Monobind Technical Support Memo
Subject; CA 15-3 Antibody Specificity
Date; September 2019

QUESTION:

Results with Monobind CA 15-3 controls and patients' sera are below the expected value as established through another method. Please find enclosed our results. Do you have any explanation?

ANSWER:

The differences noted in some individuals are related to the monoclonal antibodies used in each assay. Epitope recognition and structure variations in the CA are responsible.

I have enclosed a paper dealing with this issue which compares seven major manufacturers (automated methods). I have highlighted (in yellow) the specific sections of each paper that deal with individual differences. The methods do correlate well (correlation coefficient) when all the samples are analyzed.

Authorized by: ASshatola

Quality Representative
September 11, 2019

Performance Characteristics of Seven Automated CA 15-3 Assays

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Key Words: Imprecision; Method comparison; Immunoassay; CA 15-3

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Abstract

Measurements of serum cancer antigen (CA) 15-3 are used to monitor tumor recurrence and treatment of advanced disease. We evaluated the performance characteristics, including limit of detection, linearity, method comparison, and reference intervals, of 7 automated methods for CA 15-3, including the Access 2 (Beckman Coulter, Brea, CA), ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY), ARCHITECT i2000 and AxSYM (Abbott Diagnostics, Abbott Park, IL), Elecsys 2010 (Roche Diagnostics, Indianapolis, IN), IMMULITE 2000 (Diagnostic Products, Los Angeles, CA), and VITROS ECi (Ortho Clinical Diagnostics, Raritan, NJ) assays. The limit of detection for each assay was less than 1.0 kU/L. The maximum deviation for the target values for linearity samples was less than 10% for all methods. Method comparison studies revealed large differences for some individual samples. Overall slopes ranged from 0.50 to 1.48, and correlation coefficients were 0.90 to 0.96 when the ADVIA Centaur was the comparison method. The 97.5 percentile upper reference limit ranged from 23.3 to 51.7 kU/L. Additional standardization efforts are needed, and the availability of reference material is required. Substantial intermethod differences exist for some patient samples, indicating that redetermining the baseline is required when changing methods.

Cancer antigen (CA) 15-3 is an epitope present on episialin, which is a large mucin glycoprotein that is expressed by the mammary epithelium.[1] The circulating episialin antigen is a heterogeneous molecule. In breast cancer, particularly epithelial breast carcinoma, episialin is overexpressed and released into the circulation. CA 15-3 is not a clearly defined analyte, and a primary reference material is not available.[2] Measurements of CA 15-3 in the serum can be used as a tumor marker for surveillance of patients diagnosed with breast cancer. Serial determinations are used to monitor the treatment of advanced disease and have the potential to detect early recurrence.[3,4] It is recommended that CA 15-3 measurements not be used for breast cancer screening owing to the poor sensitivity and specificity of this test.[5] Serum CA 15-3 concentrations can be quantified by a number of commercially available automated immunoassay methods. In the present study, we examined 7 automated CA 15-3 immunoassays for limit of detection, linearity, imprecision, method comparison, and reference intervals.

Materials and Methods

The following methods were evaluated in this study: Access 2 (Beckman Coulter, Brea, CA), ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY), ARCHITECT i2000 and AxSYM (Abbott Diagnostics, Abbott Park, IL), Elecsys 2010 (Roche Diagnostics, Indianapolis, IN), IMMULITE 2000 (Diagnostic Products, Los Angeles, CA), and VITROS ECi (Ortho Clinical Diagnostics, Raritan, NJ). All methods used the manufacturer's reagents according to the instructions. Method comparison was performed using the ADVIA Centaur as the comparison method.

Limit of Detection

The *limit of detection* was defined as the mean plus 2 SD for zero calibrator material. A total of 10 replicates of the "0" material and 3 replicates of the "nonzero" calibrators specific for each instrument were measured in each run; 2 runs were performed, and the mean of the 2 runs was determined. On the Access 2, the CA 15-3 S0 (0 kU/L) and Calibrator S1 (10.6 kU/L) were used. On the ADVIA Centaur, Bayer CA 15-3 MCM1 (0 kU/L) and Cal 1 (19.7 kU/L) were used. On the ARCHITECT i2000, CA 15-3 Calibrator A (0 kU/L) and the Calibrator B (20 kU/L) were used. On the AxSYM, the CA 15-3 Calibrator zero (0 kU/L) and nonzero (15 kU/L) were used. On the Elecsys 2010, the Roche Precicontrol TSH zero (0 kU/L) and CA 15-3 Cal 1 nonzero (15 kU/L) were used. On the IMMULITE 2000, the IMMULITE multidiluent 2 (0 kU/L) and a patient sample (13 kU/L) were used. On the VITROS ECi, the Ortho High sample diluent B (0 kU/L) and CA 15-3 Cal 1 (15 kU/L) were used.

Linearity

Samples submitted for CA 15-3 clinical testing were used to assess linearity. When appropriate, these patient samples first were diluted with the manufacturer's recommended diluent until they were within the analytic measurement range of each instrument. Each sample for linearity was run in triplicate and averaged.

For the Access 2, linearity was assessed by making serial dilutions of the high patient sample with Diluent A (Beckman Coulter) to give final concentrations of 1.5%, 3.1%, 6.3%, 12.5%, 25%, 50%, and 100% (CA15-3 concentrations, 14-918 kU/L). For the ADVIA Centaur, serial dilutions were made with MD1 diluent (Bayer Diagnostics) to give final concentrations of 6.3%, 12.5%, 25%, 50%, and 80% (CA 15-3 concentrations, 13-187 kU/L). For the ARCHITECT i2000, serial dilutions were made with ARCHITECT wash buffer (Abbott Diagnostics) to give final concentrations of 3.1%, 6.3%, 12.5%, 25%, 50%, and 100% (CA 15-3 concentrations, 18-655 kU/L). For the AxSYM, serial dilutions were made with CA 15-3 assay diluent (Abbott Diagnostics) to give final concentrations of 6.3%, 12.5%, 25%, 50%, and 100% (CA 15-3 concentrations, 13-209 kU/L). For the Elecsys 2010, linearity was assessed by serial dilution with Universal diluent (Roche Diagnostics) to final concentrations of 6.3%, 12.5%, 25%, 50%, and 100% (CA 15-3 concentrations, 16-273 kU/L). For the IMMULITE 2000, linearity was assessed by serial dilutions with MD2 (Diagnostic Products) to give final concentrations of 6.3%, 12.5%, 25%, and 100% (CA 15-3 concentrations, 15-300 kU/L). For the VITROS ECi, linearity was assessed by diluting patient sample with Diluent B (Ortho Clinical Diagnostics) to obtain final concentrations of 3.1%, 6.3%, 12.5%, 25%, 50%, and 100% (CA 15-3 concentrations, 14-474 kU/L).

Imprecision

Imprecision was determined for all 7 automated methods with 3 concentrations of quality control materials. Lyphocheck Tumor Marker Control levels 1 and 2 (Bio-Rad Laboratories, Hercules, CA) quality control materials were reconstituted according to the manufacturer's package insert specifications and tested on all analyzers. In addition, manufacturers' quality control materials that were specific for each method and contained a high concentration of CA 15-3 also were used for each method, with the exception of the Access 2, for which none was available. Samples were run in duplicate for each run, with 2 separate runs per day, for 5 days, for a total of 20 replicates for each level of quality control material. Assay imprecision data were analyzed using the EP Evaluator Release 5 software (David G. Rhoads Associates, Kennett Square, PA).

Method Comparison

We used 100 patient samples previously tested for CA15-3 or CA 27.29 in the method comparison studies. These samples were from women between 32 and 90 years old, with the exception of 1 sample obtained from a 61-year-old man. The ADVIA Centaur method was chosen as the comparison method because it showed the best correlation with all of the other methods. Linear and Passing-Bablok regression analyses were performed using Analyse-it+ Clinical Laboratory, version 1.63, software (Analyse-It Software, Leeds, England).

Reference Intervals

For the reference interval studies, samples were obtained from 120 healthy women who were not taking any prescription medications and ranged in age between 20 and 65 years. All studies using samples obtained from humans were approved by the University of Utah Institutional Review Board.

Results

The limit of detection was determined for each assay and compared with the manufacturers' claimed values. For the Access 2 method, the limit of detection was 0.02 kU/L compared with the manufacturer's claim of 0.50 kU/L. For the ADVIA Centaur, the limit of detection was 0.19 kU/L, and the manufacturer's claim was 0.50 kU/L. For the ARCHITECT i2000, the limit of detection was 0.37 kU/L, and the manufacturer's claim was 0.50 kU/L. For the AxSYM, the limit of detection was 0.12 kU/L, and the manufacturer's claim was 0.30 kU/L. For the Elecsys 2010, the limit of detection was 0.09 kU/L, and the manufacturer's claim of detection was 1.0

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kU/L. For the IMMULITE 2000, the limit of detection was 0.15 kU/L, and the manufacturer's claim was 0.50 kU/L. For the VITROS ECi, the limit of detection was 0.005 kU/L, and the manufacturer's claim was 0.50 kU/L.

The target value for each linearity sample was calculated based on the samples with the lowest and highest concentrations within the analytic measurement range for each method. By platform, the maximum average deviation from the target recovery was as follows: Access 2, 6.0% at 107 and 215 kU/L; ADVIA Centaur, 8.8% at 53 and 107 kU/L; ARCHITECT i2000, 9.3% at 147 kU/L; AxSYM, 6.2% at 98 kU/L; Elecsys 2010, 4.7% at 64 kU/L; IMMULITE 2000, 7.6% at 66 kU/L; and VITROS ECi, 9.7% at 260 kU/L.

Within-run, between-run, and overall imprecision of each method were evaluated **Table 1**. Within-run imprecision ranged from 1.6% to 6.1%, and total imprecision ranged from 2.2% to 6.1% for all methods.

Method comparison studies demonstrated varying degrees of agreement with the ADVIA Centaur reference method, with slopes ranging from 0.50 to 1.48, y-intercepts ranging from –5.0 to 7.3 kU/L, and correlation coefficients ranging from 0.90 to 0.96 **Figure 1** and **Table 2**. The ARCHITECT i2000 and the AxSYM methods with slopes of 1.07 and 1.06, respectively, and correlation coefficients of 0.96 for both demonstrated the highest degree of agreement with the ADVIA Centaur comparison method. The Access 2 and the VITROS ECi, with slopes 0.50 and 1.48 and correlation coefficients of 0.90 and 0.96, respectively, demonstrated the poorest agreement with the comparison method. Results for each method comparison sample were classified as normal or elevated, and the analytic concordance of each method with the comparison method was assessed using the cutoff recommended by the manufacturer of each assay **Table 3**. The overall concordance with the comparison method ranged from 89.0% to 96.0%.

Table 2
Passing-Bablok Regression Statistics*

Method	Slope	y-Intercept	*r*
Access 2	0.50 ± 0.06	4.4 ± 2.5	0.90
ARCHITECT i2000	1.07 ± 0.06	–5.0 ± 3.0	0.96
AxSYM	1.06 ± 0.05	–1.2 ± 3.8	0.96
Elecsys 2010	1.15 ± 0.18	1.8 ± 3.2	0.92
IMMULITE 2000	1.10 ± 0.13	7.3 ± 4.3	0.93
VITROS ECi	1.48 ± 0.09	0.3 ± 3.9	0.96

* Passing-Bablok analysis was performed to compare each method with the ADVIA Centaur comparison method. Access 2, Beckman Coulter, Brea, CA; ADVIA Centaur, Bayer Diagnostics, Tarrytown, NY; ARCHITECT i2000, Abbott Diagnostics, Abbott Park, IL; AxSYM, Abbott Diagnostics; Elecsys 2010, Roche Diagnostics, Indianapolis, IN; IMMULITE 2000, Diagnostic Products, Los Angeles, CA; VITROS ECi, Ortho Clinical Diagnostics, Raritan, NJ.

When method comparison studies were analyzed using difference plots **Figure 2**, the ARCHITECT i2000, AxSYM, Elecsys 2010, and IMMULITE 2000 methods showed mean differences of 20 kU/L or less. The Access 2 had a mean difference of –94 kU/L, and the VITROS ECi has a mean difference of 94 kU/L compared with the comparison method. Generally, agreement was excellent for CA 15-3 concentrations of 100 kU/L or less, whereas at higher CA 15-3 concentrations, agreement was poorer.

Reference intervals were determined for the 7 methods. The range of CA 15-3 concentrations observed for samples from healthy women on the Access 2 was 3.6 to 68.3 kU/L with a 97.5% upper reference limit of 23.3 kU/L. On the ADVIA Centaur, the range was 3.5 to 132.7 kU/L, and the upper 97.5% reference limit was 30.8 kU/L. On the ARCHITECT i2000, the range was 3.9 to 142.1 kU/L, and the 97.5% upper reference limit was 29.2 kU/L. On the AxSYM, the range was 5.2 to 166.0 kU/L, and the 97.5% upper limit was 30.6 kU/L. On the Elecsys 2010, the range was 4.9 to

Table 1
Summary of Imprecision Data*

	Lyphocheck Low				Lyphocheck High				Manufacturer's High Control			
		CV (%)				CV (%)				CV (%)		
	Mean (kU/L)	Within-Run	Between-Run	Total	Mean (kU/L)	Within-Run	Between-Run	Total	Mean (kU/L)	Within-Run	Between-Run	Total
Access 2†	8.4	3.1	0.9	3.2	19.2	3.7	0.0	4.2	—	—	—	—
ADVIA Centaur	14.9	2.7	2.0	3.3	41.8	2.1	1.4	3.1	108.4	3.8	3.4	5.1
ARCHITECT i2000	9.8	2.9	2.8	4.0	30.3	1.9	0.8	2.2	240.0	2.2	2.5	3.3
AxSYM	12.2	3.0	0.0	3.0	35.3	3.2	0.0	4.1	144.9	4.0	3.0	5.0
Elecsys 2010	13.9	1.9	1.1	2.5	37.4	2.8	0.0	3.4	105.9	3.0	1.7	3.4
IMMULITE 2000	16.8	6.1	0.0	6.1	44.2	3.2	1.8	3.8	127.9	4.4	2.9	5.2
VITROS ECi	14.1	2.4	0.7	2.6	37.7	2.5	0.0	2.5	186.0	1.6	1.9	2.5

CV, coefficient of variation.
* Access 2, Beckman Coulter, Brea, CA; ADVIA Centaur, Bayer Diagnostics, Tarrytown, NY; ARCHITECT i2000, Abbott Diagnostics, Abbott Park, IL; AxSYM, Abbott Diagnostics; Elecsys 2010, Roche Diagnostics, Indianapolis, IN; IMMULITE 2000, Diagnostic Products, Los Angeles, CA; Lyphocheck, Bio-Rad Laboratories, Hercules, CA; VITROS ECi, Ortho Clinical Diagnostics, Raritan, NJ.
† Quality control material was not available from the manufacturer of the Access 2.

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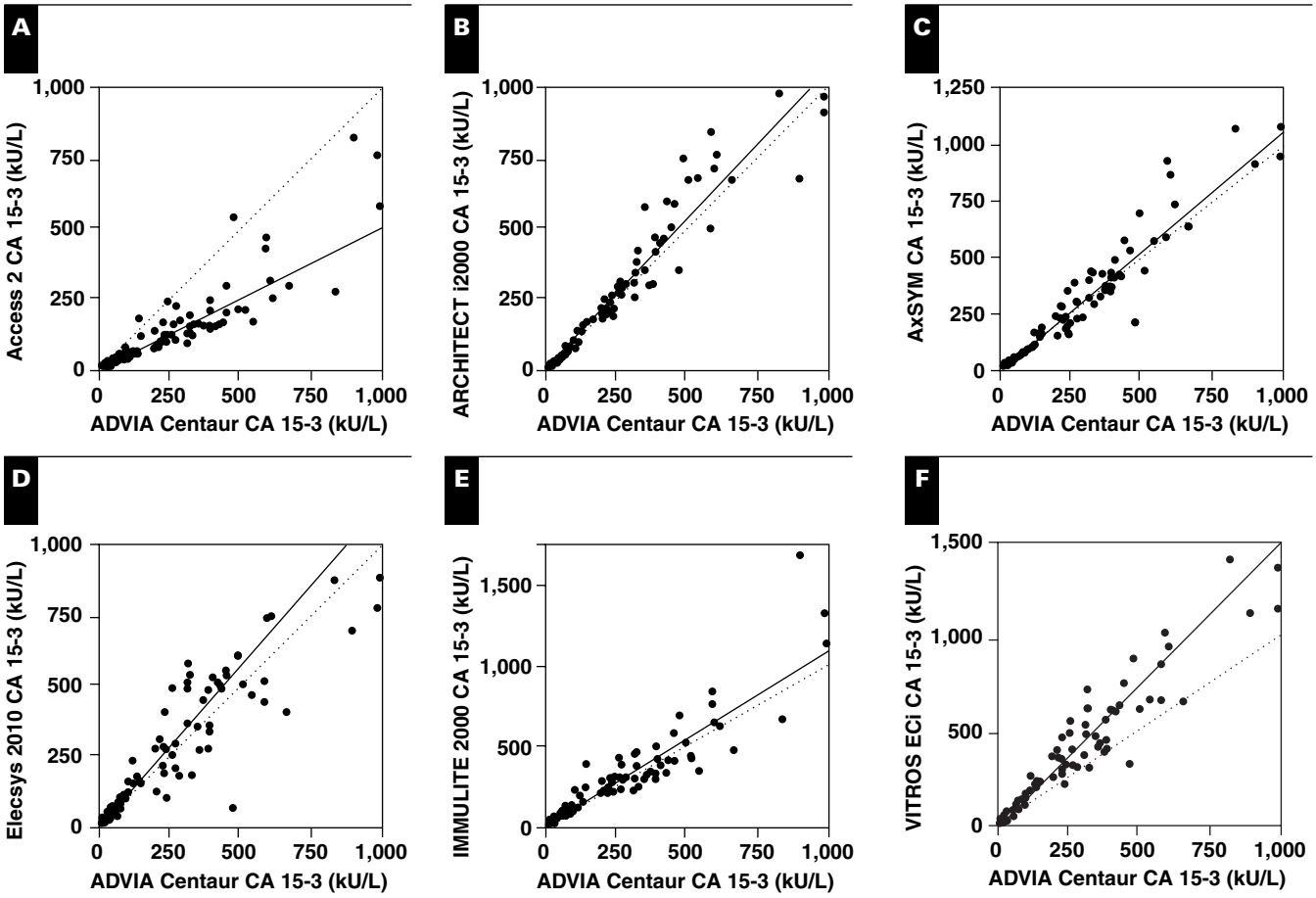

Figure 1 Comparison of cancer antigen (CA) 15-3 methods by Passing-Bablok analysis. The solid line indicates the Passing-Bablok regression line, and the dashed line indicates y = x. Regression statistics are given in Table 2. **A**, Access 2, Beckman Coulter, Brea, CA; ADVIA Centaur, Bayer Diagnostics, Tarrytown, NY; **B**, ARCHITECT i2000, Abbott Diagnostics, Abbott Park, IL; **C**, AxSYM, Abbott Diagnostics; **D**, Elecsys 2010, Roche Diagnostics, Indianapolis, IN; **E**, IMMULITE 2000, Diagnostic Products, Los Angeles, CA; **F**, VITROS ECi, Ortho Clinical Diagnostics, Raritan, NJ.

143.0 kU/L, and the 97.5% upper reference limit was 41.2 kU/L. On the IMMULITE 2000, the range was 6.6 to 160.0 kU/L, and the 97.5% upper reference limit was 42.3 kU/L. On the VITROS ECi, the range was 6.5 to 204.0 kU/L, and the 97.5% upper reference limit was 51.7 kU/L. Two samples consistently gave the highest results across all methods.

Discussion

All 7 automated CA 15-3 assays had limits of detection that were below the manufacturers' claims. If limits of ±10% of the target values are considered acceptable for CA 15-3

Table 3
Analytic Concordance With the ADVIA Centaur CA 15-3 Method*

	ADVIA Centaur CA 15-3			Overall Concordance With ADVIA Centaur (%)
	≥32.4 kU/L	<32.4 kU/L	Total	
Access 2				89.0
≥31.3 kU/L	63	0	63	
<31.3 kU/L	11	26	37	
Total	74	26	100	
ARCHITECT i2000				95.0
≥31.3 kU/L	69	0	69	
<31.3 kU/L	5	26	31	
Total	74	26	100	
AxSYM				94.0
≥31.3 kU/L	71	3	74	
<31.3 kU/L	3	23	26	
Total	74	26	100	
Elecsys 2010				89.0
≥25 kU/L	74	11	85	
<25 kU/L	0	15	15	
Total	74	26	100	
IMMULITE 2000				96.0
≥38.0 kU/L	74	7	81	
<38.0 kU/L	0	19	19	
Total	74	26	100	
VITROS ECi				93.0
≥35 kU/L	74	7	81	
<35 kU/L	0	19	19	
Total	74	26	100	

* Data are given as number of samples in each category. The cutoffs used are the manufacturers' upper reference limits. Access 2, Beckman Coulter, Brea, CA; ADVIA Centaur, Bayer Diagnostics, Tarrytown, NY; ARCHITECT i2000, Abbott Diagnostics, Abbott Park, IL; AxSYM, Abbott Diagnostics; Elecsys 2010, Roche Diagnostics, Indianapolis, IN; IMMULITE 2000, Diagnostic Products, Los Angeles, CA; VITROS ECi, Ortho Clinical Diagnostics, Raritan, NJ.

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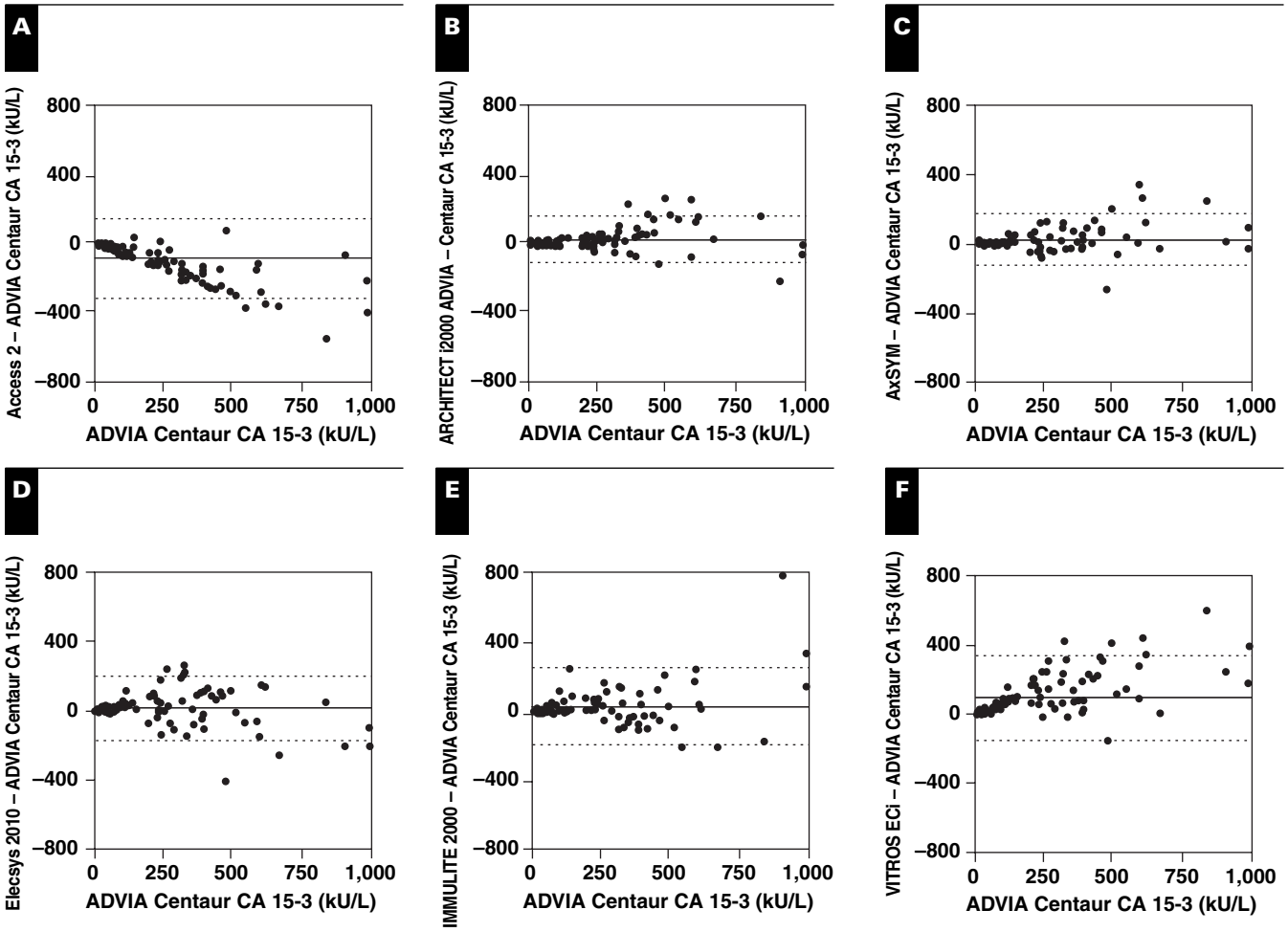

Figure 2 Comparison of cancer antigen (CA) 15-3 methods using difference plots. The solid line indicates the mean difference between methods, and the dashed lines indicate the upper and lower 95% confidence limits of the difference between methods. **A**, The mean difference was –94 kU/L (95% confidence interval [CI], –325 to 138 kU/L). **B**, The mean difference was 13 kU/L (95% CI, –120 to 147 kU/L). **C**, The mean difference was 20 kU/L (95% CI, –123 to 163 kU/L). **D**, The mean difference was 12 kU/L (95% CI, –177 to 202 kU/L). **E**, The mean difference was 31 kU/L (95% CI, –189 to 250 kU/L). **F**, The mean difference was 94 kU/L (95% CI, –153 to 340 kU/L). Access 2, Beckman Coulter, Brea, CA; ADVIA Centaur, Bayer Diagnostics, Tarrytown, NY; ARCHITECT i2000, Abbott Diagnostics, Abbott Park, IL; AxSYM, Abbott Diagnostics; Elecsys 2010, Roche Diagnostics, Indianapolis, IN; IMMULITE 2000, Diagnostic Products, Los Angeles, CA; VITROS ECi, Ortho Clinical Diagnostics, Raritan, NJ.

assay linearity, all the methods evaluated had acceptable performance. The Access 2 method had the largest analytic measurement range (0.5-1,000 kU/L), followed by the ARCHITECT i2000 (0-700 kU/L) and the VITROS ECi (0-500 kU/L). The ADVIA Centaur had the smallest analytic measurement range (0-200 kU/L), followed by the AxSYM (0-250 kU/L) and the Elecsys 2010 and IMMULITE 2000 (both 0-300 kU/L). Although the ARCHITECT i2000 has a claimed analytic measurement range to 800 kU/L, repeated attempts with 9 patient samples demonstrated nonlinear behavior, ie, an average value more than 10% from the target value, with CA 15-3 concentrations more than 700 kU/L.

The most precise method was the VITROS ECi with total coefficients of variation of less than 2.6% for all 3 concentrations of quality control material tested. The IMMULITE 2000 had the highest imprecision, with a total coefficient of variation of 6.1% for the lowest concentration of quality control material that was tested. In general, overall imprecision for each method was higher with the manufacturer's high quality control material compared with the Lyphocheck control materials. All methods demonstrated acceptable imprecision throughout the concentration range tested.

Review of the literature indicated that 4 of these automated CA 15-3 immunoassay methods, the AxSYM, Elecsys

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2010, IMMULITE 2000, and VITROS ECi had been compared previously.[6] A different comparison method was used in this study,[6] but the IMMULITE 2000 and VITROS ECi methods had the highest slopes with Passing-Bablok analysis. In our study, the ARCHITECT i2000, AxSYM, Elecsys 2010, and IMMULITE 2000 methods, on average, gave results that were comparable to the ADVIA Centaur comparison method. The Access 2 generally gave lower results and the VITROS ECi generally gave higher results than the ADVIA Centaur comparison method. Furthermore, scatter about the regression line at CA 15-3 concentrations of approximately 100 kU/L or less was much less than that observed for CA 15-3 concentrations of 100 kU/L or greater. Even for methods that showed the best agreement with the comparison method, a few results showed marked differences between methods. Therefore, the usual recommendation to follow up individual patients with a single method and to redetermine the baseline when changing methods should be followed for CA 15-3.

Reference interval studies demonstrated that an upper reference limit of 30 kU/L may be appropriate for the Access 2, ADVIA Centaur, ARCHITECT i2000, and AxSYM methods, whereas the Elecsys 2010, IMMULITE 2000, and VITROS ECi may require higher reference limits of 40 to 50 kU/L. The reference intervals determined in our study were comparable to the package insert values for the ADVIA Centaur (30.8 vs 32.4 kU/L), ARCHITECT i2000 (29.2 vs 31.3 kU/L), AxSYM (30.6 vs 31.3), and IMMULITE 2000 (42.3 vs 38.0). For the Access 2 method, we found a lower value than given in the package insert (23.3 vs 31.3 kU/L). We found higher reference intervals than given in the package inserts for the Elecsys 2010 (41.2 vs 25 kU/L) and for the VITROS ECi (51.7 vs. 35.0 kU/L). Intermethod differences were observed in both the method comparison studies and for the upper reference limits. Differences in the average values between methods suggest that additional calibration standardization is desirable. Harmonization of method calibration should produce slopes closer to 1.0 and intercepts closer to 0. The availability of a reference material would greatly facilitate this process. Our results were obtained with 1 lot of reagent and calibrators for each method. It is possible that some of the differences we found were unique to the lot of reagent or calibrator that we tested.

Substantial intermethod differences were observed for results from specific patient samples. These differences likely result from the use of different CA 15-3 monoclonal antibodies by different assays. Different antibodies presumably recognize different parts of the molecule, and antigen heterogeneity may account in part for intermethod differences. Some regulatory agencies require that the name of the tumor marker assay used be included on the patient report owing to the differences that occur among assays.

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