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Alternative antibody for the detection of CA15-3 antigen: a European multicenter study for the evaluation of the analytical and clinical performance of the Access® BR Monitor assay on the UniCel® Dxl 800 Immunoassay System

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Abstract

Background: Cancer antigen CA15-3 antigen is known as a valuable marker for the management of breast cancer.
Methods: The analytical and clinical performance of the Access® BR Monitor Immunoassay System (Beckman Coulter) was evaluated at five different European sites and compared with a reference system, defined as CA15-3 on the Elecsys® System (Roche Diagnostics).
Results: Total imprecision (%CV) of the BR Monitor ranged between 5.5% and 11.7%, and inter-laboratory reproducibility between 3.4% and 5.1%. Linearity upon dilution showed a mean recovery of 98.5% (SD±9.1%). Endogenous interferents had no influence on BR Monitor levels (mean recoveries: hemoglobin 112%, bilirubin 111%, triglycerides 108%). There was no high-dose hook effect up to 13,540 kU/L. Clinical performance investigated in sera from 1811 individuals showed a general correlation between the Access BR Monitor and Elecsys CA15-3 (R=0.797), with a slope of 1.383. CA15-3 serum levels, as measured by the BR Monitor, were low in healthy individuals (n=267, median=11.9 kU/L, 95th percentile=23.5 kU/L), higher in individuals with various benign diseases (n=549, medians=11.3–15.6 kU/L, 95th percentiles=21.6–54.6 kU/L) and even higher in individuals suffering from various cancers (n=995, medians=11.2–22.8 kU/L, 95th percentiles=30.0–429.7 kU/L). Best diagnostic accuracy for cancer detection against the relevant benign control group by the BR Monitor was found for locoregional and metastatic breast cancer, as well as for ovarian cancer [area under the curve (AUC) 0.619, 0.897 and 0.774]. Results for the reference CA15-3 assay were comparable (AUC 0.611, 0.887 and 0.818).
Conclusions: The Access BR Monitor provides accurate methodological characteristics and demonstrates an analytical and clinical correlation with Elecsys CA15-3. Best diagnostic accuracy for the BR Monitor was found in breast and ovarian cancer. Our results also suggest a clinical value of the BR Monitor in other cancers.
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Keywords: breast cancer; CA15-3; diagnosis; method comparison.

Introduction

Among all cancers, breast cancer has the highest incidence rates in women in the Western industrialized world. With more than 200,000 cases annually, it accounts for 31% of the newly diagnosed cancers among females in the United States (1). In many European countries, breast cancer still ranks as the leading cause of death among neoplastic diseases. Because breast cancer is curable in the early stages, great efforts have been made during recent years to detect the malignant disease earlier and to treat it more effectively by adjuvant and neoadjuvant therapies (2, 3).

Besides progress in radiological diagnostics, serum related markers have shown to provide valuable prognostic information and to be useful for the management of the disease, particularly in the follow-up care after the primary therapy was applied. The recurrence of disease is often mirrored in blood accurately, with a lead time of several months prior to radiological detectable tumor manifestations. The therapy efficacy can be monitored effectively by the course of serum markers, if interpreted by experts. Among breast cancer serum markers, CA15-3 antigen, CEA, and recently, HER2-neu, have proven high sensitivity for cancer detection, particularly if used in serial measurements (3–23).

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The tumor-associated antigen CA15-3 is a carbohydrate antigen of 300 kDa belonging to the milk fat globule mucin family. Assays for the determination of CA15-3 use two monoclonal antibodies binding to specific regions of the MUC 1 protein core (3, 4).

In the present study, a new assay for detection of CA15-3 antigen was evaluated for its analytical and clinical performance, and compared with an established reference method. This Access BR Monitor assay is applied on the UniCel® Dxl 800 Immunoassay System (Beckman Coulter Eurocenter S.A., Nyon, Switzerland) and uses Ma 552 as monoclonal tracer antibody, which recognizes practically the same epitope within the MUC1 protein core as the Centocor DF3 antibody (Centocor Inc., Horsham, PA, USA), and Ma 695 as monoclonal capture antibody, which detects a carbohydrate epitope of MUC 1 similar to the one recognized by the Centocor 115-D8 monoclonal antibody. As most of the currently available assays are based on the original Centocor antibody, it is challenging to compare the clinical relevance of the new assay using an alternative antibody, which in consequence has led to the new name ''Access BR Monitor assay''.

The present evaluation was performed as a European multicenter trial including five sites in various countries.

Materials and methods

Assay procedure

Access BR Monitor (CA15-3 antigen) assay on the UniCel® Dxl 800 Immunoassay System (Beckman Coulter) The Access BR Monitor assay is a paramagnetic particle, two-site immunoenzymatic (''sandwich''), chemiluminescent immunoassay for the quantitative determination of CA15-3 antigen levels in human serum and plasma using the Access Immunoassay Systems. A sample is added to a reaction vessel along with paramagnetic particles coated with polyclonal goat anti-biotin antibody, mouse monoclonal-biotin conjugate and a buffered protein solution. After incubation in a reaction vessel, separation in a magnetic field and washing remove materials not bound to the solid phase. A monoclonal-alkaline phosphatase conjugate is then added. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of CA15-3 antigen in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 1.0–1000 kU/L).

For calibration, Access BR Monitor Calibrators (Cat. No. 387647: S0–S5, 2.5 mL/vial) were used. The Access BR Monitor Calibrators are provided at six levels – zero and approximately 10, 50, 100, 500 and 1000 kU/L. Controls, Bio-Rad Lyphochek Tumor Marker Control (Cat. No. 580 Bilevel, 6×2 mL; Bio-Rad Laboratories, Munich, Germany), were run in duplicates every day of the study.

CA15-3 II assay on Elecsys 2010 Immunology System (Roche Diagnostics) The CA15-3 II assay is an electrochemoluminescence immunoassay for the quantitative determination of CA15-3 antigen levels in human serum and plasma using the Elecsys 2010/1010 and Modular Analytics E170 Immunology Systems (Roche Diagnostics GmbH, Penzberg, Germany). The assay is based on a sandwich principle with the monoclonal antibodies 115-D8 and DF3. After predilution of 20 μL of the sample, the antigen, a biotinylated monoclonal CA15-3-specific antibody, and the monoclonal CA15-3 antibody labeled with a ruthenium complex form a sandwich complex. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell buffer. Application of a voltage to the electrode then induces chemiluminescent emission that is measured by a photomultiplier tube. Results are determined via a calibration curve which is instrument-specifically generated by two-point calibration and a master curve provided by the manufacturer via the reagent pack barcode.

Samples can be accurately measured within the analytic range of 1.0–300 kU/L.

For calibration, Elecsys CA15-3 II CalSet (Cat. No. 03045846, for 4×1 mL) was used. Controls, Elecsys PreciControl Tumor Markers 1 and 2 (Cat No. 11776452, 2×3 mL), were run in duplicates every day of the study.

Analytical evaluation

The analytical performance of the Access BR Monitor assay was evaluated by all five centers in parallel, in particular imprecision, inter-laboratory reproducibility, minimum detectable concentration and linearity upon dilution. The influence of endogenous interferents and high-dose hook effect was tested in the laboratories of Munich, Barcelona and Aachen; interferences of sample type and sample storage were only tested in Munich.

Imprecision Two controls (Bio-Rad) and three human serum pools prepared by each center, including a low, medium and high concentration pool were tested in triplicate, with two runs per day for at least 10 days according to the guidelines of the CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS; document NCCLS-EP5-A). Data analysis included calculation of within-run and total imprecision and was performed by Acomed Statistics, Leipzig, Germany.

Inter-laboratory reproducibility Inter-laboratory reproducibility was evaluated using the two controls across the five evaluation sites.

Minimum detectable concentration The minimum detectable concentration was defined as the BR Monitor concentration corresponding to a signal two standard deviations above the main value of 10 replicates of the S0 calibrator tested on each of 3 days.

Linearity upon dilution A total of 22 samples, with six of them above the assay dynamic range (>1000 kU/L) and 16 of them between 200 and 1000 kU/L, were diluted with the appropriate Access BR Monitor diluent to obtain a minimum of four dilutions within the assay dynamic range. Dilutions were prepared separately in one to two steps using calibrated pipettes and were carried out in four replicates. Recov-

eries were calculated with respect to the highest concentration in the dynamic range.

Sample type interference Samples were obtained from 10 patients with unsuspicious laboratory findings, one serum in tubes with kaolin, one lithium-heparinate plasma, one EDTA plasma and one citrate plasma. Sample type interference was tested in duplicates. Recoveries were calculated with respect to the concentration in the serum sample.

Sample storage interference Samples from seven of these patients were measured natively and after storage at 4°C and –20°C for 1 day. Both storage modalities were compared with the native measurements.

Further, serum and lithium-heparinate plasma samples of the 10 patients were stored at –20°C for 6 months and measurements were compared with the original –20°C data to test the long-term stability.

Endogenous interferents The influence of bilirubin, hemoglobin and triglycerides was tested on a human serum pool with high CA15-3 concentration. This pool was diluted with a serum containing high bilirubin concentration (>0.062 μmol/L), with a serum with high hemoglobin level (ca. 0.855 mmol/L; normal serum spiked with hemolyzed blood sample) and with a serum with high triglyceride concentration (>0.006 mmol/L). These test solutions were tested with the Access BR Monitor assay and compared to the control solutions obtained from the same pool diluted in the same way with the Sample Diluent A (Catalog Number 81908) instead of the interfering substance. Each test solution and each control solution were assayed 10 times in constantly decreasing proportions. Recoveries were calculated with respect to the concentration of the undiluted serum pool. Additionally, 10 samples with high known rheumatoid factor concentration were tested in duplicate.

High-dose hook effect Serial 10-fold dilutions of five different samples with very high CA15-3 concentrations above 3000 kU/L were tested. Recoveries were calculated with respect to the highest concentration in the dynamic range.

Clinical performance

The clinical performance of the Access BR Monitor assay was evaluated by two sites (Munich and Barcelona). All clinical samples were sent to the Institute of Clinical Chemistry of the University Hospital Munich, to be tested using the Access BR Monitor assay on the UniCel® DxI 800 Immunoassay System (Beckman Coulter) and compared to the reference CA15-3 II assay on the Elecsys 2010 Immunology System (Roche Diagnostics).

Healthy individuals The normal reference interval for the BR Monitor was established from 267 samples, including 113 sera from men and 154 sera from non-pregnant women. Median age was 39.4 years (range 17–81 years). The subject inclusion/exclusion criteria were as follows:

- normal, apparently healthy subjects (evaluated clinically and by clinical chemistry parameters),
- adults older than 18 years were tested,
- no personal history of cancer disease, renal failure or liver disease.

Age and sex were mandatory for all samples enrolled. Samples with hemolysis, bilirubin or lipemia were excluded.

Individuals with benign diseases BR Monitor results were determined in a total of 549 individuals diagnosed with benign diseases, among them 148 benign breast diseases, 109 benign gynecological diseases (ovarian cysts, endometriosis, uterine leiomyoma, etc.), 155 benign gastrointestinal diseases (ulcerous colitis, Crohn's disease, liver cirrhosis, hepatitis, pancreatitis, cholelithiasis, etc.), 44 benign lung diseases (tuberculosis, sarcoidosis, pneumonia, etc.), 66 benign urological diseases (nephrolithiasis, renal failure, etc.) and 27 other benign diseases, and compared with the reference system.

Individuals with malignant diseases BR Monitor results were determined in a total of 995 individuals diagnosed with cancer diseases and compared with the reference system. The cancer diseases included 81 ovarian cancers, 57 other gynecological cancers, 62 pancreatic cancers, 26 gastric cancers, 58 hepatocellular cancers, 113 colorectal cancers, 82 lung cancers, 57 urological cancers (bladder and kidney) and 43 prostate cancers. We also studied 416 patients with breast cancer, among them 207 with locoregional and 151 with metastatic disease. For 58 patients, no exact staging information about the active disease state was available.

All samples were obtained from patients with active disease, typically before surgery as first treatment modality, or in some cases at time of recurrent disease.

Statistical analysis

The BR Monitor assay and reference method were compared using regression equations according to Passing and Bablok. Normalized differences from mean values were calculated according to Bland and Altman.

In healthy individuals, the frequency distribution for the BR Monitor and reference method was defined including 25th percentile, median, mean, upper reference limit (URL) of a normal population at 95th, 97.5th and 99th percentiles.

In all studied groups, distribution of BR Monitor and reference method concentrations were presented graphically, as well as statistically (median, range, 95th percentile).

The analysis of the sensitivity/specificity for breast cancer included receiver operating characteristic (ROC) curves, using benign breast diseases as the control group. Similarly, ROC curves were established for ovarian cancer vs. benign gynecological diseases, lung cancer vs. benign lung diseases, and colorectal cancer vs. benign gastrointestinal diseases. Further, at 95% specificity against the respective benign control group, the sensitivity for each cancer type was calculated, and also the area under the curve (AUC) of the corresponding ROC curves with the corresponding 95% confidence interval.

Results

Analytical evaluation

Imprecision Within-run imprecision of the low control (10.3–11.7 kU/L) ranged in the various centers between 5.2% and 8.1%, and of the high control (27.2–29.8 kU/L) between 6.2% and 6.8%. Within-run imprecision of the low serum pools (ranging from 8.0 to 29.8 kU/L) was between 5.3% and 6.8%, of the medium serum pools (ranging from 17.3 to 109 kU/L) between 5.3% and 7.7%, and of the high serum pools (ranging from 169 to 688 kU/L) between 4.3% and 11.6%.

Total imprecision of the low control (10.3–11.7 kU/L) ranged in the various centers from 6.6% to 10.6%, and of the high control (27.2–29.8 kU/L) from 7.6% to 9.1%. Total imprecision of the low serum pools (8.0–29.8 kU/L) was from 6.8% to 9.1%, of the medium serum pools (17.3–109 kU/L) was from 6.8% to 10.7%, and of the high serum pools (169–688 kU/L) from 5.5% to 11.7%.

Inter-laboratory reproducibility Inter-laboratory imprecision of the low control (10.3–11.7 kU/L) was found to be 5.1%, and of the high control (27.2–29.8 kU/L) 3.4%.

Minimum detectable concentration The minimum detectable concentration was found to be <1.0 kU/L in all centers. All these results were in the very low range and have no clinical relevance.

Linearity upon dilution Linearity upon dilution revealed a mean recovery of all dilutions of 98.5% in all centers, with a standard deviation of 9.1% (minimum 72.4%, maximum 145%) (Figure 1).

Sample type interference Samples from ten patients with unsuspicious laboratory findings were tested on sample type interference. BR Monitor measurements in kaolin serum, lithium-heparinate plasma and EDTA plasma were very comparable. Mean recovery in heparinate plasma was 102%, with a standard deviation of 11.4% (minimum 80.2%, maximum 120%). Mean recovery in EDTA plasma was 97.1%, with a standard deviation of 8.4% (minimum 83.8%, maximum 111%). BR Monitor values in citrate plasma were lower than BR Monitor values in serum, with a mean recovery of 84.7% and a standard deviation of 6.0% (minimum 75.0%, maximum 92.3%).

Sample storage interference Samples from seven of these patients were measured natively, after storage at 4°C and at –20°C each for 1 day. Both storage conditions tested did not affect the BR Monitor recovery. After storage at 4°C, mean recovery was 103%, with a standard deviation of 7.1% (minimum 88.0%, maximum 109%). After storage at –20°C, mean recovery was 102%, with a standard deviation of 10.2% (minimum 89.8%, maximum 117%).

In addition, serum and lithium-heparinate plasma samples of the 10 patients were stored at –20°C for 6 months and measurements were compared with the original –20°C data to test the long-term stability. Again, storage had no influence on marker levels. When serum samples were stored at –20°C for 6 months, mean recovery was 110%, with a standard deviation of 7.8% (minimum 96.9%, maximum 119%). When lithium-heparinate plasma samples were stored at –20°C for 6 months, mean recovery was 110%, with a standard deviation of 7.6% (minimum 93.9%, maximum 120%).

Endogenous interferents The potentially confounding impact of endogenous interferents, such as hemoglobin, bilirubin and triglycerides, was tested at two centers. Stepwise dilution of a serum pool having high CA15-3 level with a serum sample with high concentration of the relevant interferent and, alternatively, with sample diluent which was free of any contamination showed that neither interferent had any influence on BR Monitor level.

Dilution with hemoglobin-spiked serum resulted in a mean recovery of 113%, with a standard deviation of 19.7% (minimum 87.7%, maximum 161%). There was no trend of continuously changing BR Monitor values when increasing amounts of hemoglobin were added (Figure 2).

In the dilution series with bilirubin-rich serum, one random outlier was identified (recovery of 209%). When this value was excluded, mean recovery was 106%, with a standard deviation of 12.3% (minimum 91.3%, maximum 137%). Despite the outlier, there was no trend of continuously changing BR Monitor values when increasing amounts of bilirubin were added (Figure 2).

Dilution with triglyceride-rich serum yielded a mean recovery of 108%, with a standard deviation of 14.8% (minimum 88.7%, maximum 153%). There was no trend of continuously changing BR Monitor values when increasing amounts of triglycerides were added (Figure 2).

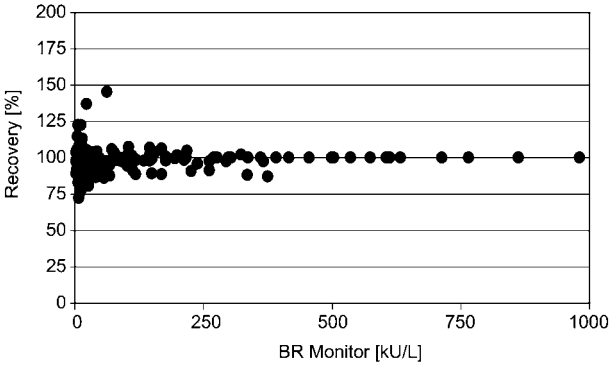

Figure 1 Linearity upon dilution.
Samples with high BR Monitor levels were diluted by one to two steps and recoveries were calculated for various dilution steps.

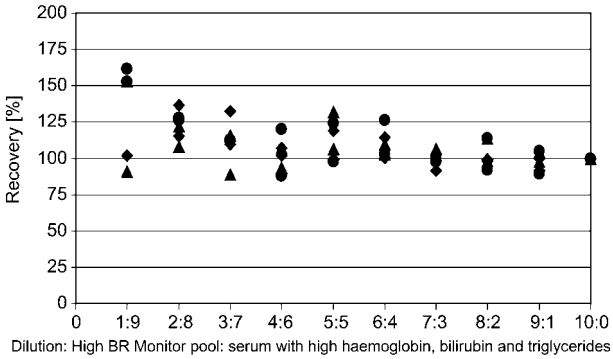

Figure 2 Influence of endogenous interferences.
Samples were spiked with various concentrations of hemoglobin (●), bilirubin (♦) and triglycerides (▲) and recoveries of BR Monitor levels were calculated for various dilution steps.

Rheumatoid factor A total of 14 serum samples with high rheumatoid factor concentrations (mean 209.4 kU/L, standard deviation 298.0 kU/L, minimum 26.7 kU/L, maximum 1131 kU/L) were tested for the potential confounding effect of rheumatoid factor on BR Monitor values. However, all BR Monitor levels were very low in the range of healthy individuals. Mean value was 17.6 kU/L, with a standard deviation of 8.6 kU/L (minimum 7.1 kU/L, maximum 42.3 kU/L).

High-dose hook effect In total, five serum samples with very high CA15-3 levels (3558–13,540 kU/L) were tested in dilution series on a potential high-dose hook effect. As illustrated graphically in Figure 3, all samples demonstrated a linear dilution response. Mean recovery in the curves was 101%, with a standard deviation of 13.6% (minimum 80.8%, maximum 136%) (Figure 3).

Clinical performance

Method comparison Comparison of the Access BR Monitor assay and the Elecsys CA15-3 assay (n = 1811) yielded a correlation coefficient of R = 0.797, with a slope of 1.383 and an intercept of –0.011.

A large number of samples (1768 out of 1811) were found to have values up to 200 kU/L. For this group, a correlation of R = 0.807, with a slope of 1.353 and an intercept of +0.302, was found. Values up to 50 kU/L were found in 1619 out of 1811 samples. For this clinically relevant group, the coefficient of correlation was R = 0.780, with a slope of 1.294 and an intercept of +0.883 (Figure 4).

Healthy individuals For the Access BR Monitor, the 95th percentile URL of a healthy population (n = 267) was determined to be 23.5 kU/L. The value distribution ranged from 2.3 to 36.8 kU/L. Mean was at 12.8 kU/L and the median was at 11.9 kU/L. The 25th percentile was calculated as 8.7 kU/L, 97.5th percentile at 28.1 kU/L and 99th percentile at 30.1 kU/L. Females had slightly higher levels (median at 12.8 kU/L, 95th percentile at 25.0 kU/L) than males (median at 11.1 kU/L, 95th percentile at 20.6 kU/L). The methods correlated reasonably well with each other (R = 0.757); however, a slope of 1.357 with lower values for the BR Monitor compared with the CA15-3 Elecsys was calculated (Figure 5, Table 1).

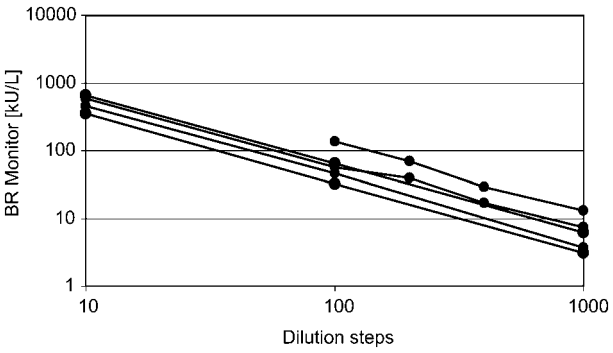

Figure 3 Influence of high dose hook effect.
Samples with extremely high BR Monitor levels >3000 kU/L were diluted by 1:10 steps.

Individuals with benign diseases Of 549 individuals diagnosed with benign diseases, patients with benign breast and gynecological diseases demonstrated the lowest levels for the Access BR Monitor and were in the range of healthy individuals (medians at 11.3 and 12.0 kU/L, 95th percentiles at 22.3 and 21.6 kU/L). Slightly higher levels were found in benign gastrointestinal, lung and urological diseases (medians at 13.7, 15.2 and 12.7 kU/L; 95th percentiles at 32.3, 32.1 and 54.6 kU/L). Particularly, renal insufficiency could cause moderate BR Monitor elevations. In all benign diseases, both methods yielded a generally acceptable correlation (R = 0.739) and a notable slope of 1.236. Details of distribution of values are listed in Table 1 and Figure 5.

Individuals with malignant diseases Of 995 individuals diagnosed with malignant diseases, patients with breast cancer yielded the highest levels for the Access BR Monitor, with values exceeding 3000 kU/L. The median value in locoregional breast cancer was calculated at 13.7 kU/L and 95th percentile at 42.3 kU/L, while in metastatic breast cancer the median was found to be 48.4 kU/L and 95th percentile at 993.8 kU/L. However, high levels were observed in patients with ovarian cancer (median at 22.8 kU/L, 95th percentile at 164.7 kU/L) and with lung cancer (median at 21.0 kU/L, 95th percentile at 161.3 kU/L). In contrast, strongly elevated BR Monitor values were only found rarely in individuals with gastrointestinal cancers, such as colorectal cancer (95th percentile at 33.4 kU/L) and gastric cancer (95th percentile at 31.3 kU/L), as well as in urological cancers, such as bladder cancer (95th percentile at 30.0 kU/L) and prostate cancer (95th percentile at 33.8 kU/L). Also in this subgroup, both methods correlated with each other (R = 0.801) and a slope of 1.389 was found. Details of distribution of values are listed in Table 1 and Figure 6.

Sensitivity for cancer disease In addition to the comparison of the absolute concentrations of both methods in various patient groups with benign and malignant diseases, the diagnostic capacity of the Access BR Monitor (CA15-3 antigen) assay on the UniCel® Dxl 800 Immunoassay System was tested by ROC curves showing the profile of sensitivity and specificity over the whole range of values and was compared to that of the CA15-3 assay on the Elecsys 2010 Immunology System. According to the guidelines of the European Group on Tumor Markers [EGTM (3)], all cancer types were compared with the respective benign disorders as the relevant control group.

Concerning their diagnostic capacity, both methods showed very comparable results for all cancer types investigated – despite the general slope in the concentrations. This good diagnostic correlation was expressed by similar values for AUC with overlapping

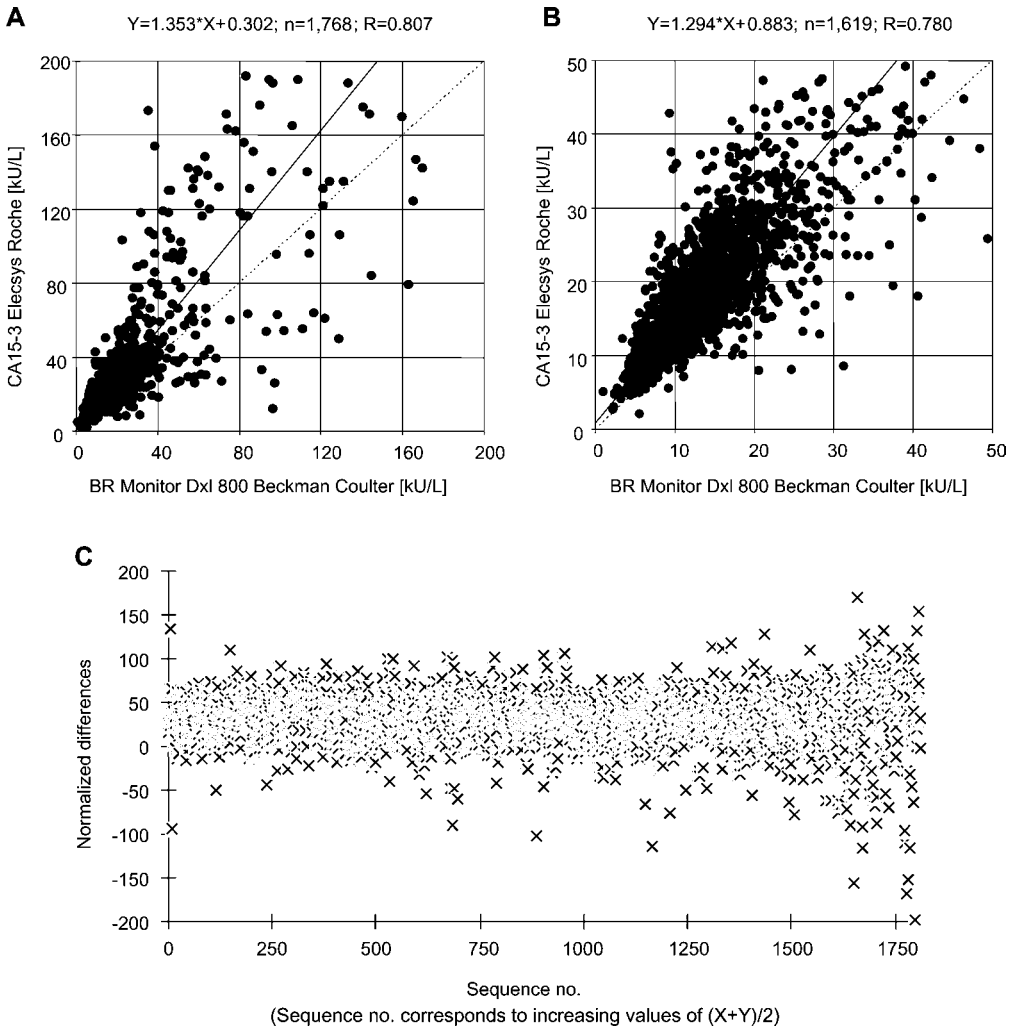

Figure 4 Method comparison of the BR Monitor with the reference method.
Correlation of Access BR Monitor (Beckman Coulter) and Elecsys CA15-3 (Roche Diagnostics) concentrations were calculated (A) for the range <200 kU/L and (B) for the range <50 kU/L. (C) Normalized differences from mean values were calculated according to Bland and Altman.

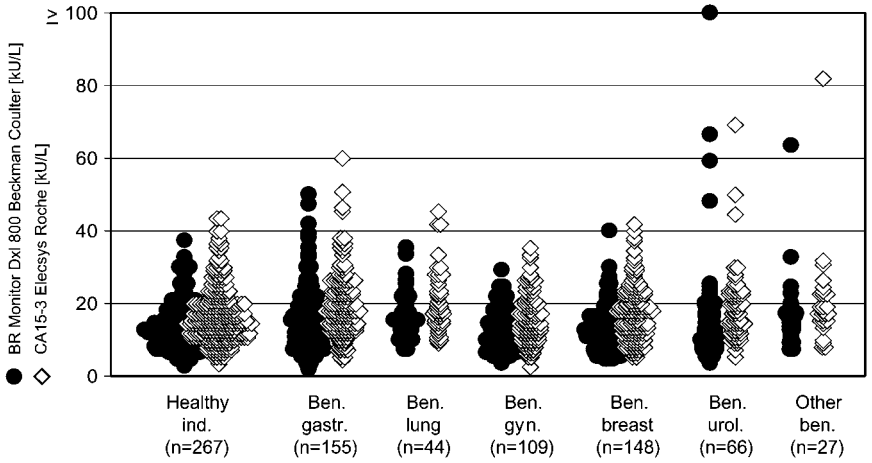

Figure 5 Value distribution of the BR Monitor and reference method in controls.
Dot plot of the (●) Access BR Monitor (Beckman Coulter) and (◇) Elecsys CA15-3 (Roche Diagnostics) concentrations in serum samples of healthy individuals and individuals with various benign diseases.

Table 1 BR Monitor concentrations in sera of cancer patients and controls.

Diagnosis	n	Method	Median, kU/L	Range, kU/L	95th percentile, U/L
Healthy individuals	267	Dxl 800	11.9	2.3–36.8	23.5
		Elecsys	15.1	3.0–42.9	31.5
Benign breast diseases	148	Dxl 800	11.3	3.9–39.3	22.3
		Elecsys	17.1	4.9–41.4	31.1
Benign gynecological diseases	109	Dxl 800	12.0	3.5–28.3	21.6
		Elecsys	14.2	2.0–35.0	27.5
Benign gastrointestinal diseases	155	Dxl 800	13.7	1.0–49.4	32.3
		Elecsys	18.0	4.1–59.4	36.3
Benign lung diseases	44	Dxl 800	15.2	6.5–35.4	32.1
		Elecsys	18.0	9.0–45.1	41.0
Benign urological diseases	66	Dxl 800	12.7	3.6–129.0	54.6
		Elecsys	17.0	4.9–68.8	39.2
Other benign diseases	27	Dxl 800	15.6	6.6–63.5	51.0
		Elecsys	17.1	8.0–81.1	61.1
Breast cancer	416	Dxl 800	16.9	4.2–3335	429.7
		Elecsys	23.0	6.0–3890	450.2
Breast cancer (locoregional)	207	Dxl 800	13.7	4.2–475.0	42.3
		Elecsys	18.9	6.4–1035	53.4
Breast cancer (metastatic)	151	Dxl 800	48.4	5.7–3335	993.8
		Elecsys	66.0	6.0–3890	1585.6
Ovarian cancer	81	Dxl 800	22.8	2.8–324.3	164.7
		Elecsys	35.5	5.5–419.0	262.5
Gynecological cancer	57	Dxl 800	16.6	4.1–94.3	60.2
		Elecsys	24.5	5.8–285.0	136.4
Gastric cancer	26	Dxl 800	11.2	2.2–35.9	31.3
		Elecsys	18.1	2.6–108.0	84.4
Hepatocellular cancer	58	Dxl 800	17.1	6.5–466.6	87.0
		Elecsys	21.8	7.3–2258	122.5
Pancreatic cancer	62	Dxl 800	14.3	2.4–388.0	56.1
		Elecsys	21.7	4.7–535.0	62.0
Colorectal cancer	113	Dxl 800	13.8	5.1–114.9	33.4
		Elecsys	20.3	7.1–118.0	48.9
Lung cancer	82	Dxl 800	21.0	5.0–1729.0	161.3
		Elecsys	23.6	7.0–241.0	134.0
Urological cancer	57	Dxl 800	14.0	5.5–51.0	30.0
		Elecsys	20.5	7.7–52.1	34.1
Prostate cancer	43	Dxl 800	15.3	4.2–38.6	33.8
		Elecsys	20.5	7.7–50.7	43.3

Median, range and 95th percentile limit of the Access BR Monitor (Beckman Coulter) for healthy individuals, patients with benign and malignant diseases. Comparison with Elecsys CA15-3 (Roche Diagnostics) concentrations.

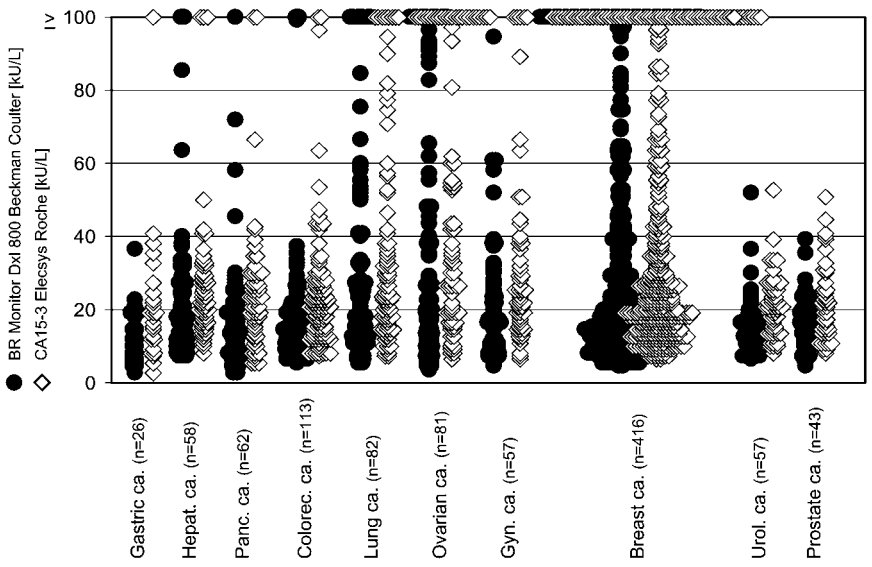

Figure 6 Value distribution of the BR Monitor and reference method in cancer patients.
Dot plot of the (●) Access BR Monitor (Beckman Coulter) and (◇) Elecsys CA15-3 (Roche Diagnostics) concentrations in serum samples of individuals with various malignant diseases.

Table 2 Diagnostic capacity of BR Monitor for various cancer diseases.

Diagnosis	n	Method	Sensitivity at 95% specificity vs. respective benign diseases	AUC	Confidence interval
Breast cancer	416	Dxl 800	38.7	0.708	0.665–0.750
		Elecsys	35.6	0.696	0.652–0.740
Breast cancer (locoregional)	207	Dxl 800	18.8	0.619	0.561–0.677
		Elecsys	16.4	0.611	0.553–0.669
Breast cancer (metastatic)	151	Dxl 800	76.2	0.897	0.859–0.935
		Elecsys	73.5	0.887	0.847–0.926
Ovarian cancer	81	Dxl 800	53.1	0.774	0.701–0.848
		Elecsys	60.5	0.812	0.746–0.879
Gynecological cancer	57	Dxl 800	36.8	0.694	0.604–0.784
		Elecsys	38.6	0.755	0.674–0.835
Gastric cancer	26	Dxl 800	3.8	0.386	0.262–0.511
		Elecsys	15.4	0.469	0.333–0.605
Hepatocellular cancer	58	Dxl 800	17.2	0.635	0.549–0.720
		Elecsys	13.8	0.647	0.564–0.730
Pancreatic cancer	62	Dxl 800	6.5	0.523	0.432–0.614
		Elecsys	11.3	0.584	0.494–0.674
Colorectal cancer	113	Dxl 800	7.1	0.519	0.449–0.589
		Elecsys	10.6	0.557	0.486–0.627
Lung cancer	82	Dxl 800	32.9	0.631	0.536–0.726
		Elecsys	26.8	0.641	0.546–0.735
Renal cancer	29	Dxl 800	0.0	0.563	0.444–0.682
		Elecsys	10.7	0.646	0.525–0.767
Bladder cancer	28	Dxl 800	3.4	0.528	0.407–0.650
		Elecsys	10.3	0.554	0.430–0.679

Survey on the diagnostic capacity of the Access BR Monitor (Beckman Coulter) for various cancer diseases when compared with their respective benign diseases as control groups and comparison with the Elecsys CA15-3 (Roche Diagnostics). Area under the curve (AUC) of the receiver operating characteristic curves and sensitivity at 95% specificity vs. the respective benign diseases indicate the discriminating power.

confidence intervals, as well as by the sensitivity for cancer detection at 95% specificity of benign diseases (Table 2). Among all cancers, breast cancer and ovarian cancer showed the highest AUC values for both methods when compared with benign breast and gynecological diseases, respectively, particularly in metastatic disease.

For patients with locoregional breast cancer, the AUC was 0.619 for the Access BR Monitor and 0.611 for the Elecsys CA15-3. The respective sensitivity at 95% specificity vs. benign breast diseases was 18.8% for the Access BR Monitor and 16.4% for the Elecsys CA15-3. For patients with metastatic breast cancer, the AUC was significantly higher at 0.897 for the Access BR Monitor and 0.887 for the Elecsys CA15-3. The respective sensitivity at 95% specificity vs. benign breast diseases was calculated at 76.2% for the Access BR Monitor and 73.5% for the Elecsys CA15-3 (Figure 7, Table 2). In ovarian cancer, the AUC was 0.774 for the Access BR Monitor and 0.812 for the Elecsys CA15-3, with a sensitivity of 53.1% (Access BR Monitor) and 60.5% (Elecsys CA15-3) at 95% specificity vs. benign gynecological diseases. In addition, the Access BR Monitor and Elecsys CA15-3, respectively, showed diagnostic power for other cancers, such as lung cancer and other gynecological cancers, too (Figure 8, Table 2).

Discussion

Several studies have shown that CA15-3 is the marker of first choice for the management of breast cancer. If no renal insufficiency is present, which can also provoke elevated CA15-3 levels, high preoperative and pretherapeutic CA15-3 concentrations are helpful in suggesting differential diagnosis of symptomatic breast cancer. Moreover, prognostic relevance of pretherapeutic CA15-3-levels for overall survival of breast cancer patients undergoing surgery and/or receiving systemic chemo- and/or radiotherapy was found repeatedly by several groups. Similarly, the usefulness of CA15-3 for therapy monitoring, as well as early detection of diseases progression in breast cancer patients, is widely recognized and accepted. In addition to breast cancer, CA15-3 is detected in ovarian, endometrial, cervical, lung and gastrointestinal cancer tissues, as well as in the serum of these patients (3–23).

In the present study, the new Access BR Monitor assay, which uses the monoclonal antibodies Ma 552 and Ma 695 for detection of the CA15-3 antigen, was tested for analytical and clinical performance. The guidelines of the EGTM (3) require a new diagnostic method to be investigated for potential influence of organ-specific and non-specific influences which might alter the metabolism of the antigen. Further, the new method has to be compared with a current accepted method to demonstrate its superiority, or at least equivalence, for the intended indication.

First, we therefore performed a thorough analytical evaluation at five European centers, to test the basic preconditions for routine application. Then, a large panel of sera from 1811 individuals was investigated. These individuals included healthy individuals,

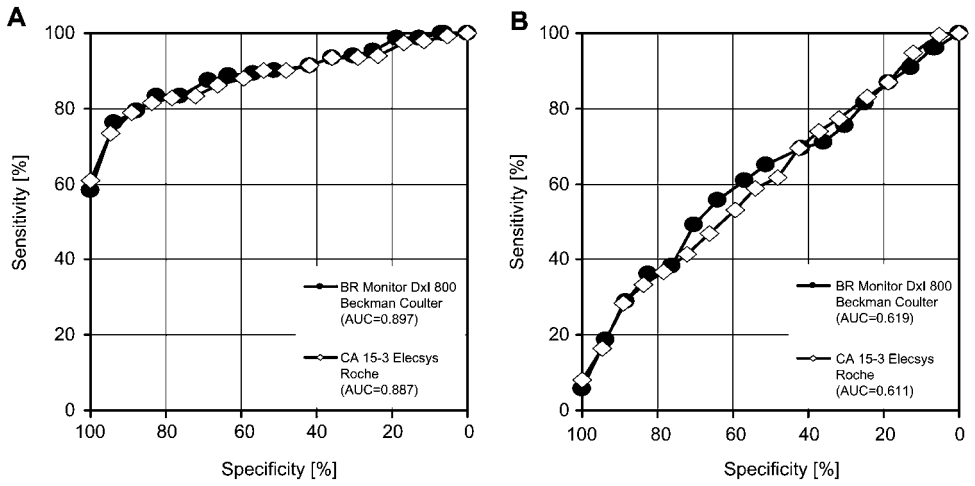

Figure 7 Diagnostic capacity of the BR Monitor and reference method for detection of metastatic and locoregional breast cancer.
Profiles of sensitivity and specificity over the whole range of cut-off values are shown by receiver operating characteristic (ROC) curves for (A) metastatic breast cancer (n = 151) and (B) locoregional breast cancer (n = 207) vs. benign breast diseases (n = 148). (●) Access BR Monitor (Beckman Coulter) and (◇) Elecsys CA15-3 (Roche Diagnostics).

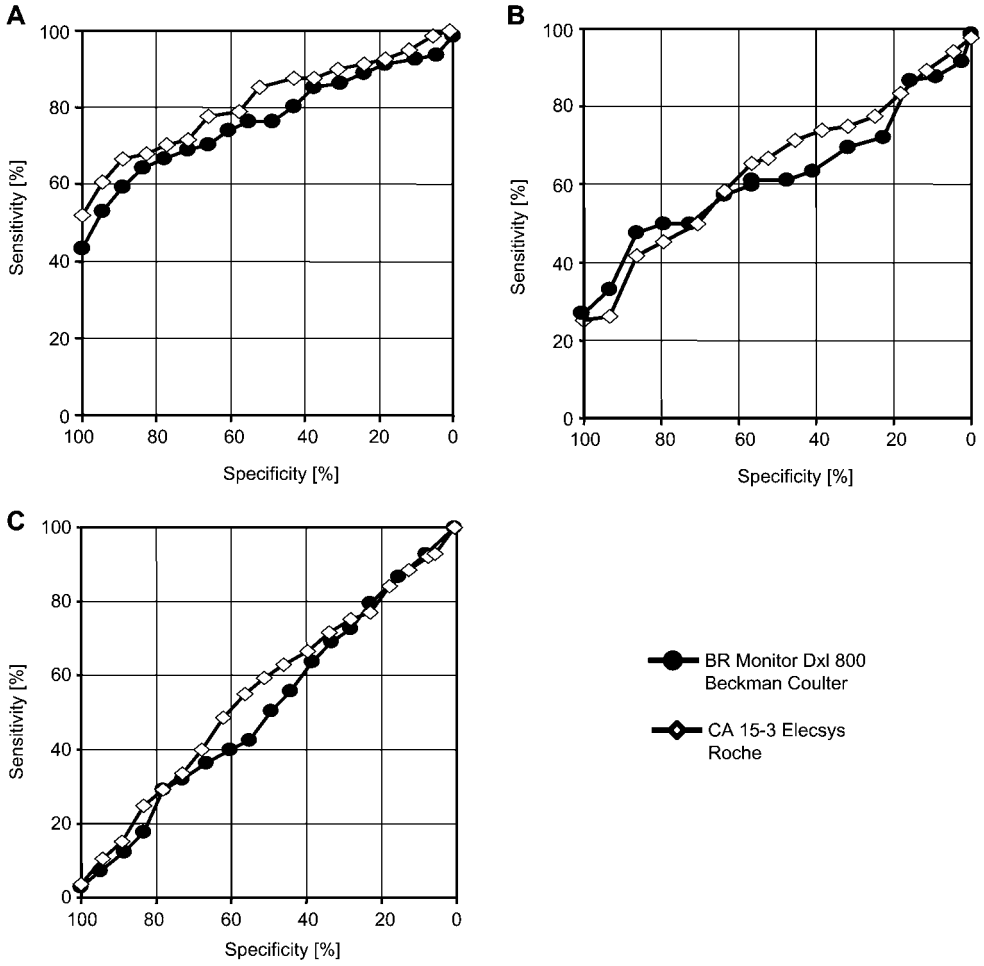

Figure 8 Diagnostic capacity of the BR Monitor and reference method for detection of ovarian cancer, lung cancer and colorectal cancer.
Profiles of sensitivity and specificity over the whole range of cut-off values are shown by receiver operating characteristic (ROC) curves for (A) ovarian cancer (n = 81) vs. benign gynecological diseases (n = 109), (B) lung cancer (n = 82) vs. benign lung diseases (n = 44) and (C) colorectal cancer (n = 113) vs. benign gastrointestinal diseases (n = 155). (●) Access BR Monitor (Beckman Coulter) and (◇) Elecsys CA15-3 (Roche Diagnostics).

patients with gastrointestinal and other benign diseases and many patients with various cancer diseases that might be relevant for differential diagnosis by CA15-3. The entire clinical evaluation of the Access BR Monitor was carried out in parallel with the Elecsys CA15-3, a current standard method, using the same sera from the same patients, to enable a fair comparison of both methods.

The analytical performance for the BR Monitor assay was accurate with a low, however, not yet optimal within-run, total and inter-laboratory imprecision. Additionally, we observed suitable recoveries during linearity upon dilution testing and no high-dose hook effect up to 13,540 kU/L. Sample type interference studies demonstrated that serum, lithium-heparinate plasma and EDTA plasma can be used interchangeably. However, BR Monitor levels in citrate plasma were approximately 15% lower than in serum. Concerning sample storage, it is important to note that freezing did not affect the marker values, and long-term storage for 6 months at –20°C still yielded stable results. Of clinical relevance is the finding that endogenous interferents, such as hemoglobin, bilirubin, triglycerides and rheumatoid factor, do not influence BR Monitor concentrations.

Comparison of the Access BR Monitor with Elecsys CA15-3 showed a reasonably good correlation for all patients and for the various subgroups investigated. However, a notable slope with 20%–40% lower concentrations for the BR Monitor was observed. Similar results have been described earlier by several authors who performed method comparisons of CA15-3 assays (11, 12). This fact shows the necessity to interpret the absolute results with care, to maintain the same method in serial investigations and to double the values when CA15-3 methods are changed.

In our study, healthy individuals had very low values, as measured with both methods. URLs were only slightly different and were in the range of the values indicated by both manufacturers (Beckman Coulter 23.5 kU/L, Roche Diagnostics 31.5 kU/L).

Levels in sera of individuals diagnosed with benign breast and gynecological diseases were similar to those of healthy individuals. Patients with benign lung, gastrointestinal and urological diseases had slightly higher BR Monitor levels. Particularly, renal insufficiency is known to potentially cause elevated levels (24, 25). In this group, single values reached more than 100 kU/L.

CA15-3 concentrations in patients suffering from various cancers were also elevated. However, in some cancer types, medians and 95th percentiles were comparable with those of benign diseases, e.g., for bladder, renal, prostate, colorectal and gastric cancer. In contrast, ovarian, lung, hepatocellular and other gynecological cancers demonstrated more pronounced CA15-3 elevations, which reached $>$1000 kU/L in some patients.

The best diagnostic accuracy of the BR Monitor for cancer detection against the relevant benign control group was found for breast cancer and for ovarian cancer. Particularly in metastatic breast cancer, a high sensitivity of 76.2% for cancer detection at 95% specificity vs. benign breast diseases was found, while it was only 18.8% in locoregional disease. These notable differential diagnostic results underline the high relevance of CA15-3 antigen for the management of breast cancer and its impact in follow-up investigations – also for those patients who had not shown an initial release during locoregional disease. Importantly, BR Monitor results also correspond very well with the diagnostic accuracy of the CA15-3 reference method for cancer detection demonstrated by the similar AUC values and the broadly overlapping confidence intervals. Though the diagnostic power of the BR Monitor is somewhat lower for other tumor types, it has to be pointed out that for lung and gynecological cancer the AUC was still higher than 0.6 and the sensitivity at 95% specificity vs. the relevant benign control group higher than 20% – once again, both methods were very comparable. This diagnostic performance in cancer types for which CA15-3 was not considered as a relevant marker suggests there may be value in including CA15-3 with other diagnostically relevant markers in future multiparametric analyses. In combination with well known markers, e.g., CA125 in ovarian cancer, as well as with CYFRA 21-1, carcinoembryonic antigen, neuron-specific enolase and progastrin-releasing peptide in lung cancer, CA15-3 and other mucin markers might be helpful to further improve the diagnostic accuracy (26, 27).

Conclusions

The Access BR Monitor is a new assay based on an alternative antibody for CA15-3 antigen detection. The Access BR Monitor provides accurate methodological characteristics for use in routine laboratory and demonstrates an acceptable analytical and clinical correlation with the Elecsys CA15-3. The BR Monitor shows a high diagnostic accuracy in breast cancer and it is a valuable marker in the management of this disease. Our results also suggest a clinical value of the BR Monitor in gynecological (ovarian and endometrial) and lung cancers. If the CA15-3 antigen method is changed, parallel measurements of CA15-3 with both methods for an appropriate time span are strongly recommended.

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