Performance Characteristics of Seven Automated CA 15-3 Assays

Patricia R. Slev, Ph.D.[1], Mindy L. Rawlins[2] and William L. Roberts, M.D., Ph.D.[1]

[1]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT

[2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT

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Corresponding author: Dr. William L. Roberts

ARUP Laboratories

500 Chipeta Way

Salt Lake City, UT 84108

Tel: (800) 242-2787 ext. 2086

Fax: (801) 584-5207

E-mail: william.roberts@aruplab.com

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 seven automated methods for CA 15-3 including the Access 2, ADVIA Centaur, ARCHITECT i2000, AxSYM, Elecsys 2010, IMMULITE 2000, and VITROS ECi assays. The limit of detection for each assay was <1.0 kU/L. The maximum deviation for the target values for linearity samples was <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 inter-method differences exist for some patient samples, indicating that rebaselining is required when changing methods.

Introduction

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 over expressed 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 due to both 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 this study we examined seven automated CA 15-3 immunoassays for limit of detection, linearity, imprecision, method comparison, and reference intervals.

Materials & Methods

The following methods were evaluated in this study: Access 2 (Beckman Coulter, Brea, CA), ARCHITECT i2000 and AxSYM (Abbott Diagnostics, Abbott Park, IL), ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY), Elecsys 2010 (Roche Diagnostics, Indianapolis, IN), IMMULITE 2000 (Diagnostic Products Corporation, 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 two standard deviations for zero calibrator material. A total of 10 replicates of the "0" material and three replicates of the "non-zero" calibrators specific for each instrument were measured in each run; two runs were performed, and the mean of the two 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, the 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 CA15-3 Calibrator zero (0 kU/L) and non-zero (15 kU/L) were used. On the Elecsys 2010, the Roche Precicontrol TSH zero (0 kU/L) and CA15-3 Cal 1 non zero (15 kU/L) were used. On the IMMULITE 2000, the IMMULITE multi-diluent 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 to for CA 15-3 clinical testing were used to assess linearity. When appropriate, these patient samples were first diluted with the manufacturer's recommended diluent until they were within the analytical 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 ranged from 14 to 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 ranged from 13 to 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 ranged from 18 to 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 ranged from 13 to 209 kU/L). For the Elecsys 2010, linearity was assessed by serial dilution with Universal diluent to final concentrations of 6.3%, 12.5%, 25%, 50% and 100% (CA 15-3 concentrations ranged from 16 to 273 kU/L). For the IMMULITE 2000, linearity was assessed by serial dilutions with MD2 (Diagnostic Products Corporation) to give final concentrations of 6.3%, 12.5%, 25%, and 100% (CA 15-3 concentrations ranged from 15 to 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 ranged from 14 to 474 kU/L).

Imprecision

Imprecision was determined for all seven automated methods with three concentrations of quality control materials. Lyphocheck Tumor Marker Control level 1 and level 2 (Bio-Rad) quality control materials were reconstituted according to 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 were also used for each method, with the exception of the Accesss 2, for which there was not one available. Samples were run in duplicate for each run, with two separate runs per day, for five 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, Kenett Square, PA**)**.

Method Comparison

One hundred patient samples previously tested for CA15-3 or CA 27.29 were used in the method comparison studies. These samples were from female subjects between 32 and 90 years of age, with the exception of one sample obtained from a 61year 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.

Reference Intervals

For the reference interval studies samples were obtained from 120 healthy female subjects that were not taking any prescription medications and ranged in age between 20 and 65 yrs. old. All studies using samples collected from humans were approved by the Institutional Review Board of the University of Utah.

RESULTS

The limit of detection was determined for each assay and compared to manufacturers' claimed values. For the Access 2 method the limit of detection was 0.02 kU/L compared with the manufacturers' 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 0.58 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 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 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 analytical measurement range for each method. By platform, the maximum average deviation from the target recovery was: 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 (Fig. 1). 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. When method comparison studies were analyzed using difference plots (Fig. 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 to the comparison method. Generally, agreement was excellent for CA 15-3 concentrations of 100 kU/L or less while at higher CA 15-3 concentrations, agreement was poorer.

Reference intervals were determined for the seven methods. The range of CA15-3 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 upper 97.5% upper limit was 30.6 kU/L. On the Elecsys 2010 the range was from 4.9 to 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 from 6.5 to 204.0 kU/L and the 97.5% upper reference limit was 51.7 kU/L.

DISCUSSION

The limit of detection for six of the seven automated CA 15-3 assays were below the manufacturers' claims. The one exception was the AxSYM method where the manufacturer's limit of detection was 0.3 kU/L but the measured limit of detection was 0.58 kU/L. Nevertheless, this method has adequate analytical sensitivity for clinical use in patients who are being monitored for recurrence.

If limits of +/- 10% of the target values are considered acceptable for CA 15-3 assay linearity, then all the methods evaluated had acceptable performance. The Access 2 method had the largest analytic measurement range (0.5-1000 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 nine patient samples demonstrated non-linear behavior, ie. average value more than 10% from the target value, with CA 15-3 concentrations >700 kU/L.

The most precise method was the VITROS ECi with total CVs of less than 2.6% for all three concentrations of quality control material tested. The IMMULITE 2000 had the highest imprecision with a total CV 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 to the Lyphocheck control materials. All methods demonstrated acceptable imprecision throughout the concentration range that was tested.

Review of the literature indicated that four of these automated CA 15-3 immunoassay methods, the AxSYM, Elecsys 2010, IMMULITE 2000 and VITROS ECi had been previously compared.[6] A different comparison method was used in this study, 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 individual patients with a single method and to rebaseline 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 while the Elecsys 2010, IMMULITE 2000, and VITROS ECi may require higher reference limits of 40 to 50 kU/L. The inter-method differences observed in both the method comparison studies and for the upper reference limits suggest that additional calibration standardization is desirable. The availability of a reference material would greatly facilitate this process.

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Figure Legend

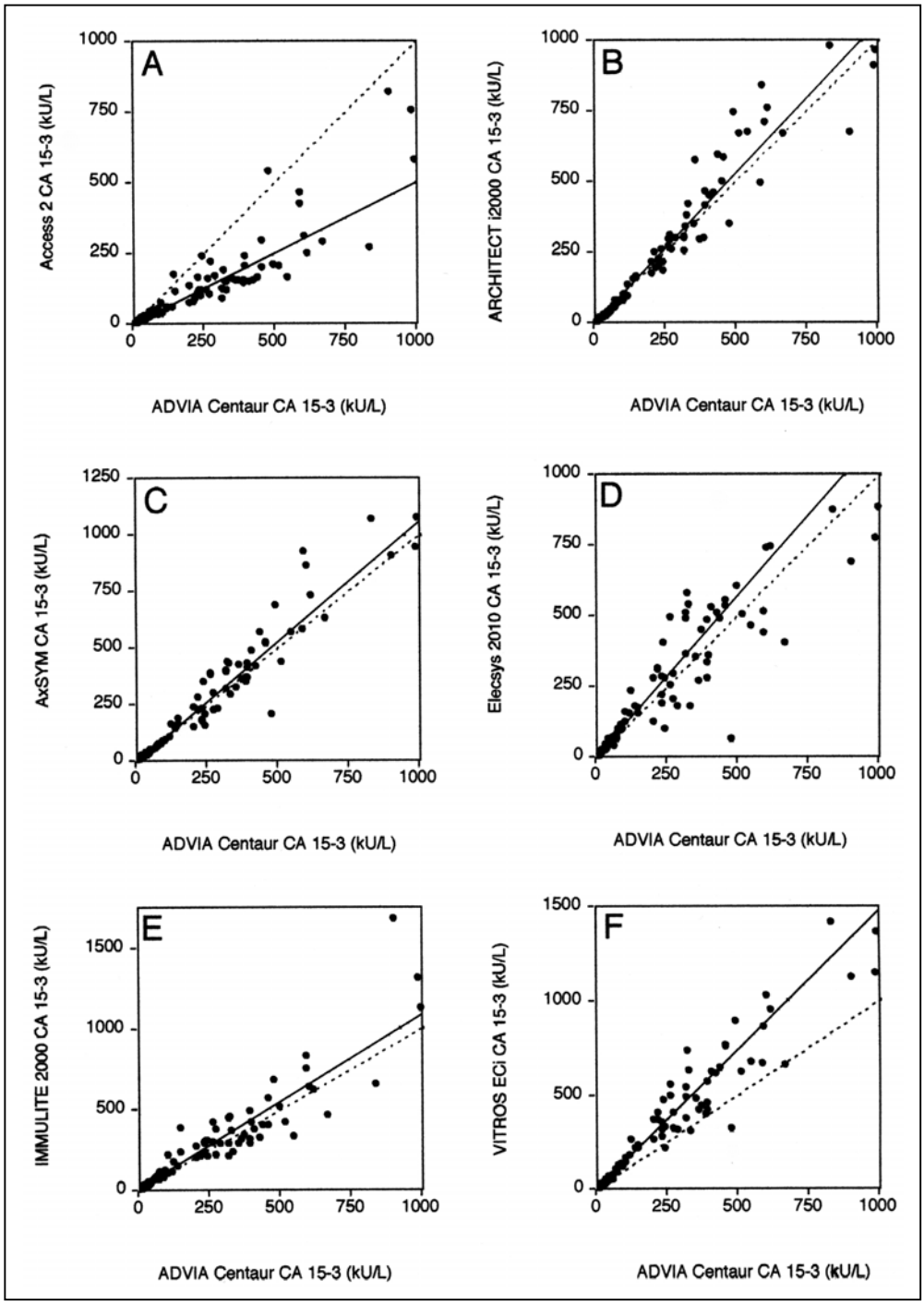
Figure 1. Comparison of CA 15-3 methods by Passing-Bablok analysis. The solid line indicates the Passing-Bablok regression line and the dashed line indicates y = x. In panel A, the slope was 0.50 (95% CI 0.45 to 0.56), the intercept was 4.4 (95% CI 2.2 to 6.9), and r = 0.90. In panel B, the slope was 1.07 (95% CI 1.01 to 1.12), the intercept was –5.0 (95% CI -8.0 to -2.1), and r = 0.96. In panel C, the slope was 1.06 (95% CI 1.01 to 1.11), the intercept was -1.2 (95% CI -5.0 to 0.8), and r = 0.96. In panel D, the slope was 1.15 (95% CI 1.04 to 1.22), the intercept was 1.8 (95% CI -1.2 to 5.0), and r = 0.92. In panel E, the slope was 1.10 (95% CI 1.02 to 1.23), the intercept was 7.3 (95% CI 3.1 to 11.6), and r = 0.93. In panel F, the slope was 1.48 (95% CI 1.39 to 1.56), the intercept was 0.3 (95% CI -3.6 to 2.3), and r = 0.96.

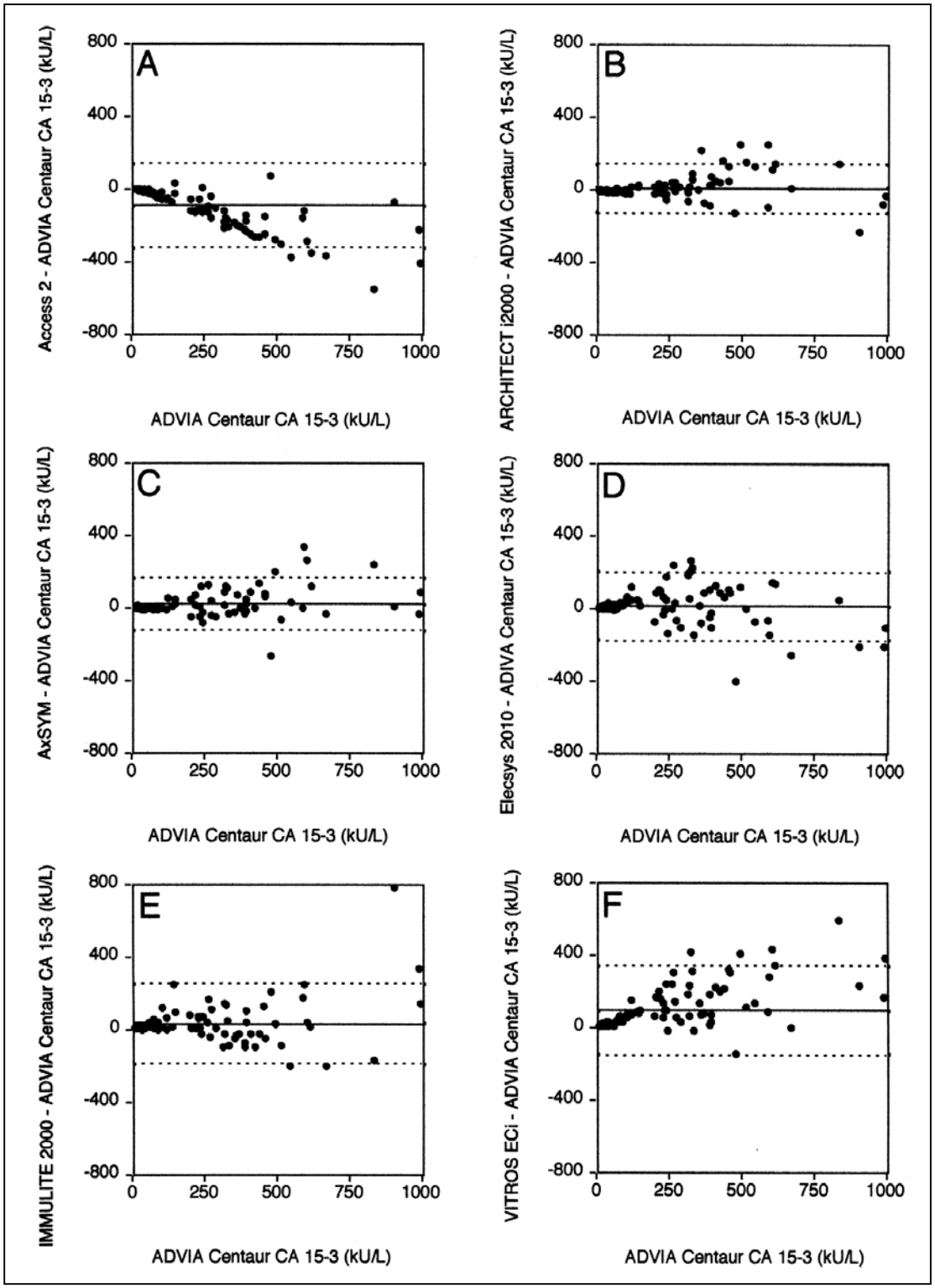
Figure 2. Comparison of CA 15-3 methods using difference plots. The solid line indicates the mean difference between methods and the dashed lines indicate the upper and lower 95% confidence limits of the difference between methods. In panel A, the mean difference was –94 kU/L (95% CI –325 to 138 kU/L). In panel B, the mean difference was 13 kU/L (95% CI –120 to 147 kU/L). In panel C, the mean difference was 20 kU/L (95% CI –123 to 163 kU/L). In panel D, the mean difference was 12 kU/L (95% CI –177 to 202 kU/L). In panel E, the mean difference was 31 kU/L (95% CI –189 to 250 kU/L). In panel F, the mean difference was 94 kU/L (95% CI –153 to 340 kU/L).

Table 1 Summary of Imprecision Data

	Lyphocheck Low				Lyphocheck High				Manufacturer's High Control			
	Mean (kU/L)	Within run CV (%)	Between run CV (%)	Total CV (%)	Mean (kU/L)	Within run CV (%)	Between run CV (%)	Total CV (%)	Mean (kU/L)	Within run CV (%)	Between run CV (%)	Total CV (%)
Access 2	8.4	3.1	0.9	3.2	19.2	3.7	0.0	4.2	NA[1]			
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

[1]NA indicates that quality control material was not available from the manufacturer of this assay.


A
Access 2 CA 15-3 (kU/L)
ADVIA Centaur CA 15-3 (kU/L)
0
250
500
750
1000
B
ARCHITECT i2000 CA 15-3 (kU/L)
ADVIA Centaur CA 15-3 (kU/L)
C
AxSYM CA 15-3 (kU/L)
ADVIA Centaur CA 15-3 (kU/L)
1250
D
Elecsys 2010 CA 15-3 (kU/L)
ADVIA Centaur CA 15-3 (kU/L)
E
IMMULITE 2000 CA 15-3 (kU/L)
ADVIA Centaur CA 15-3 (kU/L)
1500
F
VITROS ECi CA 15-3 (kU/L)
ADVIA Centaur CA 15-3 (kU/L)


A
Access 2 - ADVIA Centaur CA 15-3 (kU/L)
800
400
0
-400
-800
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250
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ADVIA Centaur CA 15-3 (kU/L)
B
ARCHITECT i2000 - ADVIA Centaur CA 15-3 (kU/L)
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ADVIA Centaur CA 15-3 (kU/L)
C
AxSYM - ADVIA Centaur CA 15-3 (kU/L)
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ADVIA Centaur CA 15-3 (kU/L)
D
Elecsys 2010 - ADIVA Centaur CA 15-3 (kU/L)
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IMMULITE 2000 - ADVIA Centaur CA 15-3 (kU/L)
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