510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k033038

B. Analyte:
CA 19-9

C. Type of Test:
Quantitative, automated chemiluminescence, two-site "sandwich" enzyme immunoassay

D. Applicant:
Beckman Coulter Inc.

E. Proprietary and Established Names:
Access GI Monitor
Access GI Monitor Calibrators on the Access Immunoassay Systems

F. Regulatory Information:

1. Regulation section:
   21 CFR 866.6010 Tumor-associated Antigen Immunological Test System
   21 CFR 862.1150 Calibrator
2. Classification:
   Class II
3. Product Code:
   NIG, System, Test, Carbohydrate antigen (CA 19-9) for monitoring and management of pancreatic cancer;
   JIT, Calibrator, Secondary
4. Panel:
   Immunology (82)

G. Intended Use:
The Access GI Monitor assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of CA 19-9 antigen levels in human serum and plasma using the Access Immunoassay Systems. This device is indicated for use in the measurement of CA 19-9 antigen to aid in the management of pancreatic cancer patients. The test is useful as an aid in monitoring of disease status in those patients having confirmed pancreatic cancer whose serum CA 19-9 antigen levels exceed 10 U/mL, the cut-off value for individuals who are Lewis blood group antigen negative. Serial testing for patient CA 19-9 antigen concentrations should be used in conjunction with other clinical methods used for monitoring pancreatic cancer.

The Access GI Monitor Calibrators are intended to calibrate the Access GI Monitor assay for the quantitative determination of CA 19-9 antigen levels in human serum and plasma using the Access Immunoassay Systems.

1. Indication(s) for use:
   as an aid in monitoring of disease status in those patients having confirmed pancreatic cancer whose serum CA 19-9 antigen levels exceed 10 U/mL, the cut-off value for individuals who are Lewis blood group antigen negative.
2. Special condition for use statement(s):
   Patients must possess the ability to express the Lewis blood group antigen or they will be unable to produce the CA 19-9 antigen even in the presence of proven malignancy. The CA 19-9 antigen is not expressed in persons with genotype Lewis^{a-b-}, which corresponds to about 5% of the population. A patient with a positive genotype for the Lewis antigen may produce varying levels of CA 19-9 antigen. Phenotyping for the presence of the Lewis blood group antigen may be insufficient to detect true Lewis antigen negative individuals.
   For prescription use only.
3. Special instrument Requirements:
   Use with automated, random access analyzers Access Immunoassay Analyzer (K922823), Access 2 (K922823/A007), Synchron LXi 725 Clinical System (K023049) and UniCel DxI 800 Access Immunoassay System (K023764). These systems were 510(k) cleared and with the same intended use and measuring method.

H. Device Description:

The Access GI Monitor reagents consist of reagent packs, calibrators, chemiluminescent substrate (dioxetane-based), wash buffer (a Tris-buffered saline solution containing surfactant and preservatives) and sample diluent (a HEPES-buffered BSA matrix containing surfactant and preservatives). Each reagent pack contains 1) the paramagnetic particles coated with goat polyclonal anti-biotin antibody in a Tris-buffered saline solution with BSA; 2) an alkaline phosphatase conjugate mouse monoclonal anti-CA 19-9 in an MES-buffered solution with BSA; 3) a biotin conjugate mouse monoclonal anti-CA 19-9 in a Tris-buffered saline solution with BSA and 4) a Tris-buffered protein solution (bovine, goat and mouse). All components in the reagent pack have <0.1% sodium azide and 0.1% ProClin 300.

The Access GI Monitor Calibrators consist of 6 calibrators with CA 19-9 concentrations of 0, 30, 90, 300, 900 and 2000 U/mL in HEPES-buffered solution, with BSA, <0.1% sodium azide and 0.5% ProClin 300.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Fujirebio Diagnostics CA 19-9™ RIA
2. Predicate K number(s):
   K020566

3. Comparison with predicate:

DEVICE	PREDICATE
A. Similarities	
Intended Use. - Quantitative analysis of CA 19-9 in human serum and plasma	Same
Antibody Type and Source – Monoclonal, mouse	Same
B. Differences	
Sample Type – Serum and plasma (lithium heparin)	Serum and plasma (EDTA, ACD and heparin)
Assay Method – Automated chemiluminescence, two-site "sandwich" enzyme immunoassay	Manual RIA, two-site "sandwich" radioimmunoassay
Solid Phase - antibody-coated paramagnetic beads	antibody-coated polystyrene beads
Detection Method - Alkaline phosphatase conjugated monoclonal antibody and a dioxetane-based chemiluminescent substrate	^{125} I conjugated monoclonal antibody
Instruments - Access Immunoassay Analyzers	Manual or semi-automated
Calibrator – Liquid, buffered bovine serum albumin matrix, 6 levels	Liquid, defibrinated normal human plasma, 6 levels
Control - Not provided	2 levels defibrinated human plasma
Antibody Specificity - Sialyl Lewis a (clone C192:22:5)	Sialyl Lewis a (1116-NS19-9)
Analytical Range – 0.8 – 2000 U/mL	0.9-240 units/mL
Assay Sensitivity - 0.8 U/mL	0.9 units/mL
Precision	
intra-assay %CV - 1.7% to 6.4%	6.5%-12.5%
inter-assay %CV - 2.4% to 5.7%	3.0% to 8.6%
total %CV -3.0% to 8.9%	6.7%-15.4%
High Dose Hook Effect – Up to 800,000 U/mL	Up to 1,250,000 U/mL

J. Standard/Guidance Document Referenced (if applicable):

None referenced.

K. Test Principle:

The Access GI Monitor assay is a two-site immunoenzymatic assay. A sample is added to a reaction vessel along with paramagnetic particles coated with polyclonal goat anti-biotin antibody (the solid phase), biotin-conjugated mouse anti-CA 19-9 monoclonal antibody (b-Mab) and a buffered protein solution. If the CA 19-9 is present in the sample, it will bind to the b-Mab which will be captured by the anti-biotin antibodies on the paramagnetic particles. After incubation, excess unbound antigen is separated from the solid phase by a magnet field and removed by washing. An alkaline phosphatase conjugated mouse anti-CA 19-9 monoclonal antibody (ap-Mab) is added and binds to the CA 19-9 captured on the solid phase. After incubation, unbound materials are removed by washing. Then the chemiluminescent substrate Lumi-Phos® 530 is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is directly

proportional to the concentration of CA 19-9 in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The within-run, between-run and total precision studies were performed using 4 in-house controls with CA 19-9 concentrations of 17.4, 110.5, 584.6 and 1664.5 U/mL. The samples were assayed in duplicates for 20 days. The following table summarized the with-in run, between-run and total precision.

Sample	Mean (U/mL)	Within-run SD	Within-run %CV	Between-run SD	Between-run %CV	Total %CV
Level 1	17.4	1.1	6.4	1.0	5.7	8.9
Level 2	110.5	2.4	2.2	3.0	2.7	3.5
Level 3	584.6	10.2	1.7	14.8	2.5	3.1
Level 4	1664.5	29.7	1.8	40.7	2.4	3.0

The %CV for within-run imprecision ranged from 1.7% to 6.4%, between-run imprecision from 2.4% to 5.7% and the total imprecision from 3.0% to 8.9%. These results are within acceptable limits.

The sponsor provided additional data on another reagent lot as requested and results showed acceptable lot-to-lot precision.

b. Linearity/assay reportable range:

Linearity was evaluated by assaying 6 serum samples containing high concentrations of CA 19-9 (602.4 to 1382.1 U/mL). Each sample was serially diluted with sample diluent to1:2, 1:4, 1:8, 1:16, 1:32 and 1:64. All concentrations were analyzed in quadruplicates. The observed results were compared to the expected results and the percent recovery was calculated. The percent recoveries ranged from 88.6% to 108.3%. The individual mean sample recoveries varied between 92.6% and 99.5%.

The assay measuring range is from 0.8 U/mL to 2000 U/mL

c. Traceability (controls, calibrators, or method):

No reference standards or method available. The Fujirebio CA 19-9 RIA assay was used to assign values to the primary reference calibrators.

d. Detection limit (functional sensitivity):

The minimal detectable concentration (MDC) is determined by assaying 10 replicates of the zero calibrator in multiple assays and is defined as the concentration of CA 19-9 that corresponds to the mean RLU's +2SD of the zero calibrator. Data showed the MDC was 0.8 U/mL.

e. Analytical specificity:
Interference was determined by spiking different concentrations of each interfering substance into aliquots of a normal sera containing low concentrations of CA 19-9. Interfering substances tested include hemoglobin, bilirubin, triglyceride, albumin, drugs and therapeutic agents. To assess interference due to rheumatoid factor and HAMA, positive patient samples were used. No significant interference was observed.

f. Assay cut-off:
Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:
Four hundred and five serum samples were tested on the Access GI Monitor assay and the Fujirebio CA 19-9 Assay. These samples were collected from male and female subjects who were either normal, with benign or malignant diseases. The CA 19-9 concentrations of the samples covered the assay range of the Fujirebio assay (0-240 U/mL). The assays were performed in duplicate. The results were analyzed by Deming regression and gave a slope of 0.9569 (95% CI; 0.91 to 1.00) and the y-intercept of 2.5726 (95% CI; -0.09 to 5.23) and a coefficient correlation (r) of 0.9007. Bland-Altman difference plot as calculated by the reviewer showed a mean difference of -6.096 U/mL (95% CI -12.2 to 0.0008). The bias between the two devices most likely reflects differences in technology and antibodies used in the assay.

b. Matrix comparison:
Eighty matched serum and lithium heparin plasma samples from healthy adult subjects were analyzed using the Access GI Monitor assay. The sample CA 19-9 concentrations ranged from 0 to 1650.9 U/mL. Results were analyzed by Deming regression which showed (Plasma) = 0.9842 (Serum) - 0.5002, the correlation coefficient (r) was 0.9995.

3. Clinical studies:

a. Clinical sensitivity:
Not applicable.

b. Clinical specificity:
Not applicable

c. Other clinical information
Serial Monitoring Analysis – Serum samples from 63 patients with confirmed pancreatic cancer were prospectively collected at two US

clinical sites. The 63 subjects consisted of 34 males and 29 females with an average age of 63.3 y (±7.5 y) and 64.6y (±15.5 y) respectively. The age range for the male subjects was from 48 y to 78 y and for the female subjects, 23 y to 83 y. The follow-up period ranged from 21 days to 66 months. Seventy-nine percent of the cohort were Caucasians, 11% Hispanics, 3% African Americans and 7% other ethnic groups. Ten patients were excluded from the analysis because they had CA 19-9 values ≤ 10 U/mL, a cut-off value for Lewis blood group non-secretors as defined by the sponsor for the Access GU Monitor assay. For the remaining 53 patients, there were 222 samples with an average number of 6.32 ± 9.0 observations per patient (ranged from 1 to 13 observation pairs). The breakdown of the patient series is summarized below.

# Samples in Series	#Observation Pairs	Frequency	%
2	1	15	28.3
3	2	11	20.7
4	3	10	18.9
5	4	8	15.0
6	5	2	3.8
7	6	1	1.9
8	7	2	3.8
9	8	1	1.9
10	9	1	1.9
11	10	1	1.9
14	13	1	1.9

At the time of diagnosis, 11.3% of the patients had Stage I disease, 30.2% Stage II, 17% Stage III and 39.6% stage IV. One patient did not have staging information at diagnosis. The average (± SD) length of time in the study was 294.7 (± 421.8) days respectively, ranging from 17 days to 2013 days. The median was 142.5 days.

Changes in CA 19-9 concentrations and changes in disease state were analyzed on a per-visit and a per-patient basis. A significant change in CA 19-9 is defined as greater than 2.5 times %CV of the assay total imprecision as established by running 5 controls (10 U/mL to 40 U/mL) for multiple days, lots, sites and instruments. A polynomial regression analysis was used to model the within, between and total imprecision. The regression model used the log (SD) as the dependent and log (mean CA 19-9 concentration for each sample) as the independent variable. Based on the study results, a 20% change was selected to cover the imprecision across the levels of CA 19-9 used. The predicate device also uses a 20% change.

Results of the 168 evaluable observation pairs are summarized below [(predicate results are in ()]. The pairs were classified as "Progression", "Stable/NED" and "Responding". The distribution of results between the Access GI Monitor assay and the predicate were

not statistically different. For the "progression" group, the Chi-Square p-value was 0.3108, for the "Stable/NED" group was 0.1828 and for the "Responding" group was 1.0000.

Change in CA 19-9	Change in Disease State			Total
	Responding	Stable/NED	Progressing	
>20%	6 (6) 27.3%	33 (23) 42.9% (29.9%)	41 (38) 59.4% (55.1%)	80 (67) 47.6% (39.9%)
=20%	3 (3) 13.6% (13.6%)	26 (36) 33.8% (46.8%)	11 (18 15.9% (26.1%))	40 (57) 23.8% (33.9%)
<20%	13 (13) 59.1% (59.1%)	18 (18) 23.4% (23.4%)	17 (13) 24.6% (18.8%)	48 (44) 28.6% (26.2%)
Total	22 (22)	77 (77)	69 (69)	168 (168)

The following table shows results grouped into "Progression" and "No Progression". "No Progression" included results from stable, responding or no evidence of disease.

Change in CA 19-9	Change in Disease State		Total
	Progression	No Progression	
Significant Change >20%	41 (38)	37 (29)	80 (67)
No Change ≤20%	28 (31)	62 (70)	88 (101)
Total	69	99	168

For the Access GI Monitor assay, total concordance was 61.3% (95%CI 58.8% to 68.3%), positive concordance 59.4% (95%CI 47.7% to 70.2%) and negative concordance 62.6% (95%CI 52.8% to 71.5%). For the predicate device, total concordance was 64.3% (95%CI 56.8% to 71.7%), positive concordance 55.1% (95%CI 43.4% to 66.2%) and negative concordance 70.7% (95%CI 61.1% to 78.8%).

The per-patient basis results are summarized below [predicate device values are in ()]. On a per-patient basis, the Access GI Monitor assay had a total concordance of 66.0% (95% CI 52.6% to 77.3%), a positive concordance 78.1% (95%CI 61.2% to 89.0%) and a negative concordance 47.6% (95%CI 28.3% to 67.6%) as compared to a total concordance of 69.8% (95% CI 56.5% to 80.5%), a positive concordance 78.1% (95%CI 61.2% to 89.0%) and a negative concordance 57.1% (95%CI 36.6 to 75.5) for the predicate device.

Change in CA 19-9	Change in Disease State		Total
	Progression	No Progression	
Significant Change (>20%)	25 (25)	11 (9)	36 (34)
No Change (≤20%)	7 (7)	10 (12)	17 (19)
Total	32 (32)	21 (21)	53 (53)

Comparison of CA 19-9 results between the Access GI Monitor assay and the predicate device showed a positive agreement of 94%, a negative agreement 83.2% and a total agreement was 87.5%.

		Fujirebio CA 19-9		
		≥20% change	<20% change	Total
Access GI Monitor	>20% change	63	17	80
	≤20% change	4	84	88
	Total	67	101	168

4. Clinical cut-off:
   Not applicable.
5. Expected values/Reference range:
   The normal reference range was established by testing serum samples from 291 subjects (150 females and 141 males). The mean and median age of the cohort was 55.4 y (ranged from 20 y to 79 y) and 60.9 y respectively. The female subjects had a mean age of 60.0 y (20 y to 79 y) and median age of 63.9 y whereas the mean age of the male subjects was 50.5 y (20 y to 78 y) and median age 50.7 y. The mean ages between the genders were statistically different but not clinically significant. The table below shows the CA 19-9 results analyzed by gender. The 95th percentile (35 U/mL) of the CA 19-9 results was used as the upper reference limit for the Access assay.

Subject	#Subjects	CA 19-9 Concentration (U/mL)			
		Mean ± SD	Median	95th Percentile	Range
Males	141	11.0 ± 12.2	7.3	34.9	0.0-85.1
Female	150	12.0 ± 12.1	8.4	36.0	0.1-71.1
Combined	291	11.5 ± 12.1	7.7	35.4	0.0-85.1

The following table shows the CA 19-9 assay result distribution with 94.5% of the subjects had <35 U/mL CA 19-9.

Subject	Number of Subjects	CA 19-9 Concentrations (U/mL)			
		0-35	35.1-70	70.1-100	> 100
Male	141	134	6	1	0
Female	150	141	8	1	0
Combined	291	275	14	2	0

Using each assay's upper reference limit, the Access GI Monitor assay results were compared to that of the Fujirebio CA 19-9 assay. The % positive agreement was 71.4% (15/21), % negative agreement 99.6% (269/270) and % total agreement 97.6% (284/291). The discordance between the two assays was not statistically different from zero (p = 0.125).

		Fujirebio CA 19-9		
		≥37 U/mL	<37 U/mL	Total
Access GI Monitor	≥35 U/mL	15	1	16
	<35 U/mL	6	269	275
	Total	21	270	291

In addition to the normal cohort, 522 serum samples from patients with benign conditions and 469 from patients with malignant diseases were tested. The CA 19-9 concentrations for the cohorts are summarized below.

Cohorts	# Subjects	CA 19-9 concentration U/mL		
		Mean ± SD	Median	Range
Normal	291	11.5 ± 12.1	7.7	35.4
Pancreatic	100	18.4 ± 16.5	15.1	0.0-131.5
Chronic heart disease/ Hypertension	85	11.9 ± 9.9	9.0	0.1-42.1
Gastrointestinal	147	14.0 ± 12.5	9.5	0.0-68.6
Genitourinary	190	14.2 ± 13.0	11.0	0.0-77.6
Biliary/Gallbladder	25	530.5 ± 1065.0	26.7	0-3836
Breast	37	40.6 ± 165.3	11.8	0-1016
Gastrointestinal	142	897.5 ± 8474.3	13.8	0-100780
Genitourinary	111	103.5 ± 876.4	12.6	0-9250
Liver	84	86.4 ± 351.7	10.1	0-2271
Lung	70	1391.0 ± 10913.7	17.3	0-91320
Pancreatic Cancer	40	7164.9 ± 307.1	43330	0.0-139130

The table below summarizes the distribution of subjects according to CA 19-9 concentrations. Results showed all cancer groups had higher mean CA 19-9 values than the benign disease cohorts. Patients with pancreatic cancer had mean values five times greater than the other cancer groups.

Cohorts	# Subjects	CA 19-9 Concentrations (U/mL)			
		0-35	35.1-70	70.1-100	> 100
Normal	291	275	14	2	0
Benign Conditions					
Pancreatic	100	90	92	0	1
Chronic heart disease/ Hypertension	85	81	4	0	0
Gastrointestinal	147	140	7	0	0
Genitourinary	190	174	15	1	0
Non-pancreatic Malignant Conditions					
Biliary/Gallbladder	25	13	0	0	12
Breast	37	35	0	1	1
Gastrointestinal	142	102	21	0	19
Genitourinary	111	95	9	4	3
Liver	84	67	9	0	8
Lung	70	52	11	1	6
Pancreatic Cancer	40	10	2	5	23

Serum samples from 40 patients (25 males and 15 females) with pancreatic cancer (stages I to IV) were also analyzed. Twelve patients had treatment prior to their specimen draw date. The mean and median age of the combined cohort was 63.4 and 64.6 yrs respectively (ranged from 38 to 85 yrs). The CA 19-9 concentrations by the Access GI Monitor assay and the predicate device are summarized below. Non-parametric Wilcoxon analysis demonstrated the median values between the two assays were not statistically different (p-value of 0.4914).

Assay	Number of Subjects	CA 19-9 Concentration (U/mL)					
		Mean	Median	5^{th} Percentile	95^{th} Percentile	95% CI (Median)	Range
Fujirebio	40	12757.8	406.4	4.7	98280	79.2 to 1730.6	2.2-174340
Access	40	7164.9	307.1	0.01	43330	76.5 to 1406.8	0.0-139130

The distribution of subjects according to CA 19-9 levels are summarized below.

Assay	Number of Subjects	CA 19-9 Concentrations (U/mL)			
		0-35	35.1-70	70.1-100	> 100
Fujirebio	40	11	2	1	26
Access	40	10	2	5	23

M. Conclusion:

Based on the review of information provided in this 510 (k), the Access GI Monitor appears to be Substantially Equivalent to the marketed device Fujirebio CA 19-9 RIA regulated under 21 CFR 866.6010, product code NIG, Immunology Device Panel 82, Class II. The calibrator is reviewed following regulation 21 CFR 862.1150 and product code, JIT, Class II.