510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k080194

B. Purpose for Submission:
New device

C. Measurand:
Carcinoembryonic antigen (CEA)

D. Type of Test:
Quantitative, Enzyme Linked Fluorescent assay (ELFA)

E. Applicant:
bioMerieux, Inc.

F. Proprietary and Established Names:
VIDAS® CEA (S)

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.6010 Tumor-associated antigen immunological test system
2. Classification:
   Class II
3. Product code:
   DHX, System, Test, Carcinoembryonic Antigen
4. Panel:
   Immunology (82)

H. Intended Use:
1. Intended use(s):
   VIDAS® CEA (S) is an automated quantitative test for use on the VIDAS instruments, for the quantitative measurement of Carcinoembryonic antigen (CEA) in human serum using the ELFA technique (Enzyme linked Fluorescent Assay). The VIDAS CEA (S) assay is indicated as an aid in the monitoring of cancer patients in whom changing concentrations of CEA are observed.
2. Indication(s) for use:
   Same as Intended Use
3. Special conditions for use statement(s):
   Prescription use only
4. Special instrument requirements:
   VIDAS Instrument systems

I. Device Description:
The device is an *in vitro* diagnostic device using an automated fluorescent immunoassay test principle for the quantitative measurement of CEA on VIDAS instruments using human serum specimens. Each VIDAS CEA lot contains 60 tests. The kit is comprised of 60 CEAS strips, 60 CEAS SPRs (Solid Phase Receptacle), CEAS control, CEAS calibrator, CEAS diluent, one MLE (Master Lot Entry) card and one package insert.
- The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device. It is coated with anti-CEA monoclonal immunoglobulins (mouse).

- CEAS trip consists of 10 wells.
  Description of the CEAS strip:

Well number	Reagents
1	Specimen sample
2-4	Empty
5	Goat anti-CEA labeled with alkaline phosphatase in TRIS buffer, mouse IgG, goat IgG, calf serum, inactivated alkaline phosphatase, and sodium azide (1 g/L)
6-7	Washing solution 1
8	Diluent: TRIS buffer, mouse IgG, goat IgG, calf serum, inactivated alkaline phosphatase, and sodium azide (1 g/L)
9	Washing solution 2
10	Optical well with substrate: 4-methyl-umbelliferyl phosphate, diethanolamine, and sodium azide (1 g/L)

- CEA control 1 (2 mL, lyophilized) containing recombinant human CEA in bovine serum albumin protein and a buffer
- CEA calibrator 1 (2 mL liquid, ready-to-use) containing recombinant CEA in bovine serum albumin protein and a buffer
- Diluent (50 mL, liquid) reagent containing bovine serum albumin protein and sodium azide (buffer not specified, if a buffer is used).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Tosoh ST AIA-Pack CEA
2. Predicate 510(k) number(s):
   k023893
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	VIDAS CEA (S) Assay	Tosoh ST AIA-Pack CEA
Technology	based on enzyme immunoassay technology using 2 antibodies in a sandwich immunoassay format	Same
Indications for use	aid in the monitoring of cancer patients in whom changing concentrations of CEA are observed	Same
Detection	Fluorescence	Same

Differences		
Item	Device	Predicate
	VIDAS CEA (S) Assay	Tosoh ST AIA-Pack CEA
Calibrator Standardization	Traceable to Carcinoembryonic Antigen 1st International Reference Preparation provided by the National Institute of Biological Standards and Controls (code 73/601)	Traceable to WHO 1st International Reference Preparation 73/601

Differences		
Item	Device	Predicate
Measurement range	0.5 to 200 ng/mL	0.5 to 100 ng/mL
Limit of detection	< 0.5 ng/mL	0.5 ng/mL
Intra-assay precision	Mean 3.4 ng/mL - %CV 2.7% to 4.4% Mean 25 ng/mL - %CV 3.5% to 4.4% Mean 160 ng/mL - %CV 3.7% to 5.3%	Mean 4.6 ng/mL - %CV 4.3% Mean 19.7 ng/mL - %CV 3.6% Mean 79.1 ng/mL - %CV 3.1%
Inter-run precision	Mean 3.4 ng/mL - %CV 0 to 1.3% Mean 25 ng/mL - %CV 0 to 1.6% Mean 160 ng/mL - %CV 0 to 1.0%	Mean 5.3 ng/mL - %CV 3.9% Mean 22.8 ng/mL - %CV 3.3% Mean 91.5 ng/mL - %CV 3.2%
Instrument platforms	VIDAS and miniVIDAS	AIA Instrument Systems
Solid phase	Antibody coated solid phase receptacle	Antibody coated magnetic beads
Antibody	Mouse monoclonal anti-CEA and goat polyclonal anti-CEA antibodies	Two mouse monoclonal anti-CEA antibodies
Sample volume	200 uL	100 uL

K. Standard/Guidance Document Referenced (if applicable):
CLSI EP5 Evaluation of Precision Performance of quantitative measurement methods
CLSI EP17 Protocols for the determination of limits of detection and limits of quantitation
CLSI EP7 Interference testing in clinical chemistry
CLSI EP6 Evaluation of linearity of quantitative measurement procedures: A statistical approach
CLSI C28 How to define and determine reference intervals in the clinical laboratory

L. Test Principle:
The assay principle is a two-step immunoassay sandwich method measuring a fluorescent signal. A solid-phase receptacle (SPR) serves as solid support to which anti-CEA monoclonal antibody (derived from mouse) is coated. Serum specimen, calibrator, or control samples are incubated in the SPR to allow capture of bound material to the solid phase. Unbound components are washed away during a washing step. Alkaline-phosphatase labeled anti-CEA polyclonal antibody (goat derived) is added and incubation begun. During incubation labeled anti-CEA binds to solid-phase captured CEA. Unbound material is washed away during a second wash step. The substrate (4-methyl-umbelliferyl phosphate is added to the SPR and cycled in and out. During this incubation the enzyme catalyzes a reaction in which a fluorescent product is produced (4-methyl-umbelliferone) and measured at 450 nm by the VIDAS analyzer. The intensity of fluorescence is proportional to the concentration of CEA present in the sample. Fluorescence intensity is converted to a concentration by comparison with a signal generated by known concentrations of CEA in calibrators. The final concentration is printed by the analyzer.

M. Performance Characteristics (if/when applicable):
1. <u>Analytical performance:</u>
   a. *Precision/Reproducibility:*
   Three serum samples were assayed in duplicate in 40 runs, 2 runs per day for 20 days with 2 reagent lots at 3 sites. Kit controls and 2 levels of a commercially available tumor marker control (Bio-Rad) were run in each run as quality control checks. The mean CEA concentration, standard deviation, and coefficient of variation (%CV) were calculated for each stated source of variation. The %CV for day-to-day variation ranged from 1.2% to 4.5% across sites, lots, and CEA concentrations. The %CV for run-to-run variation ranged from 0% to 1.6% across sites, lots, and CEA concentrations. The %CV for intra-assay variation ranged from 2.7% to 5.3% across sites, lots, and CEA concentrations. The total variability across all sites and samples ranged from 3.9% to 6.5% across sites, lots, and CEA concentrations. The sponsor concludes that the %CV of total variation across sites and samples was ≤ 5.6%.

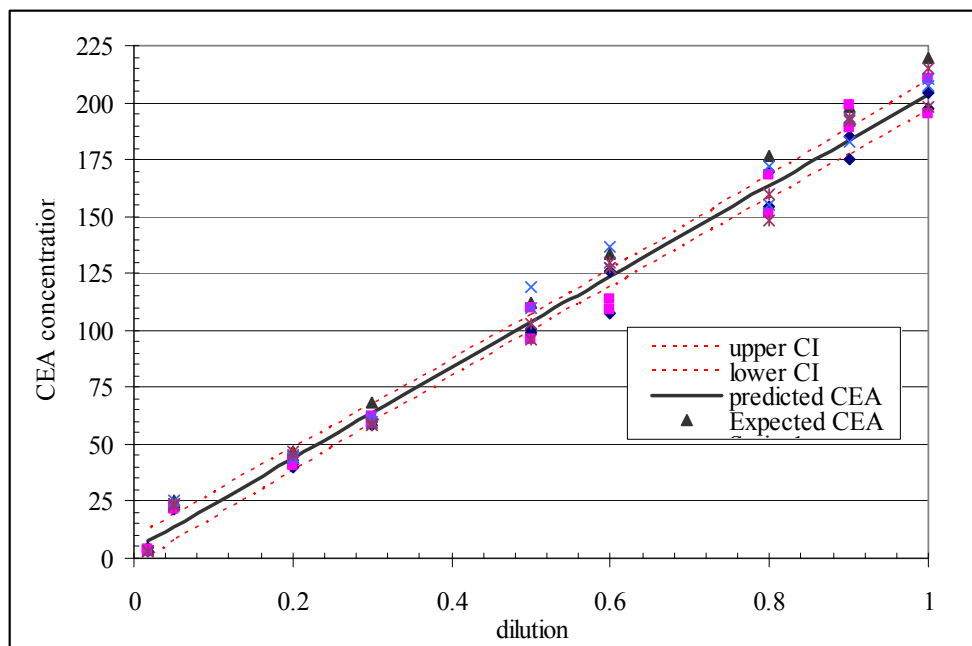| Inter-run imprecision	Mean 3.4 ng/mL	%CV 2.7% to 4.4%
	Mean 25 ng/mL	%CV 3.5% to 4.4%
	Mean 160 ng/mL	%CV 3.7% to 5.3%
Intra-run imprecision	Mean 3.4 ng/mL	%CV 0 to 1.3%
	Mean 25 ng/mL	%CV 0 to 1.6%
	Mean 160 ng/mL	%CV 0 to 1.0%

   b. *Linearity/assay reportable range:*
   A high concentration spiked serum pool and a low concentration natural serum pool were mixed in varying amounts to evaluate the linearity of the assay in its full measurement range. The analysis and study protocol utilized CLSI evaluation protocol EP6-A. Nine samples were generated by mixing a pool supplemented with a high CEA concentration and a pool with low CEA concentration. Each dilution was tested in 4 assay runs with 2 lots of assay kit. The mean CEA concentration in ng/mL and %CV of replicates across runs were calculated. The sample %CV was compared with the expected %CV from the precision analysis. If the observed %CV was less than the upper 95% confidence limit of the expected %CV, the results were included for polynomial regression analysis. For acceptance criteria, the criteria resemble the CLSI acceptance criteria. In these acceptance criteria, parameter estimates of the second and third order polynomial regression are not significant and the linearity is indicated for the first order parameter estimates. If these conditions are present then the assay is deemed linear.
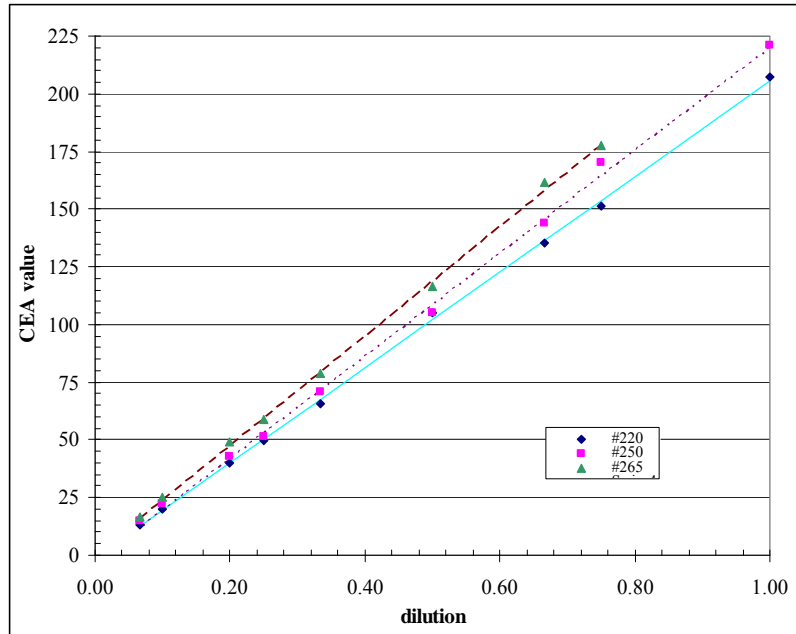
   In the sponsor's description, there was deviation from linearity (parameter coefficients were different from 0) for both lots studied but the deviation was acceptable since the difference in linear and polynomial models is the same magnitude as assay variability. The sponsor shows 2 graphs. The sponsor asserts that the assay is linear from 0.500 to 200.00 ng/mL.

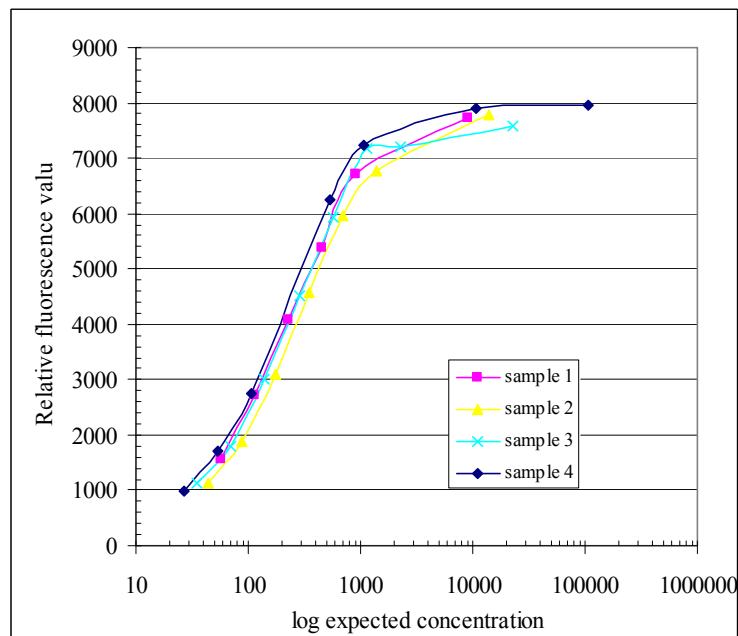   A graphical representation is as follows:

Three samples with very high CEA values were diluted with kit diluent to yield CEA concentrations across the assay measurement range. Each sample was diluted 8 times. Dilutions were tested in triplicate with 2 kit lots and 1 diluent lot. The mean CEA concentration at each dilution and the %CV of replicates at each dilution were calculated. Using CLSI protocol EP6-A, the coefficient estimates for the first, second, and third order polynomials were calculated to determine if the models for the second and third order polynomials had statistically significant coefficients. If the coefficients are not significant, the first order linear model is deemed linear on dilution.

The linearity demonstrates linearity on dilution with the assay diluent in the assay measurement range. The graphs of the three samples are as follows:

Hook effect

Four specimens (3 with very high CEA values and one specimen spiked with a very high CEA concentration) were assessed after dilution over a large measurement range. At least one dilution was within the CEA assay range. Dilution was made using CEA negative serum. A hook effect would be apparent in a graphic representation of relative fluorescence units with increasing CEA concentration when the fluorescence signals decreases after reaching a maximum CEA concentration. Tested samples were graphically plotted and a recreation of the graph based on sponsor supplied data is as follows:

The RFV (relative fluorescence value) signals do not decrease at high CEA concentrations and is indicative of the lack of a hook effect. The graph does indicate a flattening of fluorescence signals above 1000 ng/mL but no decrease in signal up to approximately 100,000 ng/mL. While no formal analysis is performed the graph is supportive of the lack of a hook effect.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Assay calibrators are traceable to reference standards established at the sponsor and to Carcinoembryonic Antigen 1st International Reference Preparation provided by the National Institute of Biological Standards and Controls (code 73/601).

d. *Detection limit:*
Limits of blank, detection, and quantitation were determined using 2 kit lots on 2 instruments (one per lot) using CLSI protocol EP17-A. Seven low CEA samples were tested using two lots on two VIDAS instruments. Tested CEA values ranged from approximately 0.05 to 0.3 ng/mL. Samples ranging from 0.2 to 0.3 ng/mL were used to estimate the limit of detection while 3 samples of lower CEA value (0.05 to 0.08 ng/mL) were used to estimate the limit of quantitation. Acceptance criteria for the limit of blank, detection, and quantitation were described as follows:

- Limit of blank – highest measurement result which has a 95% probability to be observed for a blank sample. It is the 95th percentile of a blank distribution
- Limit of detection – lowest amount of analyte that can be detected with 95% probability, though not quantified at an exact value.
- Limit of quantitation – lowest actual amount of analyte that can be reliably detected and at which total error meets lab requirements for accuracy. Total error specification $\pm$ 100% for doses < 0.5 ng/mL.

The results are noted to support a claim for a limit of detection and limit of quantitation of < 0.5 ng/mL. Therefore, the lowest value for the range of the assay is 0.5 ng/mL.

e. *Analytical specificity:*
Hemoglobin, triglyceride, bilirubin, human albumin, rheumatoid factor, HAMA, and 27 drug interferents were evaluated for interference when added to a human serum pool containing measured amounts of CEA.

Three samples, one CEA negative and two positive CEA (spiked into a negative sample) were prepared and split into 2 aliquots. One aliquot was spiked with hemoglobin, triglyceride, or bilirubin and the other with a corresponding volume of buffer. Five intermediate CEA concentrations, derived from the highest and lowest CEA concentrations, were prepared by mixing varying amounts of the two aliquots. Hemoglobin concentrations ranged from 0 to 305 umol/L. Triglyceride concentrations ranged from 0 to 30 g/L. Bilirubin concentrations ranged from 0 to 510 umol/L. Aliquots of each concentration were tested in single replicates in 2 runs. The linear regression coefficients were calculated for the mean CEA concentration at each interfering substance concentration. The hypothesis that the slope of the best fit line was zero was tested. If the slope is not equivalent with 0 with a probability < 0.05

then there is interference in the assay from the tested substance and testing must be repeated with lower concentrations of interfering substance. As acceptance criteria, there must be no effect from hemoglobin for concentrations less than 300 umol/L, no effect from triglycerides for concentrations less than 30 g/L, and no effect from bilirubin at concentrations less than 510 umol/L.

For hemoglobin concentrations ranging from 0 to 305 umol/L, three different CEA concentrations were within the CEA specification range in the presence and absence of hemoglobin. The 95% confidence intervals (CI) of the slope of the best fit line of CEA concentration and hemoglobin concentration all included zero in the confidence interval (-0.0001 to 0.0023 for CEA value 3 ng/mL; -0.0097 to 0.0124 for CEA value 44.8 ng/mL; -0.0626 to 0.0756 for CEA concentration 135.4 ng/mL).

For triglyceride concentrations ranging from 0 to 30 g/L, three different CEA concentrations were within the CEA specification range in the presence and absence of triglyceride. Additionally, the 95% CIs of the slope of the best fit line of CEA concentration and triglyceride concentration all included zero in the confidence interval (-0.0083 to 0.0069 for CEA value 3 ng/mL; -0.0645 to 0.0205 for CEA value 26.1 ng/mL; -0.422 to 0.140 for CEA concentration 74.4 ng/mL).

For bilirubin concentrations ranging from <2 to 595 umol/L, three different CEA concentrations were within the CEA specification range in the presence and absence of bilirubin. Additionally, the 95% CIs of the slope of the best fit line of CEA concentration and bilirubin concentration all included zero in the confidence interval (-0.0002 to 0.0006 for CEA value 3.6 ng/mL; -0.0038 to 0.0025 for CEA value 28.8 ng/mL; -0.0254 to 0.0115 for CEA concentration 94.4 ng/mL).

Interference with human albumin, rheumatoid factor, and human anti-mouse antibodies (HAMA) was evaluated at a separate site during clinical study testing for precision. A modified variation of CLSI document EP7-A2 was used. The 3 interferents were added to a human serum pool containing known CEA concentration (24.6 ng/mL ± 3.3 ng/mL; range 21.3 to 27.9). The acceptance criterion was no interference if the test sample %recovery was within the range 85% to 115% (i.e. ± 15% of 100% recovery). No interference was seen with human albumin or HAMA up to 150 g/L albumin and up to 912 IU/mL HAMA. Decreased CEA recovery (55%) was seen in the presence of rheumatoid factor at 2400 IU/mL. The sponsor states that the RF concentration exceeds any value expected in usual serum samples. **The limitations section of the labeling will reflect this interference.**

Interference with various chemotherapeutic drugs was evaluated at a separate site during clinical study testing for precision. A modified variation of CLSI document EP7-A2 was used. Twenty-seven drug interferents were added to a human serum pool containing a known CEA concentration (24.6 ng/mL ± 3.3 ng/mL; range 21.3 to 27.9) and tested in three assay runs. The drugs tested represent drugs typically used in treatment and over-the-counter (OTC) drugs. The following drugs were tested:

Tested interfering drugs

5-fluorouracil	Acetaminophen
N-acetyl-L-cysteine	Acetylsalycilic acid
Ampicillin	Ascorbic acid
Bleomycin	Carboplatin
Cefoxitin	Cisplatin
Cyclophosphamide	Cyclosporine
Dactinomycin	Doxocycline
Doxorubicin	Etoposide
Ibuprofen	Levodopa
Methotrexate	Metronidazole
Mitomycin C	Naprosyn
Paclitaxel	Phenylbutazone
Rifampicin	Vinblastine
Vincristine	

The acceptance criterion is a ratio of test sample to control between 0.9 and 1.10. The sponsor notes that rifampicin tested at 2 concentrations had significant interference (ratio of 0.03 at 1 mg/mL and 0.04 at 0.766 mg/mL). No significant interference was noted for other tested drugs. All %recoveries were between 0.9 and 1.1 with the exception of N-acetyl-L-cysteine at 2 ng/mL and acetylsalicyclic acid at 1 mg/mL (%recoveries ranged from 0.85 to 1.15). **For rifampicin, a statement is included in the Limitations section of the labeling.** The recovery for N-acetyl-L-cysteine at 2 ng/mL and acetylsalicyclic acid at 1 mg/mL will be claimed at 85% - 115% recovery.

Cross-reactivity with beta-HCG, CA125, CA27.29, CA19-9, AFT, Prostate-specific antigen (PSA), and Prostatic Acid Phosphatase (PAP) was assessed using samples serving as calibrators in respective TOSOH assays at its designated concentration. A human serum-based diluent served as a blank. The samples and concentrations tested are:
- Beta-HCG - 206 mIU/mL
- CA125 - 100 U/mL
- CA27.29 - 21.6 U/mL
- CA19-9 - 423 U/mL
- AFP - 201 ng/mL
- PSA - 52 ng/mL
- PAP - 19.3 ng/mL

All samples gave CEA values less than 2 ng/mL, the acceptance criterion for this analysis.

f. *Assay cut-off:*
The sponsor defines a 12.2% percentage change in CEA values as significant. The significant change is 2.5 times the total imprecision of the CEA assay across sites, lots, and concentration (expressed as %CV = 4.8%). The sponsor chose this value to ensure that the change in CEA value is not attributed to assay variation.

2. Comparison studies:

a. *Method comparison with predicate device:*
   Using 1307 samples representing paired values between 0.5 and 100 ng/mL were tested with the proposed and predicate assays. Deming regression analysis of comparison gave a slope of 0.941 (95% CI 0.824 to 1.058) and an intercept of -1.291 (95% CI -1.681 to -0.90). The slope was not significantly different from 1.0 (95% CI of slope includes 1.0) indicating the lack of proportional bias in assay result between the proposed and predicate assay. The intercept is significantly different from 0 (95% CI does not include 0) indicating the presence of constant bias between the two assays.

   Using 311 samples collected from colon cancer subjects undergoing serial surveillance monitoring for disease progression, Deming regression analysis yielded a slope of the best fit line of 0.82 (95% CI 0.527 to 1.107) and an intercept of 13.3 (95% CI 0.27 to 26.31). This analysis indicates the presence of constant bias of 13.3 ng/mL in serial surveillance monitoring samples when using the proposed assay compared with the predicate assay. Proportional bias is not present in this comparison.

b. *Matrix comparison:*
   Not applicable since only serum specimens are utilized.

3. Clinical studies:

   a. *Clinical Sensitivity:*
   b. *Clinical specificity:*
   c. *Other clinical supportive data (when a. and b. are not applicable):*
      Samples utilized in this study were obtained from retrospective sample banks at M.D. Anderson Cancer Center. Specimens for the study were from subjects with colorectal cancer. Serial sets must include a minimum of 3 draws (4 draws or more desired) per subject. Samples were blood draws performed at or after diagnosis throughout as much of the clinical course as possible. Clinical information detailing the disease status for each sample and information on types of therapy, if any, received with dates of administration was collected. Three hundred and two specimens from 79 subjects were collected. There were 223 evaluable observation pairs. The mean number of serial specimens per subject was 3.8 (median 2.0). Of the serial samples from 79 subjects, 43% of subjects had 3 visits, 36% had 4 visits, 18% had 5 visits, and 4% had 6 visits. The mean age at diagnosis was 61 years. Eighty-one percent of subjects were Caucasian, 5% Asian, 7% Hispanic, and 7% African-American. Approximately 75% are stage III and IV subjects. Stage 0, I, and II represent the remaining percentage. The National Cancer Institute's fact sheet for colorectal cancer (http://seer.cancer.gov/statfacts/html/colorect.html) notes the following information: *The stage distribution based on historic stage shows that 40% of colon and rectum cancer cases are diagnosed while the cancer is still confined to the primary site (localized stage); 36% are diagnosed after the cancer has spread to regional lymph nodes or directly beyond the primary site; 19% are diagnosed after the cancer has already metastasized (distant stage) and for the remaining 5% the staging information was unknown. The corresponding 5-year relative survival rates were: 89.7% for localized; 68.4% for regional; 10.8% for distant; and 36.6% for unstaged.*

Localized stage as described in this way is stage I and II. Regional stage corresponds to stage III, and distant stage corresponds to stage IV. Therefore the stage distribution for stage III and IV would be 55%. The fact that the sponsor's study utilized 70% subjects as stage III and IV indicates a significant over-sampling of these subjects in the study.

The outcome of interest was defined as progression of disease from time point i (clinical visit i, i=1 to n-1) to a succeeding time point j (clinical visit j, j=i+1 to n). The number of clinical visits for which samples and data are available is defined as n. The visit number made by a study subject is at the time of diagnosis or after diagnosis and prior to death, loss to follow-up or remission of disease. The sponsor statistically defines w_{ij} as a variable representing disease progression and has 2 values as follows:

    1 if there is disease progression from visit i to visit j

    0 if no progression (stable disease, response to therapy) from visit i to visit j

Disease progression is determined by the subject's physician and is based on any or a composite of physical signs/symptoms, results of lab tests for colorectal cancer, radiographic findings (CAT scans, PET scans, MRI, x-ray, or ultrasound), or patient reported symptoms.

The sponsor defines the variable v_{ij} as 1 if the difference in value of the test assay at visit i (x_i) and value of the test assay at a later visit j (x_j) is greater than or equal to 12.2% (i.e. v_{ij} = 1 if $(x_j - x_i) \geq 12.2\%$). The variable v_{ij} is 0 if the difference is otherwise (i.e. v_{ij} = 0 if $(x_j - x_i) < 12.2\%$). The sponsor chose this value to ensure that the change in CEA value is not attributed to assay variation and is statistically significant. In the same way, the percentage change in the predicate device was defined as 2.5 times the %CV of total imprecision as stated in the predicate package insert. This value was 2.5 x 4% = 10% change.

To determine an association between the variables w (disease progression) and v (change in CEA value) a 2 x 2 contingency table can be constructed to find an association between variables. Items in each of the 4 cells represent pairs of v and w (1 and/or 0) for visits for all subjects (or for subjects only). The total concordance from the 2 x 2 contingency table (equivalent with total agreement), positive concordance, and negative concordance can be calculated. In each situation, it is assumed that agreement is with physician determined disease progression or no progression. No specification for concordance values was present. A similar definition and association will be sought for the predicate device for comparison purposes.

The table and calculation of concordance values for the association of the proposed test with disease state across all patient visits for all subjects is the following:

VIDAS CEA	Change in disease state (variable w) to		
Change in CEA (variable v)	Progression	No progression	Total
≥ 12.2%	43	54	97
< 12.2%	21	105	126
Total	64	159	223
Total concordance C	43+105/223= **0.664**	Lower 95% CI = 0.595	Upper 95% CI = 0.727
Positive concordance C₊	43/64= **0.672**	Lower 95% CI = 0.549	Upper 95% CI = 0.778
Negative concordance C₋	105/159= **0.660**	Lower 95% CI = 0.583	Upper 95% CI = 0.737

The probability of no association between disease status and change in VIDAS CEA (≥ 12.2%) was less than 0.0001. The difference in positive concordance (0.672) and 1 minus the negative concordance (1-0.660) was 0.332 (95% CI of difference 0.196 to 0.469). The test is informative on a per visit basis.

Comparison of the concordance values for the proposed and predicate assays was available from analysis of the line listings for monitored colorectal cancer subjects. For comparison of the positive concordance (concordance of %change in CEA among subjects with progression) the following table results:

Progression positive subjects

VIDAS CEA Assay	Tosoh CEA			
	CEA change >10%	CEA change < 10%	Total	
change ≥12.2%	40	6	46	(0.667)
change < 12.2%	4	19	23	
total	44	25	69	
	(0.638)			
difference in positive concordance	-0.029	± 0.0457	p = 0.5259	
95% CI	-0.1186	to 0.0606		

The difference in positive concordance value was 2.9%, the proposed assay having a higher concordance value. However, the difference is not statistically significant (95% CI of difference is -0.119 to 0.061).

For comparison of the negative concordance (concordance of %change in CEA among subjects without progression) the following table results:
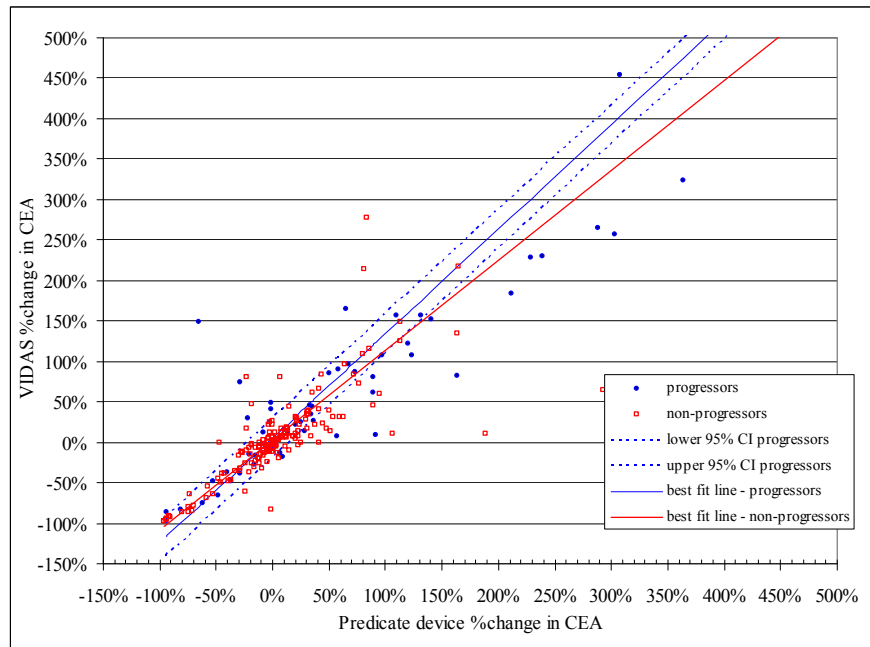
Progression negative subjects

VIDAS CEA Assay	Tosoh CEA			
	CEA change >10%	CEA change < 10%	Total	
change ≥12.2%	43	10	53	
change < 12.2%	16	85	101	(0.656)
total	59	95	154	
		(0.617)		
difference in negative concordance	0.039	± 0.0330	p = 0.2372	
95% CI	-0.0256	to 0.1036		

The difference in negative concordance value was 3.9%, the proposed assay having a higher concordance value. However, the difference is not statistically significant (95% CI of difference is -0.026 to 0.104).

Comparison of the %change in CEA value in the clinical study population of subjects was performed. A graphical representation of the %CEA change in the proposed and predicate assay is shown in the following graph.

Note from the graph that the linear correlation between %CEA change is modestly good. Among non-progressing subjects, the slope of the best fit line is 1.14 (95% CI 1.005 to 1.274), a value significantly different from 1.0. The intercept of the line for non-progressing subjects was 0.026 (95% CI -0.100 to 0.152), a value not

significantly different from 0. Among progressing subjects, the slope of the best fit line is 1.22 (95% CI 1.160 to 1.281), a value different from 1.0. The intercept of the line for progressing subjects was 0.094 (95% CI -0.115 to 0.304), a value not different from 0. The 95% CIs of the regression line on the graph is the interval for the progressing subjects. This indicates that the slopes of the best fit line for non-progressing and progressing subjects are equivalent up to approximately 100% change, despite the difference in the slopes from 1.0. This fact suggests similar, possibly equivalent, slopes and equivalent %CEA changes in both assays.

ROC analysis was performed of the proposed test with disease state classifications for all subjects with any progression/no progression and any change in CEA value (223 events in 80 subjects). There is no cutoff value for %change in CEA that is published or clinically accepted as a reasonable choice. Therefore, clinicians can choose a cutoff value that they believe is acceptable for the clinical situation of the patient they are managing. The following table summarizes various sensitivity values and the resulting specificities and cutoff values at a chosen sensitivity value for the proposed device:

VIDAS				
cutoff	Sensitivity	specificity	lower 95% CI of specificity	upper 95% CI of specificity
-48.9%	90.6%	11.3%	6.8%	17.3%
0.0%	75.0%	50.3%	42.3%	58.3%
12.3%	67.2%	66.0%	58.1%	73.4%
24.3%	62.5%	75.5%	68.0%	81.9%
47.0%	53.1%	86.8%	80.5%	91.6%
184.2%	25.0%	95.6%	91.1%	98.2%

Note from the table that at high sensitivity there is low specificity (~12%) and the cutoff %change value is negative. At a cutoff value of 12.3%, very near the sponsor choice of cutoff, the specificity of the proposed assay is 66%. At this specificity for the predicate assay, the sensitivity is equivalent (66.7%) with the proposed assay but the cutoff value for %change in CEA is different, -0.8% or essentially 0%.

4. Clinical cut-off:
   The sponsor defines a 12.2% percentage change in CEA values as significant. The significant change is 2.5 times the total imprecision of the CEA assay across sites, lots, and concentration (expressed as %CV = 4.8%). The sponsor chose this value to ensure that the change in CEA value is not attributed to assay variation.

5. Expected values/Reference range:
   Estimation and empirical distributions of CEA values in various populations of subjects was performed. A normal healthy population of subjects was recruited for assessment of CEA values. Approximately 150 each of male and female subjects aged 18-80 years of age who were apparently healthy (by self report) and were not sick on the day of serum sampling were tested using the proposed assay. Additionally approximately 75 current/recent male and female smokers were recruited and tested in the proposed assay. Cumulative distributions and order statistics were calculated in normal healthy

individuals. Confidence intervals for the 5th, 90th, and 95th order statistic were constructed. The results for these normal healthy subjects for the 5th and 95th percentiles are as follows:

Population	Percentile	CEA Estimate (ng/mL)	Lower 95% confidence limit	Upper 95% confidence limit
Female non-smokers	5th	0.50	0.50	0.50
	95th	1.77	1.34	2.44
Female smokers	5th	0.50	0.50	0.50
	95th	3.12	2.42	3.53
Male non-smokers	5th	0.50	0.50	0.50
	95th	2.78	2.19	3.23
Male smokers	5th	0.50	0.50	0.53
	95th	5.10	3.63	6.44

The empirical distribution of CEA in normal healthy individuals is fundamentally different and the labeling should reflect the differences.

The following benign disease cohort of 423 subjects was collected for analysis of the CEA distribution:

Benign urogenital disease 54
Benign diseases of gastrointestinal tract and lung 110
Diabetes 106
Heart disease/hypertension/liver disease 108
Benign breast disease 45

Subjects were males and females 18-80 years of age who had no history of malignancy (other than non-invasive skin cancer) diagnosed with the above benign diseases. ANOVA analysis was performed on the CEA values and also included factors for disease group (5 levels), smoking status (3 levels) and age category (< 56 years and > 56 years). Analysis indicated a significant smoking and age effect. Empirical distributions and 95% confidence intervals of the 5th, 90th, and 95th percentiles was developed and is summarized as follows (90th percentile value not shown):

Population	Percentile	CEA Estimate (ng/mL)	Lower 95% confidence limit	Upper 95% confidence limit
Never smoked and younger than 56 years	5th	0.50	0.50	0.50
	95th	2.38	2.04	3.59
Past smokers and younger than 56 years	5th	0.56	0.50	0.76
	95th	3.16	2.55	3.96
Present smokers and younger than 56 years	5th	0.68	0.50	0.75
	95th	5.21	3.34	6.03
Never smoked and older than 56 years	5th	0.50	0.50	0.53
	95th	3.17	2.52	3.63
Past smoker and older than 56 years	5th	0.59	0.53	0.68
	95th	3.12	2.79	3.73
Present smoker and older than 56 years	5th	0.86	0.71	1.37
	95th	11.29	4.66	16.69

The following malignant disease cohort of 514 subjects was collected for analysis of the CEA distribution:

    Lung/liver cancer   102

    Gall bladder/biliary/gastric/pancreatic cancer   59

    Prostate/testicular/bladder cancer   147

    Colorectal cancer   151

    Breast cancer 55

Banked serum samples from the above subjects who were 18-80 years of age, had a no history of other malignancy (except non-invasive skin cancer), and diagnosed with only one of the above cancers were selected and analyzed for CEA values. ANOVA analysis was performed on the CEA values and also included factors for disease group (5 levels), smoking status (4 levels) and age at diagnosis. The results indicated no differences in CEA level. Empirical distributions and 95% confidence intervals of the 5th, 90th, and 95th percentiles was developed and is summarized as follows (90th percentile value not shown):

Population	Percentile	CEA Estimate (ng/mL)	Lower 95% confidence limit	Upper 95% confidence limit
Malignant diseases	5th	0.50	0.50	0.60
	95th	73.82	39.71	114.80

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.