Performance Characteristics of Seven Automated CA 15-3 Methods

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

[1]Department of Pathology, University of Utah, Salt Lake City, UT and [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT

Abstract

CA 15-3 is a tumor marker that is used clinically to monitor breast cancer therapy and disease progression. In this study, seven automated immunoassays for CA15-3 (Access 2, ADVIA Centaur, ARCHITECT i2000, AxSYM, Elecsys 2010, IMMULITE 2000, VITROS ECi) were evaluated for the following parameters: limit of detection, linearity, imprecision, method comparison and reference intervals. The limit of detection was < 1.0 kU/L for each method. The analytic measurement ranges of the assays varied widely (see Table 1). Linearity studies demonstrated that the maximum average deviation from the target recovery ranged from 6.1% for the Access 2 to 12.3% for the ARCHITECT i2000. Total imprecision at a concentration of 14 kU/L ranged from a CV of 2.5% for the Elecsys 2010 to 6.0% for the IMMULITE 2000; at a concentration of 35 kU/L it ranged from 2.2% for the ARCHITECT i2000 to 4.2% for the Access 2; and at a concentration of 152 kU/L it ranged from 2.5% for the VITROS ECi to 5.2% for the IMMULITE 2000 (see Table 2). Method comparison studies were conducted using 100 samples and variable agreement with the ADVIA Centaur comparison method was found. Slopes ranged from 0.50 to 1.48 and correlation coefficients from 0.90 to 0.96 (see Figure 1). The ARCHITECT i2000 and the AxSYM methods with slopes of 1.07 and 1.06 respectively, and correlation coefficients of 0.96 demonstrated the highest agreement with the ADVIA Centaur comparison method. The upper 97.5% reference limits were determined using 120 samples (see Table 3). The upper 97.5% reference limits found for the Elecsys 2010, IMMULITE 2000 and VITROS ECi were above the manufacturer's reference limits. Overall, all methods performed well. However, both method comparison and reference interval studies suggest differences that may be due to calibration, assay design, and/or the antibodies used.

Introduction

CA 15-3 is a tumor marker that is recommended for monitoring breast cancer therapy and disease progression, particularly management of metastasis. It is not recommended, however, for breast cancer screening. The CA 15-3 antigen is a mucin glycoprotein encoded by MUC1. MUC1 proteins or mucins are normally expressed on the apical side of glandular epithelia of various organs and serve to protect and lubricate surrounding cells. In breast cancer, particularly epithelial breast carcinoma, mucins become overexpressed and released into circulation. Elevated serum CA 15-3 levels can be detected by a number of commercially available automated immunoassays : Beckman-Coulter Access 2 (Beckman Coulter, Brea, CA), Abbott AxSYM (Abbott Diagnostics, Abbott Park, IL), Bayer ADVIA Centaur (Bayer Diagnostics, Tarrytown, NY), Abbott ARCHITECT i2000 (Abbott Diagnostics, Abbott Park, IL), Roche Elecsys 2010 (Roche Diagnostics, Indianapolis, IN), DPC IMMULITE 2000 (Diagnostics Products Corporation, Los Angeles, CA) and Ortho-Clinical Diagnostics VITROS ECi (Ortho-Clinical Diagnostics, Raritan, NJ) methods. The aim of these studies was to evaluate these seven automated methods for the following parameters: limit of detection, linearity, imprecision, reference intervals and method comparison with the ADVIA Centaur as the comparison method.

Methods

The limit of detection was determined by performing two separate runs and averaging the mean. In each run, there were 10 replicates of the "0" material and two replicates of the "non-zero" calibrator specific for each instrument and calculating 2 SD for the "0" material.

One hundred samples submitted to our clinical laboratory and previously tested for CA 15-3 or CA 27.29 were used to assess linearity. Three such individual samples were run in triplicate on each analyzer. When appropriate, individual patient samples were first diluted with the manufacturer's recommended diluent until they were within the previously reported analytical measurement range specific for each instrument.

Imprecision was estimated for all seven methods by using the standard commercially available Biorad Lyphocheck Tumor Marker Control (Low, or level 1), BioRad Lyphocheck Tumor Marker Control (High or level 2) and a high manufacturer quality control material specific for each instrument (exception: Access 2, not available). Samples were run in duplicate, on two separate runs per day, for five days, for a total of 20 replicates for each control level. The imprecision levels were analysed using the EP Evaluator Release 5 software (David G. Rhoads Associates, Kenett Square, PA).

One hundred patient samples that were previously tested for CA15-3 or CA 27.29 on the ADVIA Centaur and represented female subjects between 32-90 yrs. of age (exception, one 61yr. old male) were used in the method comparison studies. Passing-Bablock regression analysis were calculated using the Analyse-it+ Clinical Laboratory version 1.63 software.

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 that used samples collected from human subjects were approved by the Institutional Review Board of the University of Utah.

Results

Statistical analysis determined that the limit of detection for the seven automated immunoassays were as follows: Access 2 average limit of detection was 0.02 kU/L which was within the manufacturers' claim of 0.50 kU/L; ADVIA Centaur average limit of detection was 0.19 kU/L and the manufacturer's claim was 0.50 kU/L; ARCHITECT i2000 limit of detection was 0.24 kU/L and the manufacturer's claim was 0.50 kU/L; AxSYM limit of detection was 0.58 kU/L and the manufacturer's claim was 0.30 kU/L; Elecsys 2010 limit of detection was 0.09 kU/L and the manufacturer's claim of detection was 0.50 kU/L. IMMULITE 2000 limit of detection was 0.15 kU/L and the manufacturer's claim was 0.50 kU/L; VITROS ECi limit of detection was 0.01 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. The maximum deviation for a mean recovery from 100% ranged from a low of 6.1% for the Access 2 to a high of 12.3% for the Architect i2000. The Access 2 had a maximum average deviation from the mean target recovery of 6.1% (107.4 kU/L)), the ADVIA Centaur had a maximum average deviation from the mean target recovery of 8.8% (52.8 kU/L), the AxSYM had a maximum deviation of 6.4% (50.0 kU/L), the ARCHITECT i2000 had a maximum average deviation of 12.3%, the Elecsys 2010 had a maximum average deviation from the mean target recovery of 4.7% (64.4 kU/L), the IMMULITE 2000 had a maximum average deviation from the mean target recovery of 11.6% (14.8 kU/L) and the VITROS ECi had a maximum average deviation from the mean target recovery of 9.7% (259.7 kU/L).

Table 1. Manufacturers' Analytical Measurement Range

Method	AMR (kU/L)
Access 2	0.5 - 1000
ADVIA Centaur	0.5 - 200
ARCHITECT i2000	0 – 700
AxSYM	0 – 250
Elecsys 2010	0 - 300
IMMULITE 2000	0 – 300
VITROS ECi	0 - 500

Table 2. Summary of Imprecision Data

Method	Mean CA 15-3 (kU/L)	Within Run CV (%)	Between Run CV (%)	Total CV (%)
Access 2				
Level 1	8.4	3.1	0.9	3.2
Level 2	19.2	3.7	0.0	4.2
Level 3		none available		
ADVIA Centaur				
Level 1	14.9	2.7	2.0	3.3
Level 2	41.8	2.1	1.4	3.1
Level 3	108.4	3.8	3.4	5.1
ARCHITECT i2000				
Level 1	9.8	2.9	2.8	4.0
Level 2	30.3	1.9	0.8	2.2
Level 3	240.0	2.2	2.5	3.3
AxSYM				
Level 1	12.2	3.0	0.0	3.0
Level 2	35.3	0.0	2.4	4.0
Level 3	144.9	4.0	3.0	5.0
Elecsys 2010				
Level 1	13.6	1.9	1.1	2.5
Level 2	37.4	2.8	0.0	3.4
Level 3	105.6	3.0	1.7	3.4
IMMULITE 2000				
Level 1	16.8	6.1	0.0	6.0
Level 2	44.2	3.2	1.8	3.8
Level 3	127.9	4.4	2.9	5.2
Vitros ECi				
Level 1	14.1	2.4	0.7	2.6
Level 2	37.7	2.5	0.0	2.5
Level 3	186.0	1.6	1.9	2.5

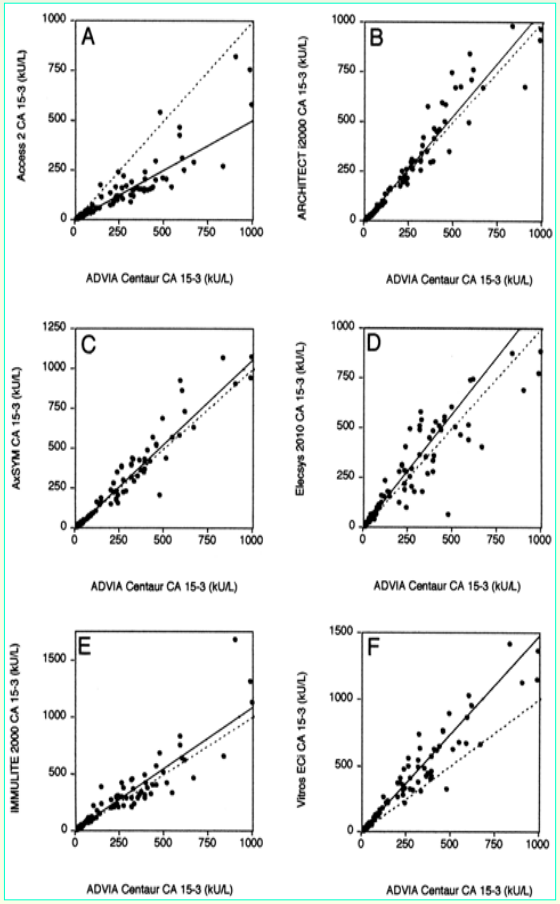

Figure 1. Comparison of six automated CA 15-3 immunoassay methods. The ADVIA Centaur was used as the comparison method. The dashed lines indicate (x=y) and the solid lines indicate the Passing-Bablock regression. In panel A, the Access 2 method slope was of 0.50, the intercept was 4.4 and r=0.90. In panel B, the ARCHITECT i2000 method slope was 1.07, the intercept was –5.0 and r=0.96. In panel C, the AxSYM method slope was 1.06, the intercept was –1.2 and r=0.96. In panel D, the Elecsys 2010 method slope was 1.15, the intercept was 1.8 and r=0.92. In panel E, the IMMULITE 2000 method slope was 1.10, the intercept was 7.3 and r=0.93. In panel F, the VITROS ECi slope was 1.48, the intercept was 0.3 and r=0.96.

Table 3. Summary of Reference Intervals

Method	97.5 Percentile reference limit (kU/L)	Manufacturer's reference limit (kU/L)
Access 2	23.3	31.3
ADVIA Centaur	30.8	32.4
ARCHITECT i2000	29.2	31.3
AxSYM	30.6	31.3
Elecsys 2010	41.2	31.0
IMMULITE 2000	42.3	38.0
VITROS ECi	51.7	35.0

Conclusions

The limit of detection for six of the seven automated methods for measuring CA 15-3 levels were all within acceptable ranges and below manufacturers' claims. The one exception was the AxSYM where the manufacturer's limit of detection was 0.30 kU/L and the measured limit of detection was 0.58 kU/L. This should not pose a problem in clinical use.

If 10% is used as the acceptable cutoff, then all the methods evaluated for linearity had acceptable maximum deviation from mean recovery values with the exception of the ARCHITECT i2000 and the IMMULITE 2000 where the maximum average deviation from target recovery was 12.3% and 11.6%, respectively.

All methods demonstrated acceptable imprecision, with total imprecision levels less than 6.1% for all methods at all three quality control materials tested.

The method comparison studies demonstrated varying degrees of agreement with the ADVIA Centaur reference method as indicated by the slopes ranging from 0.50 to 1.48, Y intercepts ranging from -5.0 to 7.3 and correlation coefficients ranging from 0.90 to 0.96. The ARCHITECT i2000 and the AxSYM with slopes of 1.07 and 1.06 respectively, showed the best agreement. The ARCHITECT i2000 and the AxSYM, both with correlation coefficients of 0.96 were also the two methods that demonstrated the highest degree of correlation with the ADVIA Centaur comparison method. The IMMULITE 2000 and the Elecsys 2010 with slopes of 1.10 and 1.15 and correlation coefficients of 0.93 and 0.92 demonstrated poorer agreement with the reference method. The Access and the VITROS ECi methods with slopes 0.50 and 1.48 and correlation coefficients of 0.90 and 0.96 respectively, demonstrated considerable bias.

The upper 97.5% reference limits found were below the manufacturer's reference limits for the Access 2, ADVIA Centaur, ARCHITECT i2000 and the AxSYM. The exceptions were the Elecsys 2010, IMMULITE 2000 and VITROS ECi.Overall, all methods performed well and are clinically acceptable. However, both method comparison and reference interval studies suggest differences between the methods that may be due to calibration, assay design, and/or the antibodies used. These methods cannot be used interchangeably.

Acknowledgements

Support for this study was provided by Abbott Diagnostics and by the ARUP Institute for Clinical and Experimental Pathology.