.79	0.25	1.87%
HB4	400	15.83	0.24	2.13%
HB5	500	16.07	0.19	3.70%

Table S7c. Interference of lipemia to Architect CA72-4 assay.

Lipemia (LM, FTU)		CA72-4 (U/mL)		
		Mean	S.D.	Interference
Control	0	14.93	0.31	
LM1	420	14.84	0.25	-4.24%
LM2	840	14.82	0.17	-4.37%
LM3	1260	14.83	0.12	-4.32%
LM4	1680	14.69	0.18	-5.21%
LM5	2100	14.56	0.10	-6.02%

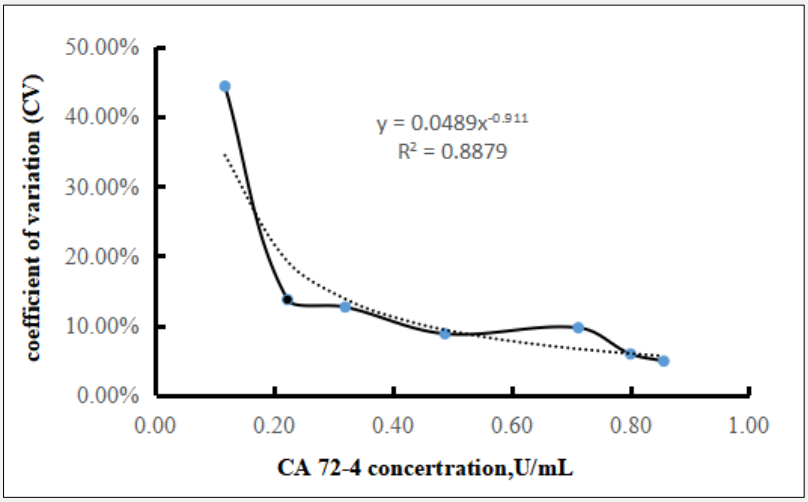
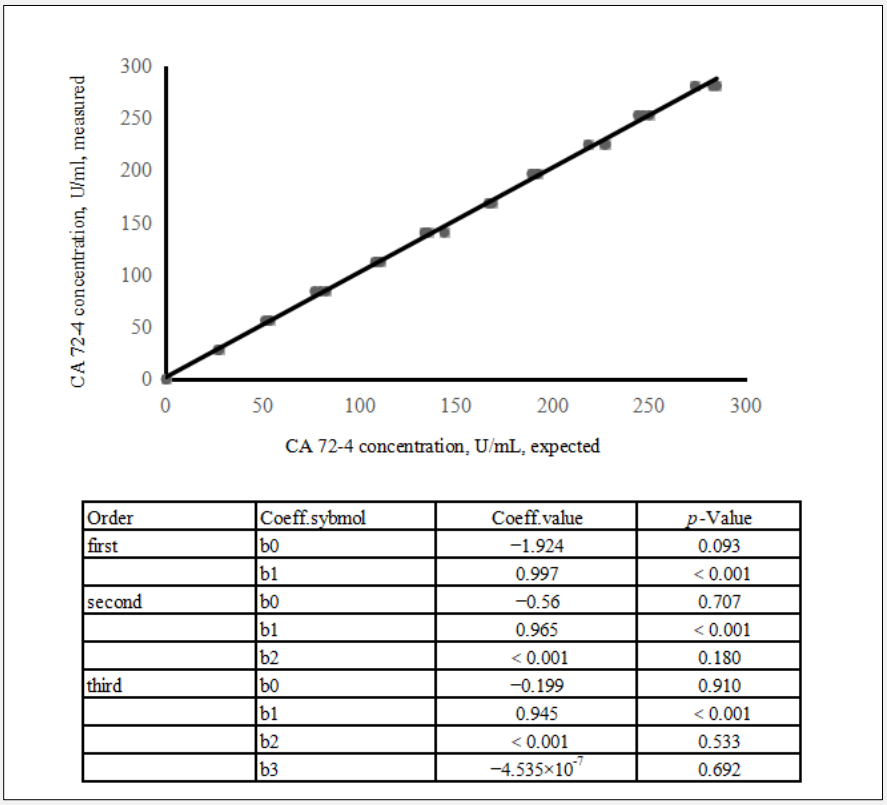


Figure S1. Assessment of LoQ for the Architect CA 72-4 assay.



Order	Coeff.sybmol	Coeff.value	*p*-Value
first	b0	−1.924	0.093
	b1	0.997	< 0.001
second	b0	−0.56	0.707
	b1	0.965	< 0.001
	b2	< 0.001	0.180
third	b0	−0.199	0.910
	b1	0.945	< 0.001
	b2	< 0.001	0.533
	b3	-4.535×10^{-7}	0.692

Figure S2. Assessment of method linearity for the Architect CA 72-4 assay.

For evaluation of the Architect CA 72-4 assay, a serum pool with a mean concentration of 280.41 U/mL was diluted with a serum pool of 0.08 U/mL. None of the non-linear coefficients was statistically significant; thus, a linear equation models the data best.