

Alternative antibody for the detection of CA19-9 antigen: a European multicenter study for the evaluation of the analytical and clinical performance of the Access® GI Monitor assay on the UniCel® Dxl 800 Immunoassay System

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Abstract

Background: Gastrointestinal cancer antigen CA19-9 is known as a valuable marker for the management of patients with pancreatic cancer.

Methods: The analytical and clinical performance of the Access® GI Monitor assay (Beckman Coulter) was evaluated on the UniCel® Dxl 800 Immunoassay System at five different European sites and compared with a reference method, defined as CA19-9 on the Elecsys System (Roche Diagnostics).

Results: Total imprecision (%CV) of the GI Monitor ranged between 3.4% and 7.7%, and inter-laboratory reproducibility between 3.6% and 4.0%. Linearity upon dilution showed a mean recovery of 97.4% (SD+7.2%). Endogenous interferents had no influence on GI Monitor levels (mean recoveries: hemoglobin 103%, bilirubin 106%, triglycerides 106%). There was no high-dose hook effect up to 115,000 kU/L. Clinical performance investigated in sera from 1811 individuals showed a good correlation between the Access® GI Monitor and Elecsys CA19-9 ($R=0.959$, slope=1.004, intercept=+0.17). GI Monitor serum levels were low in healthy individuals ($n=267$, median=6.0 kU/L, 95th percentile=23.1 kU/L), higher

in individuals with various benign diseases ($n=550$, medians=5.8–13.4 kU/L, 95th percentiles=30.1–195.5 kU/L) and even higher in individuals suffering from various cancers ($n=995$, medians=8.4–233.8 kU/L, 95th percentiles=53.7–13,902 kU/L). Optimal diagnostic accuracy for cancer detection against the relevant benign control group by the GI Monitor was found for pancreatic cancer [area under the curve (AUC) 0.83]. Results for the reference CA19-9 assay were comparable (AUC 0.85).

Conclusions: The Access® GI Monitor provides very good methodological characteristics and demonstrates an excellent analytical and clinical correlation with the Elecsys CA19-9. The GI Monitor shows the best diagnostic accuracy in pancreatic cancer. Our results also suggest a clinical value of the GI Monitor in other cancers.

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Introduction

Pancreatic cancer accounts for only 2% of the newly diagnosed cancers per year. Because the overall median survival is only 3–5 months with a 1-year survival rate of less than 10% (1), pancreatic cancer ranks as the fourth leading death cause among cancer diseases (2). More than 80% of the patients are initially diagnosed at advanced stages of disease, when treatment options are limited to systemic therapies to control disease-related symptoms and prolong survival (3, 4). The diagnosis of pancreatic cancer is usually established based on imaging techniques and subsequent histological confirmation. Desmoplastic stroma reaction is a well-known confounder, particularly of computed tomography, which makes it difficult to differentiate normal pancreas, local inflammation and fibrosis from malignant tissue (5). Therefore, other surrogate markers for differential diagnosis and estimation of treatment efficacy are needed. For these purposes, several studies have suggested the determination of the gastrointestinal cancer antigen CA19-9 concentration (6–9).

The tumor-associated antigen CA19-9 is the sialylated hapten of the human Lewis Le^a blood group antigen (10), first described by Koprowski et al. in 1979 (11). Individuals with a Le^(a-b) phenotype (lacking

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the Lewis-antigen glycosyltransferase) are unable to synthesize and release CA19-9 (12). Koprowski et al. defined CA19-9 using the monoclonal antibody 1116-NS-19-9, which was produced by a mouse spleen hybridoma immunized with a human colorectal carcinoma cell line (11, 13).

CA19-9 is detected in colorectal, gastric and pancreatic cancer tissues (80%–90%), but also in liver, gall bladder, lung, breast and gynecological cancers (8, 9, 14–16). In serum, only low concentrations of the high molecular, mucinous CA19-9 (molecular weight 900,000 Da) are found physiologically. In contrast, very high levels (up to 100,000 kU/L) have been reported in human body secretions, such as bile fluid, urine, sputum, milk, amniotic fluid and ovarian cysts (8–10). However, some benign diseases have been associated with elevated CA19-9 antigen levels, including particularly non-malignant gastrointestinal diseases with a cholestatic component, such as liver cirrhosis, cholangitis, hepatitis and pancreatitis (8, 9, 17–19).

For the diagnosis of pancreatic cancer, CA19-9 is the marker of choice with a sensitivity of 70%–90% (8, 20–22), particularly in tumors of the pancreatic head. Because there is no direct correlation of marker levels with tumor volume, CA19-9 is often (approximately 80%) already released in early tumor stages and reaches concentrations of more than 500 kU/L in 30% of the cases. Very high levels (>10,000 kU/L) are indicative of distant metastases. However, due to the lacking tumor and organ specificity, the early detection of pancreatic cancer by a one-time determination of CA19-9 is not possible (8, 9).

The prognostic value of CA19-9 levels for overall survival has been shown in various studies for pancreatic cancer patients undergoing surgery, as well as for those receiving systemic chemo- and/or radiotherapy: pre-therapeutic low CA19-9 concentrations are associated with a significantly longer survival. Cut-offs used showed a considerable variation between 200 kU/L and 2000 kU/L and were lower in patients with early stage pancreatic cancer undergoing surgery (6, 23–25).

The usefulness of CA19-9 for estimation of therapy efficacy is currently under investigation. Several studies have reported a close correlation between CA19-9 kinetics and therapy response, even if they used different definitions of CA19-9 decrease as criterion for therapy efficacy (6, 26–31). Stemmler et al. demonstrated CA19-9 decreases during chemotherapy – independent from their degree – were associated with longer survival, whereas increasing levels were linked with poor prognosis (31).

In the present study, a new assay for detection of CA19-9 antigen was evaluated for its analytical and clinical performance, and compared with an established reference method. This Access GI Monitor assay is applied on the UniCel® Dxl 800 Immunoassay System (Beckman Coulter Eurocenter S.A., Nyon, Switzerland) and uses the monoclonal antibody C192 as tracer and capture antibody, which recognizes practically the same epitope (same specificity) as the

Centocor 1116-NS-19-9 monoclonal antibody (Centocor Inc., Horsham, PA, USA). 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 GI Monitor assay”.

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

Materials and methods

Assay procedure

Access GI Monitor (CA19-9 antigen) assay on the UniCel® Dxl 800 Immunoassay System (Beckman Coulter) The Access GI Monitor assay is a paramagnetic particle, two-site immunoenzymatic (“sandwich”), chemiluminescent immunoassay for the quantitative determination of CA19-9 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 CA19-9 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 0.8–2000 kU/L).

For calibration, Access GI Monitor Calibrators (Cat. No. 387688: S0–S5, 2.5 mL/vial) were used. The Access GI Monitor Calibrators are provided at six levels – zero and approximately 30, 90, 300, 900 and 2000 kU/L. Controls, Bio-Rad Lyphochek Tumor Marker Controls (Cat. No. 580 Bilevel, 6×2 mL; Bio-Rad Laboratories, Munich, Germany), were run in duplicates every day of the study.

CA19-9 assay on Elecsys 2010 Immunology System (Roche Diagnostics GmbH) The CA19-9 assay is an electrochemoluminescence immunoassay for the quantitative determination of CA19-9 antigen levels in human serum and plasma using the Elecsys 2010/1010 and Modular Analytics E170 Immunology Systems (Roche Diagnostics, Penzberg, Germany). The assay is based on a sandwich principle with the monoclonal antibody 1116-NS-19-9: 10 µL of sample, a biotinylated monoclonal CA19-9-specific antibody, and the monoclonal 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 cal-

ibration 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 0.6–1000 kU/L.

For calibration, Elecsys CA19-9 CalSet (Cat. No. 11776215, 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 GI 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 (32), 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 GI monitor concentration corresponding to a signal two standard deviations above the mean value of 10 replicates of the S0 calibrator tested on each of 3 days.

Linearity upon dilution A total of 32 samples, with 13 of them above the assay dynamic range (>2000 kU/L) and 19 of them between 1000 and 2000 kU/L, were diluted with the appropriate Access GI 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. Recoveries were calculated with respect to the highest concentration in the dynamic range.

Sample type interference Samples were obtained from 10 patients with unsuspecting 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 CA19-9 concentrations. The serum pool was diluted with a serum containing high bilirubin concentrations (>0.062 μmol/L), with a serum with high hemoglobin (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 GI 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 11 different samples with very high CA19-9 concentrations above 30,000 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 GI 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 GI Monitor assay on the UniCel® Dxl 800 Immunoassay System (Beckman Coulter) and compared to the reference CA19-9 assay on the Elecsys 2010 Immunology System (Roche Diagnostics).

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

Individuals with malignant diseases GI Monitor results were determined in a total of 995 individuals diagnosed with cancer diseases and were compared with the reference method. The cancer diseases included 62 pancreatic cancers, 26 gastric cancers, 58 hepatocellular cancers, 113 colorectal cancers, 82 lung cancers, 81 ovarian cancers, 57 other gynecological cancers, 416 breast cancers, 57 urological cancers (bladder and kidneys) and 43 prostate cancers.

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 GI 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 GI Monitor and reference method was defined including 25th percentile, median, mean and upper reference limit (URL) of a normal population at 95th, 97.5th and 99th percentiles.

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

The analysis of the sensitivity/specificity for pancreatic cancer included receiver operator characteristics (ROC) curves, using benign gastrointestinal diseases as the control group. Similarly, ROC curves were established for lung cancer vs. benign lung diseases, colorectal cancer vs. benign gastrointestinal diseases, breast cancer vs. benign breast diseases, and ovarian cancer vs. benign gynecological 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.

Results

Analytical evaluation

Imprecision Within-run imprecision of the low control (8.6–9.3 kU/L) ranged in the various centers between 2.6% and 5.2%, and of the high control (32.2–35.2 kU/L) between 2.6% and 4.4%. Within-run imprecision of the low serum pools (ranging from 4.8 to 44.0 kU/L) was between 2.3% and 4.4%, of the medium serum pools (ranging from 106 to 404 kU/L) between 2.1% and 4.7%, and of the high serum pools (ranging from 447 to 1652 kU/L) between 2.4% and 5.0%.

Total imprecision of the low control (8.6–9.3 kU/L) ranged in the various centers between 4.4% and 7.7%, and of the high control (32.2–35.2 kU/L) between 4.6% and 7.7%. Total imprecision of the low serum pools (4.8–44.0 kU/L) was between 4.8% and 6.4%, of the medium serum pools (106–404 kU/L) between 3.7% and 6.3%, and of the high serum pools (447–1652 kU/L) between 3.4% and 6.1%.

Inter-laboratory reproducibility Inter-laboratory imprecision of the low control (8.6–9.3 kU/L) was found to be 3.6%, and of the high control (32.2–35.2 kU/L) 4.0%.

Minimum detectable concentration In four centers, the minimum detectable concentration was found to be <0.8 kU/L, and in one center <1.05 kU/L. All these results are in the very low range which has no clinical relevance.

Linearity upon dilution Mean recovery of all dilutions in all centers was 97.4%, with a standard deviation of 7.2% (minimum 79.7%, maximum 121%) (Figure 1).

Sample type interference Samples from 10 patients with unsuspected laboratory findings were tested on sample type interference. GI Monitor measurements in kaolin serum and lithium-heparinate plasma were very comparable. Mean recovery in heparinate plasma was 100%, with a standard deviation of 3.4% (minimum 94.4%, maximum 106%). GI Monitor values in EDTA plasma and citrate plasma were very comparable and lower than GI Monitor values in serum. For EDTA plasma, mean recovery was 77.5%, with a standard deviation of 2.4% (minimum 72.9%, maximum 80.3%). For citrate plasma, mean recovery was 79.6%, with a standard deviation of 3.9% (minimum 73.9%, maximum 85.8%).

Sample storage interference Samples from seven of these patients were measured natively, after storage at 4°C and at –20°C for 1 day. Both storage conditions tested did not affect the GI Monitor values. After storage at 4°C, mean recovery was 100.0%, with a standard deviation of 4.3% (minimum 91.5%, maximum 104.3%). After storage at –20°C, mean recovery was 100%, with a standard deviation of 3.8% (minimum 94.9%, maximum 104.5%).

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 was stored at –20°C for 6 months, mean recovery was 100.7%, with a standard deviation of 6.9% (minimum 91.5%, maximum 109%). When lithium-heparinate plasma was stored at –20°C for 6 months, mean recovery was 105%, with a standard deviation of 10.6% (minimum 89.7%, maximum 126.9%).

Endogenous interferents The potentially confounding impact of endogenous interferents, such as hemoglobin, bilirubin and triglycerides was tested at two

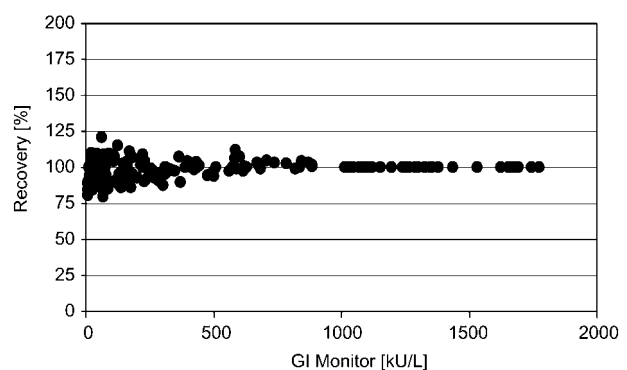


Figure 1 Linearity upon dilution. Samples with high GI Monitor levels were diluted by one to two steps and recoveries were calculated for various dilution steps.

centers. Stepwise dilution of a serum pool having high CA19-9 levels with a serum sample with high concentrations of the relevant interferent and, alternatively, with sample diluent which was free of any contamination showed that neither interferent had any influence on GI Monitor levels.

Dilution with hemoglobin-spiked serum resulted in a mean recovery of 103%, with a standard deviation of 10.3% (minimum 78.6%, maximum 123%). There was no trend of continuously changing GI Monitor values when increasing amounts of hemoglobin were added (Figure 2).

Dilution with bilirubin-rich serum showed a mean recovery of 106%, with a standard deviation of 7.5% (minimum 84.5%, maximum 115%). There was no trend of continuously changing GI Monitor values when increasing amounts of bilirubin were added (Figure 2).

In the dilution series with triglyceride-rich serum, one random outlier was identified (recovery of 186%). If this value was excluded, mean recovery was 106%, with a standard deviation of 11.1% (minimum 89.1%, maximum 131%). Despite the outlier, there was no trend of continuously changing GI Monitor values when increasing amounts of triglycerides were added (Figure 2).

Rheumatoid factor In total, 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 on a potential confounding effect on GI Monitor values. However, all GI Monitor levels were very low in the range of healthy individuals. Mean value was 8.9 kU/L, with a standard deviation of 8.6% (minimum 0.8 kU/L, maximum 33.0 kU/L).

High-dose hook effect In total, 11 serum samples with extremely high CA19-9 levels (18,011–114,920 kU/L) were tested in dilution series on a potential high-dose hook effect. As illustrated graphically in Figure 3, in all samples a linear dilution response was observed. Mean recovery in the curves

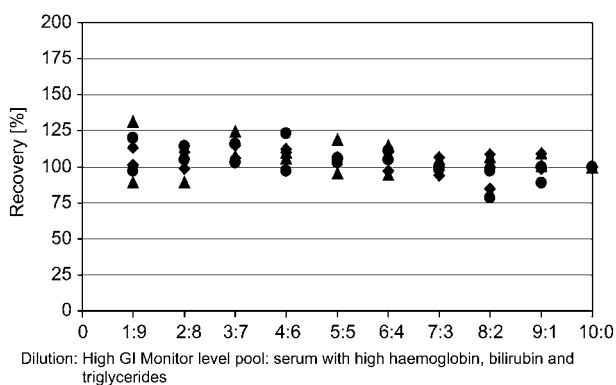


Figure 2 Influence of endogenous interferences. Samples were spiked with various concentrations of hemoglobin (◆), bilirubin (▲) and triglycerides (●) and recoveries of GI Monitor levels were calculated for various dilution steps.

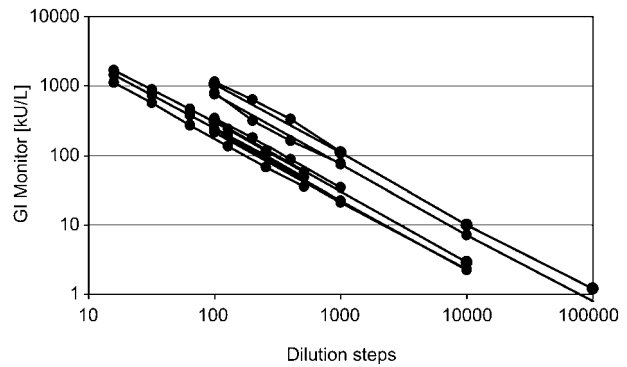


Figure 3 Influence of high-dose hook effect. Samples with extremely high GI Monitor levels (>18,000 kU/L) were diluted by 1:10 steps.

was 101%, with a standard deviation of 7.1% (minimum 79.7%, maximum 116%) (Figure 3).

Clinical performance

Method comparison Comparison of the Access GI Monitor (CA19-9 antigen) assay on the UniCel® Dxl 800 Immunoassay System and the CA19-9 assay on the Elecsys 2010 Immunology System, calculated on all serum samples (n=1811), yielded a correlation coefficient of $R=0.959$, with a slope of 1.004 and an intercept of +0.17.

A large number of samples (n=1765) were found to have values up to 1000 kU/L. For this clinically relevant group, an excellent correlation was still found. The coefficient of correlation was $R=0.962$, with a slope of 1.006 and an intercept of +0.16. Finally, in patients with GI Monitor values in the clinical decision range up to 100 kU/L (n=1636), the coefficient of correlation was $R=0.935$, with a slope of 1.023 and an intercept of +0.05 (Figure 4).

Healthy individuals For the Access GI Monitor, the 95th percentile URL of a healthy population (n=267) was found at 23.1 kU/L. The value distribution ranged from <0.8 to 87.0 kU/L. Mean was at 8.3 kU/L, median at 6.0 kU/L. The 25th percentile was calculated at 3.7 kU/L, 97.5th percentile at 28.9 kU/L and 99th percentile at 39.6 kU/L. Females had slightly higher levels (median at 6.8 kU/L, 95th percentile at 25.2 kU/L) than males (median at 5.3 kU/L, 95th percentile at 17.3 kU/L). Both methods showed a very comparable distribution and a good correlation ($R=0.954$, slope 1.124, intercept of -0.41) (Figure 5, Table 1).

Individuals with benign diseases Of 549 individuals diagnosed with benign diseases, patients with benign gastrointestinal diseases showed the highest levels for the Access GI Monitor up to 500 kU/L (median at 13.4 kU/L, 95th percentile at 195.5 kU/L). Two patients with end-stage renal disease had GI Monitor values higher than 200 kU/L. The lowest levels were found in benign breast diseases (median at 5.8 kU/L, 95th percentile at 30.1 kU/L), which were in the range of healthy individuals. For all benign diseases, both methods showed comparable results and a good correlation ($R=0.967$, slope 0.979, intercept of +0.62).

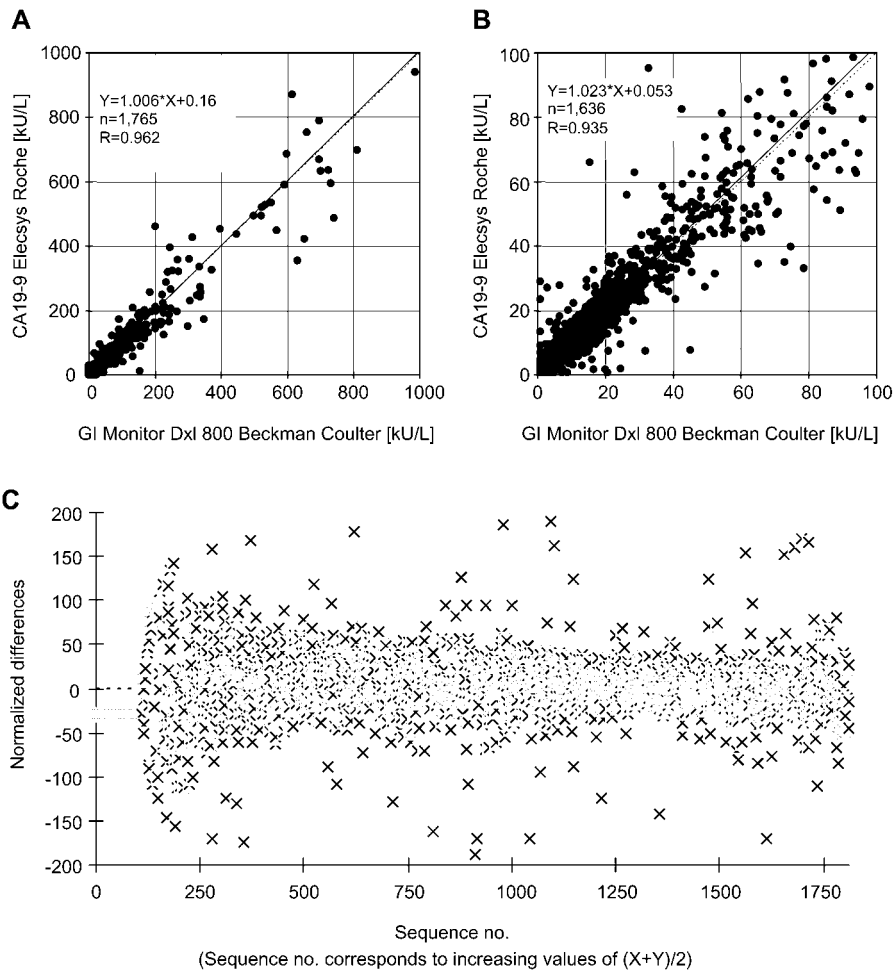


Figure 4 Method comparison of the GI Monitor with the reference method. Correlation of Access GI Monitor (Beckman Coulter) and Elecsys CA19-9 (Roche Diagnostics) concentrations were calculated (A) for the range < 1000 kU/L and (B) for the range < 100 kU/L. (C) Normalized differences from mean values were calculated according to Bland and Altman.

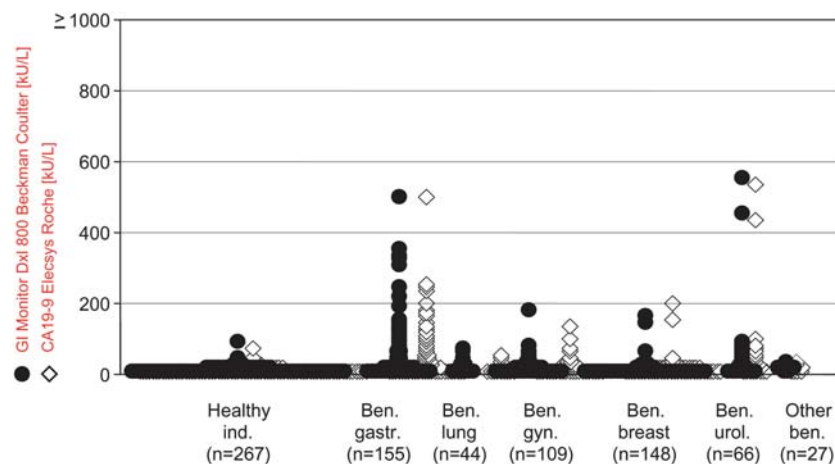


Figure 5 Value distribution of the GI Monitor and reference method in controls. (●) Dot plot of Access GI Monitor (Beckman Coulter) and (◇) Elecsys CA19-9 (Roche Diagnostics) concentrations in serum samples of healthy individuals and individuals with various benign diseases.

Details of value distribution are listed in Table 1 and Figure 5.

Individuals with malignant diseases Of 995 individuals diagnosed with malignant diseases, patients with pancreatic cancer showed the highest levels for the

Access GI Monitor (median 233.8 kU/L, 95th percentile at 13,902 kU/L), with maximum levels of more than 15,000 kU/L. Some extremely elevated values were found in single individuals with colorectal, ovarian or gynecological cancers too, but median and 95th percentiles were considerably lower than in pancreatic

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

Diagnosis	n	Method	Median, kU/L	Range, kU/L	95th percentile, kU/L
Healthy individuals	267	Dxl 800	6.0	0.8–87.0	23.1
		Elecsys	5.9	0.6–69.1	24.0
Benign gastrointestinal diseases	155	Dxl 800	13.4	0.8–497.0	195.5
		Elecsys	13.4	0.6–495.0	173.4
Benign lung diseases	44	Dxl 800	10.5	0.8–66.0	59.7
		Elecsys	7.2	0.6–51.9	46.7
Benign gynecological diseases	109	Dxl 800	7.4	0.8–179.9	51.9
		Elecsys	8.7	0.6–132.0	53.1
Benign breast diseases	148	Dxl 800	5.8	0.8–158.0	30.1
		Elecsys	8.7	0.6–193.0	31.7
Benign urological diseases	66	Dxl 800	13.8	0.8–551.5	84.2
		Elecsys	13.5	0.6–534.0	91.4
Other benign diseases	27	Dxl 800	11.8	0.8–34.8	30.4
		Elecsys	10.7	0.6–30.0	27.6
Gastric cancer	26	Dxl 800	9.3	0.8–811.0	644.2
		Elecsys	13.4	1.3–699.0	572.0
Hepatocellular cancer	58	Dxl 800	55.8	0.8–397.8	209.6
		Elecsys	61.7	0.6–461.0	331.4
Pancreatic cancer	62	Dxl 800	233.8	2.5–15,489	13,902
		Elecsys	205.5	4.5–19,483	12,952
Colorectal cancer	113	Dxl 800	33.5	0.8–39,325	11,014
		Elecsys	35.0	0.6–25,104	9224
Lung cancer	82	Dxl 800	18.9	0.8–855.2	652.3
		Elecsys	17.3	0.6–1074	732.8
Ovarian cancer	81	Dxl 800	15.2	0.8–72,602	1298.3
		Elecsys	16.6	0.6–95,184	1703.3
Gynecological cancer	57	Dxl 800	20.2	0.8–20,245	3174.1
		Elecsys	22.5	0.6–18,200	3009.6
Breast cancer	416	Dxl 800	11.0	0.8–4934	84.3
		Elecsys	11.2	0.6–4422	82.0
Urological cancer	57	Dxl 800	8.4	0.8–6883	102.1
		Elecsys	10.1	0.6–4089	118.1
Prostate cancer	43	Dxl 800	8.4	2.7–130.0	53.7
		Elecsys	9.5	2.5–123.0	50.4

Median, range and 95th percentile limit of the Access GI Monitor (Beckman Coulter) for healthy individuals, patients with benign and malignant diseases. Comparison with Elecsys CA19-9 (Roche Diagnostics) concentrations.

cancer. Except prostate cancer, all cancer types were associated with GI Monitor values up to 400 kU/L and greater. In general, results of both methods were very comparable and a good correlation was found ($R=0.961$, slope 1.004, intercept of +0.17). Details of value distribution 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 GI Monitor (CA19-9 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 CA19-9 assay on the Elecsys 2010 Immunology System. According to the guidelines of the European Group on Tumor Markers [EGTM, (33)], 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. This good diagnostic correlation was

expressed by the similar values for the AUC with overlapping confidence intervals, as well as by the sensitivity for cancer detection at the 95% specificity of benign diseases (Table 2). Among all cancers, pancreatic cancer showed the highest AUC value for both methods when compared with benign gastrointestinal diseases (Access GI Monitor: AUC 0.827, Elecsys CA19-9: AUC 0.850) and the highest sensitivity at 95% specificity of benign gastrointestinal diseases (Access GI Monitor: sensitivity 53.2%, Elecsys CA19-9: sensitivity 58.1%). Most importantly, for GI Monitor values above 500 kU/L, a sensitivity of 40.3% at a specificity of 100% was reached, suggesting a diagnostic value of high GI Monitor levels in the setting of suspicious pancreatic lesions (Figure 7, Table 2). Otherwise, the Access GI Monitor and Elecsys CA19-9, respectively, showed diagnostic power for other cancers, such as hepatocellular cancer, colorectal cancer, lung cancer, ovarian cancer, breast cancer and other gynecological cancers, too (Figure 8, Table 2).

Sensitivity for cancer disease in patients with normal bilirubin values The evaluation of the diagnostic power of the GI Monitor and Elecsys CA19-9, respectively, was performed in a subgroup of patients with

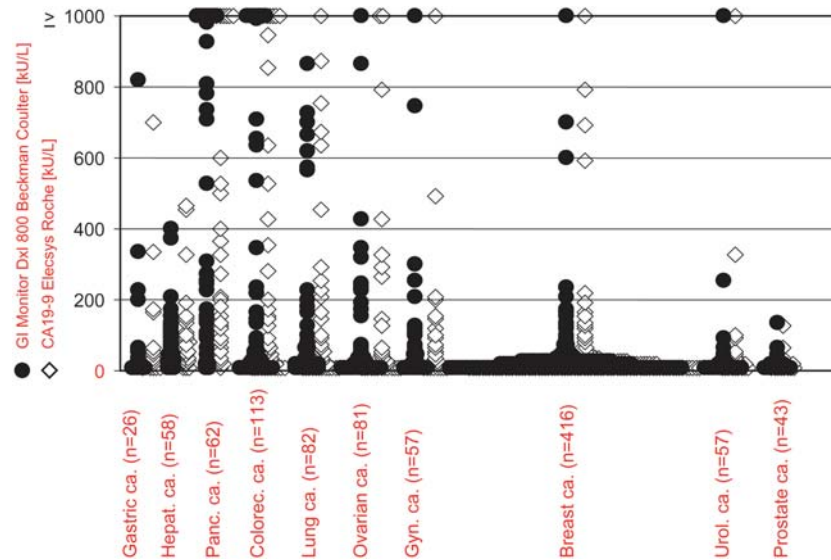


Figure 6 Value distribution of the GI Monitor and reference method in cancer patients.

(●) Dot plot of Access GI Monitor (Beckman Coulter) and (◇) Elecsys CA19-9 (Roche Diagnostics) concentrations in serum samples of individuals with various malignant diseases.

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

Diagnosis	n	Method	Sensitivity at 95% specificity vs. respective benign diseases	AUC	Confidence interval
Pancreatic cancer	62	Dxl 800	53.2	0.827	0.760–0.894
		Elecsys	58.1	0.850	0.789–0.911
Gastric cancer	26	Dxl 800	15.4	0.440	0.310–0.570
		Elecsys	7.7	0.482	0.354–0.610
Hepatocellular cancer	58	Dxl 800	6.9	0.682	0.600–0.763
		Elecsys	6.9	0.718	0.640–0.795
Colorectal cancer	113	Dxl 800	25.7	0.626	0.557–0.696
		Elecsys	25.7	0.633	0.564–0.702
Lung cancer	82	Dxl 800	26.8	0.689	0.596–0.782
		Elecsys	31.0	0.715	0.625–0.805
Ovarian cancer	81	Dxl 800	22.2	0.613	0.530–0.697
		Elecsys	24.7	0.614	0.530–0.698
Gynecological cancer	57	Dxl 800	31.6	0.666	0.570–0.762
		Elecsys	36.8	0.650	0.552–0.748
Breast cancer	416	Dxl 800	16.3	0.674	0.625–0.723
		Elecsys	15.9	0.600	0.552–0.649
Renal cancer	29	Dxl 800	3.6	0.416	0.299–0.533
		Elecsys	0.0	0.429	0.312–0.546
Bladder cancer	28	Dxl 800	6.9	0.436	0.315–0.558
		Elecsys	10.3	0.460	0.336–0.585

Survey on the diagnostic capacity of the Access GI Monitor (Beckman Coulter) for various cancer diseases when compared with their respective benign diseases as control groups and comparison with the Elecsys CA19-9 (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.

normal bilirubin levels ($< 17.103 \mu\text{mol/L}$), because the well-known in vivo influence of cholestasis on serum values of CA19-9 antigen often corresponds to elevated serum bilirubin levels. While median and 95th percentile levels remained constant in most benign and malignant subgroups, lower levels of the GI Monitor were observed in patients with benign gastrointestinal diseases (all patients: $n = 155$, median 13.4 kU/L, 95th percentile 195.5 kU/L; patients with normal bilirubin levels: $n = 89$, median 10.6 kU/L, 95th percentile 123.8 kU/L), in patients with hepatocellular cancer

(all patients: $n = 58$, median 55.8 kU/L; patients with normal bilirubin levels: $n = 25$, median 37.6 kU/L) and colorectal cancer (all patients: $n = 113$, median 35.0 kU/L; patients with normal bilirubin levels: $n = 83$, median 28.7 kU/L), but not in patients with pancreatic cancer (all patients: $n = 62$, median 233.8 kU/L; patients with normal bilirubin levels: $n = 37$, median 244.8 kU/L). This means that the bilirubin levels were relevant for GI Monitor values, and in a very similar way, also for Elecsys CA19-9 values, in patients with benign gastrointestinal diseases, as well as in those

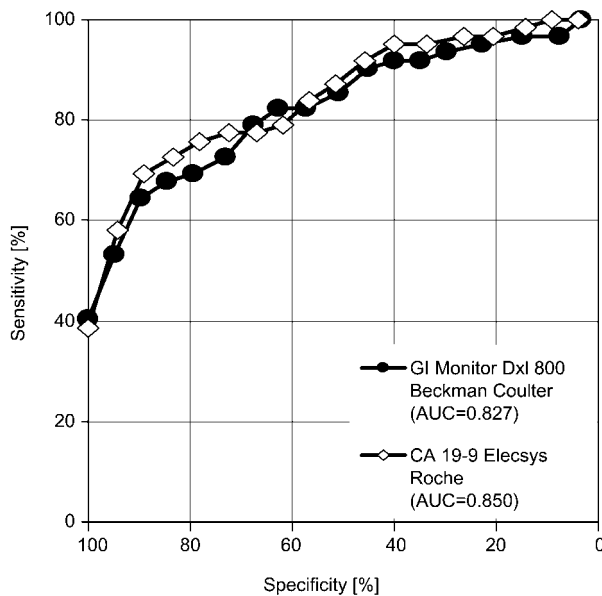


Figure 7 Diagnostic capacity of the GI Monitor and reference method for detection of pancreatic cancer. Profiles of sensitivity and specificity over the whole range of cut-off values are shown by receiver operating characteristic (ROC) curves for pancreatic cancer ($n=62$) vs. benign gastrointestinal diseases ($n=155$). (●) Access GI Monitor (Beckman Coulter) and (◇) Elecsys CA19-9 (Roche Diagnostics).

with hepatocellular and colorectal cancers, but not in patients with pancreatic cancer. Thus, the diagnostic power of the GI Monitor for most cancer diseases remained unchanged, but was considerably improved for pancreatic cancer vs. benign gastrointestinal diseases by considering only sera with normal bilirubin content (Access GI Monitor: AUC 0.910, Elecsys CA19-9: AUC 0.913; Figure 9). Then, the sensitivity at 95% specificity for pancreatic cancer vs. benign gastrointestinal diseases reached 67.6% for the Access GI Monitor and 70.3% for Elecsys CA19-9.

Discussion

Several studies have shown that CA19-9 is the marker of first choice for diagnosis of pancreatic cancer (8, 20–22). If no cholestatic gastrointestinal diseases, such as liver cirrhosis, cholangitis, cholecystolithiasis are present – which can also provoke elevated CA19-9 levels (8, 9, 17–19) – preoperative and pretherapeutic CA19-9 concentrations are helpful in suggesting differential diagnosis of pancreatic cancer. Moreover, prognostic relevance of pretherapeutic CA19-9-levels for overall survival of pancreatic cancer patients undergoing surgery and/or receiving systemic chemo- and/or radiotherapy was found repeatedly by several groups (6, 23–25). Similarly, the usefulness of CA19-9 for therapy monitoring as well as early detection of disease progression, in pancreatic cancer patients is widely recognized and accepted (6, 26–31).

Besides pancreatic cancer, CA19-9 is detected in colorectal, gastric, liver, gallbladder, lung, breast and ovarian cancer tissues, as well as in the serum of

these patients (8–10). Diagnostic and/or prognostic value of CA19-9 was reported, particularly for cholangiocellular cancer (34), colorectal cancer (35, 36) and gastric cancer (37, 38).

In the present study, the new Access GI Monitor assay, which uses the monoclonal antibody C192 for detection of the CA19-9 antigen, was tested on its analytical and clinical performance. The guidelines of the EGTM (33) 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, patients with gastrointestinal and other benign diseases and many patients with various cancer diseases that might be relevant for differential diagnosis by CA19-9. The entire clinical evaluation of the Access GI Monitor was carried out in parallel with the Elecsys CA19-9, 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 GI Monitor assay was very good with a low within-run, total and inter-laboratory imprecision. Additionally, we observed high recoveries during linearity upon dilution testing, and no high-dose hook effect up to 114,920 kU/L. Sample type interference studies demonstrated that serum and lithium-heparinate plasma can be used interchangeably. However, GI Monitor levels in EDTA plasma and citrate plasma were approximately 20%–30% 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 GI Monitor concentrations in vitro. However, as mentioned above, it is well known that cholestatic diseases which are often associated with high bilirubin levels can in vivo cause considerable elevations of CA19-9 antigen in serum (8, 9, 17–19).

Comparison of the Access GI Monitor with Elecsys CA19-9 showed a very good correlation for all patients and for the various subgroups investigated. This observation is all the more valuable as the slope and intercept were only minimal, meaning that the absolute values of both methods were very comparable. Earlier studies which compared various CA19-9 methods reported significant systematic differences among various systems (39). They also found no similar clinical efficacy, though different cut-off values for the various systems were used (39). Nevertheless, it has to be pointed out that in single patients considerable differences in the concentrations were

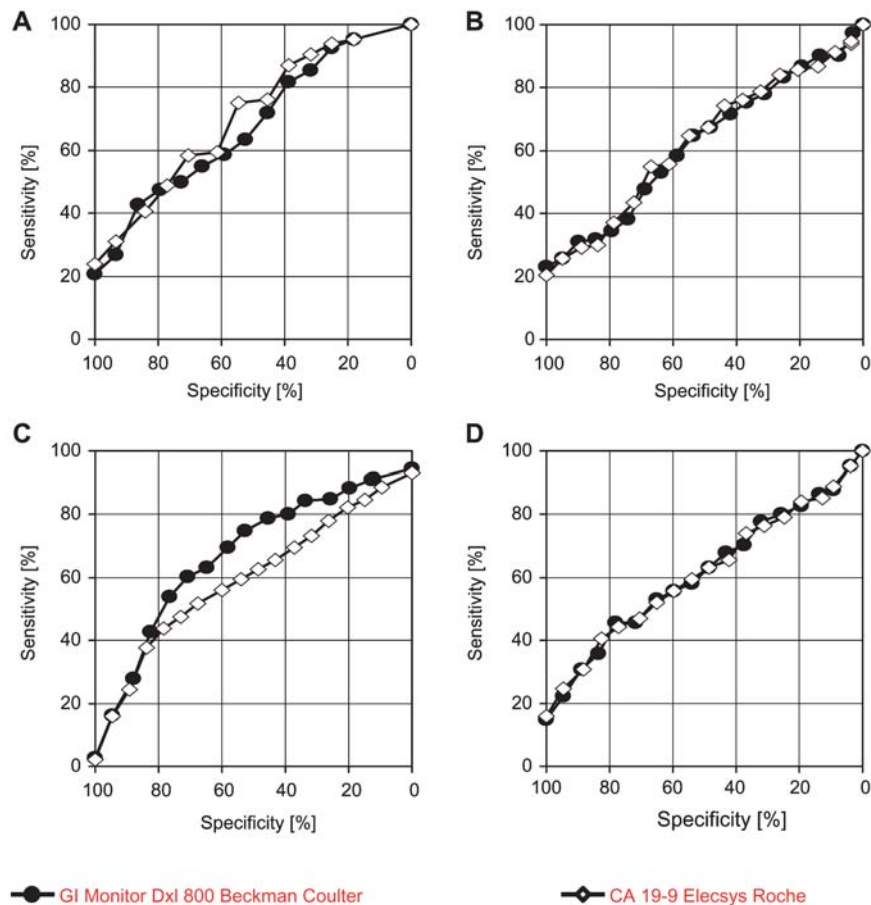


Figure 8 Diagnostic capacity of the GI Monitor and reference method for detection of lung cancer, colorectal cancer, breast cancer and ovarian cancer.

Profiles of sensitivity and specificity over the whole range of cut-off values are shown by receiver operating characteristic (ROC) curves for (A) lung cancer ($n=82$) vs. benign lung diseases ($n=44$), (B) colorectal cancer ($n=113$) vs. benign gastrointestinal diseases ($n=155$), (C) breast cancer ($n=416$) vs. benign breast diseases ($n=148$), and (D) ovarian cancer ($n=81$) vs. benign gynecological diseases ($n=109$). (●) Access GI Monitor (Beckman Coulter) and (◇) Elecsys CA19-9 (Roche Diagnostics).

observed showing the necessity to plan carefully the potential change of the CA19-9 antigen methods and to measure CA19-9 antigen in parallel with both methods if kinetic interpretations are carried out.

In our study, healthy individuals had very low concentrations, as measured with both methods. Cut-offs for the URLs were very comparable and were in the range of the values indicated by both methods (Beckman Coulter 35 kU/L, Roche Diagnostics 27 kU/L).

Concentrations in sera of individuals diagnosed with benign gastrointestinal, lung, breast, gynecological diseases and other disorders were higher than in healthy individuals. However, the differences were only slight in benign lung, breast, gynecological, urological and other benign diseases, even if single individuals (e.g., with end-stage renal disease) reached higher values. As expected, the highest CA19-9 levels were observed in benign gastrointestinal diseases, such as liver cirrhosis, cholangitis, hepatitis and pancreatitis, where single individuals reached values up to 500 kU/L.

CA19-9 concentrations in patients suffering from various cancers were also elevated. However, in some cancer types, the medians and 95th percentiles were comparable with those of benign diseases, e.g.,

for bladder, renal and prostate cancer. In contrast, gastrointestinal, urological and gynecological cancer demonstrated greater CA19-9 elevations, which reached $>20,000$ kU/L in some patients.

The best diagnostic accuracy of the GI Monitor for cancer detection against the relevant benign control group was found for pancreatic cancer (AUC 0.827, sensitivity at 95% specificity vs. benign gastrointestinal controls 53.2%). Most impressively, for GI Monitor values above 500 kU/L, a sensitivity of 40.3% at a specificity of 100% was reached, meaning that, for those levels and in this subgroup of patients, the GI Monitor had diagnostic properties. This excellent differential diagnostic result cannot be achieved by other established tumor-associated antigens and underlines the high relevance of CA19-9 antigen in pancreatic cancer. Importantly, GI Monitor results also correspond very well with the diagnostic accuracy of the CA19-9 reference method for cancer detection, as shown by similar AUC values and overlapping confidence intervals. Though the diagnostic power of the GI Monitor is lower for other tumor types, it has to be pointed out that for colorectal, lung, ovarian and gynecological cancer the AUC was still higher than 0.6 and the sensitivity at 95% specificity vs. the rele-

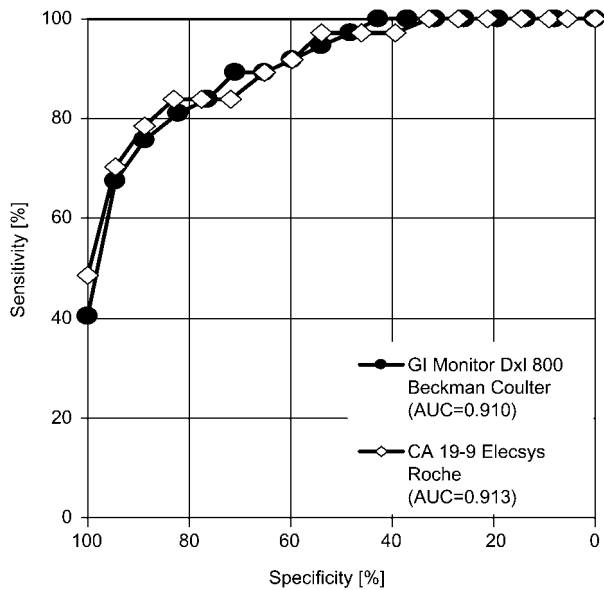


Figure 9 Diagnostic capacity of the GI Monitor and reference method for detection of pancreatic cancer with normal bilirubin levels.

Profiles of sensitivity and specificity over the whole range of cut-off values are shown by receiver operating characteristic (ROC) curves for pancreatic cancer ($n=37$) vs. benign gastrointestinal diseases ($n=89$) with normal bilirubin levels (<10 mg/L). (●) Access GI Monitor (Beckman Coulter) and (◇) Elecsys CA19-9 (Roche Diagnostics).

vant benign control group was higher than 20% – once again, both methods were very comparable even if small differences were observed in some ROC curves, possibly due to slightly different epitope binding sites or different compositions of the assays. This diagnostic performance in cancer types for which CA19-9 was not considered as a relevant marker suggests there may be value in including CA19-9 with other diagnostically relevant markers in future multiparametric analyses. In combination with well-known markers, e.g., CYFRA 21-1, carcinoembryonic antigen, neuron-specific enolase and progastrin-releasing peptide in lung cancer, CA19-9 and other mucin markers might be helpful to further improve the diagnostic accuracy (40).

The diagnostic power of the GI Monitor was also analyzed in a subgroup of patients with normal bilirubin levels below 10 mg/L, to study the in vivo influence of cholestasis on serum values of CA19-9 antigen more precisely. In patients with benign gastrointestinal diseases and hepatocellular and colorectal cancers, the findings of lower GI Monitor levels confirmed an influence of cholestasis on GI Monitor values. In contrast to this, GI Monitor levels remained unchanged in all other benign and malignant diseases, including pancreatic cancer. As a consequence, the diagnostic power of the GI Monitor for pancreatic cancer vs. benign gastrointestinal diseases was further improved. It is noteworthy to mention that bilirubin levels had a similar influence on the Access GI Monitor and Elecsys CA19-9 assays, and the subgroup analysis showed very comparable results for both methods.

Conclusions

The Access GI Monitor is a new assay based on an alternative antibody for CA19-9 antigen detection. The Access GI Monitor provides very good methodological characteristics for use in routine laboratory and demonstrates an excellent analytical and clinical correlation with the Elecsys CA19-9. The GI Monitor shows a high diagnostic accuracy in pancreatic cancer and it is a valuable marker in the management of this disease. Our results also suggest a clinical value of the GI Monitor in gastrointestinal (colorectal and gastric), gynecological (ovarian and endometrial) and lung cancers. If the CA19-9 antigen method is changed, parallel measurements of CA19-9 with both methods for an appropriate time span are strongly recommended.

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