510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K042732

B. Purpose for Submission:
New assay on instrument

C. Measurand:
CA 15-3

D. Type of Test:
Chemiluminescent microparticle immunoassay

E. Applicant:
Fujirebio Diagnostics Inc.

F. Proprietary and Established Names:
Proprietary name: Architect CA 15-3 assay
Common name: Tumor marker immunological test system

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.6010 Tumor-associated antigen immunological test system
   21 CFR 862.1150 Calibrator
   21 CFR 862.1660 Quality Control Material (assayed and unassayed),
2. Classification:
   class II
3. Product code:

assay	MOI, System , Test, Immunological Antigen, Tumor
calibrator	JIT, Calibrator Secondary
control	JJX, Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:
   Immunology Devices (82)

H. Intended Use:
1. Intended use(s):
   The Architect CA 51-3 assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of DF3 defined antigen in human serum and plasma on the Architect *i* systems. The Architect CA 15-3 assay is to be used as an aid in the management of stage II and III breast cancer patients. Serial testing for patient CA 15-3 assay values should be used in conjunction with other clinical methods for monitoring breast cancer.

2. Indication(s) for use:
   The Architect CA 15-3 assay is to be used as an aid in the management of stage II and III breast cancer patients. Serial testing for patient CA 15-3 assay values should be used in

conjunction with other clinical methods for monitoring breast cancer.

3. Special conditions for use statement(s):
   prescription use only
4. Special instrument requirements:
   ARCHITECT *i* Systems – ARCHITECT *i* 2000 and ARCHITECT *i* 2000_{SR}.

I. Device Description:

The components of the reagent kit include the following:

- Microparticle reagent bottle – 1 bottle containing 6.6 ml for 100 tests, or 4 bottles containing 27 ml for 500 tests, having 115D8 antibody coated microparticles in TRIS buffer with protein stabilizers and anti-microbial agent.
- Conjugate reagent bottle – 1 bottle containing 5.9 ml for 100 tests, or 26.3 ml for 500 tests, having DF3 antibody conjugated to acridinium in phosphate buffer with protein stabilizers and anti-microbial agent.

Other components required but not supplied for the assay include the following:

- Pre-trigger solution containing 1.32% (w/v) hydrogen peroxide
- Trigger solution containing 0.35N sodium hydroxide
- Wash buffer containing phosphate buffered saline solution and anti-microbial agent
- Multi-assay manual diluent containing phosphate buffered saline and anti-microbial agent
- Architect System analyzer and software (Addition A, version 1.0)

Calibrator kit consists of 6 bottles of CA 15-3 calibrators, each bottle (4 ml each) with a separate concentration in TRIS buffer, protein stabilizers, and anti-microbial agent. Nominal calibrator concentrations are 0, 20, 80, 160, 400, and 800 U/ml.

Control kit consists of 2 bottles of CA 15-3 controls, each bottle (8 ml each) containing different concentrations in TRS buffer, protein stabilizers, and anti-microbial agent. Nominal concentrations are 40 U/ml (range 27.2 – 52.8 U/ml) and 250 U/ml (range 170 – 330 U/ml).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Abbott AxSYM CA 15-3 assay
2. Predicate 510(k) number(s):
   K963926
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Classification and product code	Class II, MOI	Class II, MOI
Product usage	Clinical and hospital labs	Clinical and hospital labs
Intended Use	The Architect CA 15-3 assays is a chemiluminescent microparticle immunoassay for the quantitative determination of DF3 defined antigen in human serum and plasma on the Architect i system. The Architect CA 15-3 assay is to be used as an aid in the management of Stage II and stage III breast cancer patients. Serial testing for patient CA 15-3 assay values should be used in conjunction with other clinical methods for monitoring breast cancer.	The AxSYM Ca 15-3 assay is a microparticle enzyme immunoassay for the quantitative measurement of CA 15-3 assay values in human serum and plasma (EDTA) to aid in the management of Stage II and Stage III breast cancer patients. Serial testing for patient CA 15-3 assay values should be used in conjunction with other clinical methods for monitoring breast cancer.
Analyte detected	Breast cancer-associated mucin antigen encoded by the MUC 1 gene	Breast cancer-associated mucin antigen encoded by the MUC 1 gene
Capture antibody	115D8 mouse monoclonal	115D8 mouse monoclonal
Conjugate antibody	DF3 mouse monoclonal	DF3 mouse monoclonal
Calibrators	6 levels (0 – 800 U/ml)	6 levels (0 – 250 U/ml)
Controls	2 levels (40 and 250 U/ml)	2 levels (35 and 150 U/ml)
Interpretation of results	Standard curve	Standard curve

Differences		
Item	Device	Predicate
Principle of operation	Chemiluminescent microparticle immunoassay	Enzyme microparticle immunoassay
Type of specimen	Human serum or plasma (EDTA, Li and Na heparin)	Human serum or plasma (EDTA)

Note from the table that both proposed and predicate devices have a similar, but not identical, assay technology. The capture and conjugate antibodies used in the assay are the same. But the type of signal produced is different. The assays have the same number of calibrators and controls, though the concentrations of the calibrators and controls differ. It is not clear at this point if the assays have the same assay cutoff. The assays differ slightly in the type of specimens used.

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

FDA Guidance document – "Guidance document for the submission of tumor associated antigen premarket notifications, [510(k)], to FDA"
NCCLS Document EP5-A – "Evaluation of precision performance of clinical chemistry devices – Approved guideline"
NCCLS Document EP6-P2 – "Evaluation of the linearity of quantitative analytical methods – Proposed guideline, second edition"
NCCLS Document EP7-A – "Interference testing in clinical chemistry devices – Approved guideline"
NCCLS Document EP9-A2 – "Method comparison and bias estimation using patient samples – Approved guideline, second edition"
NCCLS Document EP14-A – "Evaluation of matrix effects – Approved guideline"
NCCLS Document C28-A2 – "How to define and determine reference intervals in the clinical laboratory – Approved guideline, second edition"
ISO 14971:2000 – Medical devices – "Application of risk management to medical devices"
EN 375:2001 – "Information supplied by the manufacturer with in vitro diagnostic reagents for professional use"
EN 591:2001 – "Instructions for use for in vitro diagnostic instruments for professional use"
EN 13612:2002 – "Performance evaluation of in vitro diagnostic medical devices"
EN 13640:2002 – "Stability testing of in vitro diagnostic reagents"
EN 13641:2002 – "Elimination or reduction of risk of infection related to in vitro diagnostic reagents"
EN 980:1997 – "Graphical symbols for use in the labeling of medical devices"
ISO 15223:2000 – "Medical devices – Symbols to be used with medical device labels, labeling and information to be supplied"

L. Test Principle:

The assay is a chemiluminescent microparticle immunoassay to detect DF3-defined antigen, also known as CA 15-3, present in human serum and plasma. Sample, calibrator or control material and an anti-CA 15-3 antibody coated paramagnetic microparticle are mixed and incubated. If present, CA 15-3 antigen binds to the microparticles. After washing of microparticles, acridinium-labeled anti CA 15-3 antibody is mixed with the microparticles. Fluorescent solutions are added to the reaction mixture. The resulting chemiluminescence is measured by the analyzer as relative light units (RLU). The amount of fluorescent light measured is proportional to the amount of CA 15-3 antigen present in the sample. The concentration of CA 15-3 antigen in the sample is determined by comparing the fluorescence of a defined concentration of CA 15-3 from a calibrator-determined standard curve with the fluorescence of the sample.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>
   a. *Precision/Reproducibility:*
      Two separate studies of precision were conducted both using NCCLS EP5-A guidelines. In the first study, 5 defibrinated plasma based samples of pooled specimens were tested in duplicate at 2 separate times per day for 20 days using 2

Architect instruments with a 2 lots of reagents and a single calibration per instrument. Total and within-run precision was calculated from the standard deviation and %coefficients of variation. Sample concentrations ranged from 30 U/ml to 675 U/ml. The %CV for within-run imprecision ranged from 1.7% to 4.7%, simple average 2.8%. The %CV for total imprecision ranged from 2.2% to 5.1%, simple average 3.6%. The data support a claim in the label of <8%CV.

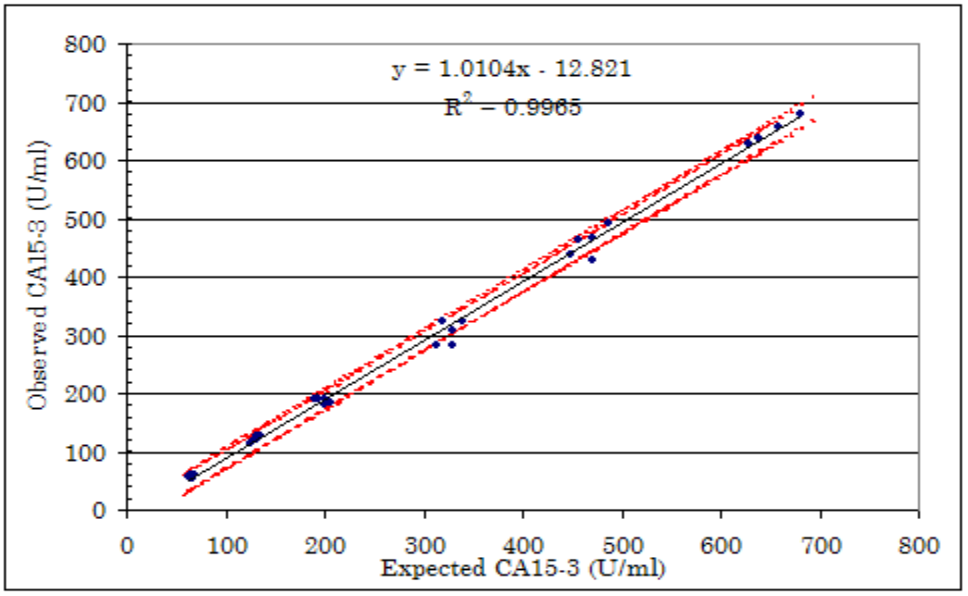
In the second study, 5 defibrinated plasma based samples of pooled specimens were tested in quadruplicate at 2 separate times of the day for 13 days using 3 Architect analyzers using 3 lots of reagent and a single calibration per instrument. Total and within-run precision were calculated similarly to the above experiment. Sample concentrations ranged from 25 U/ml to 600 U/ml. The %CV for within-run imprecision ranged from 0% to 2.5%, simple average 0.5%. The %CV for total imprecision ranged from 2.6% to 8.5%, simple average 4.0%. The data support a claim in the label of <8%CV.

To assess equivalence of precision for the *i*2000 and *i*2000$_{sr}$ Architect family members, 80 replicates of each of 5 defibrinated plasma panels (2 replicates per run, 2 runs per day for 20 consecutive days) were tested on one instrument of each family member. The %CV for total imprecision ranged from 2.2% to 4.4% for the Architect *i*2000$_{sr}$ and ranged from 3.1% to 5.1% for the *i*2000 family member. The acceptance criterion was a %CV for total imprecision of less than 8%. The acceptance criterion was met for both instrument family members.

b. Linearity/assay reportable range:

Aliquots of 10 serum specimens were supplemented with CA 15-3 and serially diluted with assay diluent. Diluted and undiluted samples were tested in duplicate in the assay. The percent recovery, expressed as the ratio of observed to expected CA 15-3 concentration x 100, was calculated. The applicant concludes that the samples demonstrated linearity over range of the assay and support a claim in the package insert of 100% ± 15%.

Information shown by the applicant can be utilized for analysis using NCCLS document EP6. Analysis of the data using the method and criteria presented in the guideline indicate that the assay is linear from 50 U/ml – 700 U/ml. A graph of the linearity analysis is as follows:



Note from the graph that the 95% confidence interval of the linear regression line contains 95% of the replicate data points for all tested concentrations. The percentage coefficient of variation of a y value for a given x value is 4%. The slope of the best fit regression line is not different from a slope of 1.0. The reportable range claimed by the applicant is 0-800 U/ml.

It would be reasonable to assume that deviation from linearity at concentrations between 700-800 U/ml on dilution may be expected, but probably not clinically important since a further dilution may be performed to obtain an accurate result. It is unclear if deviation from linearity at concentrations below 50 U/ml on dilution would be expected. Nor is it clear if there would be a clinically significant error in Ca 15-3 concentration when below 50 U/ml on dilution.

To assess dilution linearity using the automatic dilution protocol of the Architect instrument, 10 serum samples were supplemented with CA 15-3 to concentrations within the reportable range of the assay. Samples were manually diluted or instrument-diluted using the automatic instrument dilution protocol. Undiluted and diluted samples were tested in the assay. The percentage recovery compared with the undiluted concentration was calculated using a formula the applicant shows. Manual or auto-diluted samples were compared with the respective undiluted sample in the calculation. Additionally, the observed diluted concentration of the manual and auto-diluted samples were compared with each other by calculating the percentage recovery. The results are shown in an attachment. The attachment is a table showing he sample number, undiluted CA 15-3 concentration, the observed diluted concentration, concentration correcting for the dilution factor, and percent recovery. The applicant concludes that the automatic dilution protocol provides acceptable dilution linearity. The percent recovery of the auto-diluted samples to the manual dilution ranged from 92% to 100%.

No analysis was performed by the applicant to note if the corrected undiluted concentration from manual or automatic dilution was equivalent with the undiluted

concentration, though the percent recovery would appear to indicate small differences. For the manual dilution, the %recovery ranged from 91% to 100%, mean 93% ± standard error 1%. The mean difference in corrected diluted concentration and undiluted concentration was -46.3 U/ml ± standard error 8.7 U/ml. The relative mean difference (difference as a percentage of the undiluted concentration) was -6.7% ± standard error 1.2%. For automatic dilution, the mean difference in corrected diluted concentration and undiluted concentration was -75.4 U/ml ± standard error 6.7 U/ml. The relative mean difference was -10.9% ± standard error 0.8%. For the manual dilution, 90% of the differences relative to the undiluted concentration ranged from -3.0 U/ml to -108.3 U/ml with 90% confidence. For the automatic dilution, 90% of the differences relative to the undiluted concentration ranged from -53.3 to -123.8 U/ml with 90% confidence. For both manual and automatic dilution, the observed calculated concentration was consistently lower than the undiluted concentration.

The mean difference from undiluted concentration for the automatic dilution protocol was almost 50% higher than the mean difference of the manual dilution from the undiluted concentration. The difference between manual and automatic dilution protocols relative to the undiluted concentration was significantly higher for the automatic protocol ($p = 0.008$, general t-test). Therefore, the automatic dilution protocol appeared to be worse than manual dilution relative to the undiluted concentration, though the relative differences from undiluted concentration were more modest (-7% for manual dilution vs. -11% for automatic dilution).

When comparing the corrected observed concentration for the automatic dilution with the corrected observed concentration for the manual dilution, the mean difference was -5.8 U/ml ± standard error 1.3 U/ml (90% of the differences ranged from -0.1 U/ml to -10.8 U/ml with 90% confidence). The relative difference in corrected observed concentrations for the manual and automatic dilution was -4.2% ± standard error 0.9% (90% of differences ranged from -0.1% to -8.0% with 90% confidence). The probability for the hypothesis that the difference in observed corrected concentration between automatic and manual dilution equaled zero was 0.001. This further suggests that the automatic dilution was an additional 4% lower than the concentration for the manual dilution with its own error.

The applicant has concluded that 96% recovery of the automatic dilution relative to the manual dilution is acceptable. When stated another way, the applicant states that a 4% lower concentration for the automatic dilution relative to manual dilution is acceptable. In my own analysis, the relative difference of -4% was significantly lower than the manual dilution. It is unclear to me if an automatic dilution consistently 4% lower than manual dilution would be acceptable. If a CA 15-3 concentration were as high as 680 U/ml (the observed mean CA 15-3 concentration of the 10 samples tested by the applicant), then a consistently lower concentration of 4-5% might be clinically meaningless. The applicant has not noted what conditions trigger the automatic dilution protocol. Presumably such a trigger would be when the undiluted CA 15-3 concentration is greater than the upper limit of the working range of the assay (800 U/ml). If this were the case and if the corrected result was found in a clinical situation of monitoring cancer recurrence or response to therapy, it could be argued that the error from the automatic dilution protocol would have no clinical effect (or no worse

effect than manual dilution). While this assumption is reasonable, it assumes many situations which may be untrue. In the absence of definitive data, it is not possible to make firm conclusions about the clinical effects of a dilution error 4% more than would occur with manual dilution. Therefore, it is not possible to make any conclusions about the acceptability of the data or the applicant's claim.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Calibrators used to standardize the assay have values expressed as U/ml. A unit is defined by the applicant using a reference preparation maintained by the applicant. No internationally recognized reference preparation is available at the moment.

Assay standardization

Calibrators used to standardize the assay have values expressed as U/ml. A unit is defined by the applicant using a reference preparation maintained by the applicant. No internationally recognized reference preparation is available at the moment. A stock solution of CA 15-3 is evaluated using the CA 15-3 radioimmunoassay. Based on the assay, values are volumetrically calculated using the amount of antigen stock and a standard calibrator matrix. The calibrators for the Architect assay are made at 1/10 the labeled concentration but are used at 10 times the sample volume. Three radioimmunoassay using three kit lots are performed by three separate operators. The applicant notes that the characteristics of the tissue culture antigen and antigen in patient samples is such that CA 15-3 values are different for each of the proposed assay, predicate assay, and the radioimmunoassay. To compensate for the difference, primary CA 15-3 calibrators and patient samples having CA 15-3 are measured in the appropriate system and concentrations adjusted based on the correlation between the AxSYM and Architect systems whose values are determined with the radioimmunoassay-determined values. The applicant notes that 162 serum samples (not stated but presumably from cancer patients) were assayed in triplicate on 2 AxSYM systems and 2 Architect systems using 2 reagent kit lots per assay platform. Sample values determined on the Architect utilized calibrators whose value was assigned with the radioimmunoassay. Results from the Architect were correlated (using Passing-Bablok linear regression) with values from the AxSYM assay. The applicant notes that the "upper end" of the slope of the best fit line is inconsistent with values under 500 U/ml. So a preliminary linear regression analysis using values from 0-500 U/ml was made. The equation of the best fit line was $y = 0.76x + 0.85$. The sponsor then states that the equation of the best fit line for all samples was $y = 0.77x – 0.2$. The applicant states that due to the small number of samples with values of 500-800 U/ml, the direct evaluation of one calibrator, calibrator F, was difficult. The applicant determined the final value after dilution of the calibrator to determine its value relative to the other calibrators. The applicant notes that recovery of the diluted calibrator varied from 95% to 100% with no apparent trending. The values of all calibrators were calculated using the slope of the best fit line for serum values from 0-500 U/ml. The calibrators were "physically adjusted". The adjusted calibrators were used to generate a new calibration curve from which values for the serum samples were re-calculated. A second "physical adjustment" was made after re-assignment of calibrator values from the new correlation with AxSYM values. The sponsor then states the following:"After the second adjustment, the physically

adjusted calibrators were assayed to establish new standard curves that were used to re-evaluate the Architect sample values using the original RLU values for the samples." It is very unclear what the applicant has done in this process. It is noted that the relationship of the slope between the 2 assay systems was specified to be 1 ± 2% (between 0.98 and 1.02). To verify the appropriateness of value assignment, the applicant assayed 118 serum samples using 1 lot of AxSYM reagent and 2 Architect reagent lots. Samples were tested in single replicates only. The sponsor notes that linear regression analysis indicated that the slope of the best fit of AxSYM and Architect values was within the specification (1 ± 10%, 0.90 to 1.10). The primary calibrators derived from this method will be used as the reference of all future Architect calibrator lots.

The scheme of the applicant is not clear to me, especially the multiple correlations and re-adjustments after each correlation. The simplest description appears to be that values for the Architect are linked with values for the AxSYM assay. The use of serum samples with CA 15-3 antigen that may or may not be conformationally or biologically related to the cell culture derived antigen appears unnecessary or only marginally valuable scientifically.

d. Detection limit:

To determine the lowest measurable concentration distinguishable from zero with 95% confidence, calibrator A (0 U/ml CA 15-3) was tested in 10 replicates on each of three instruments using 2 lots of reagents and 2 lots of calibrator (n = 24 runs). In addition, 2 replicates of calibrator B (20 U/ml) was tested similarly. The mean values and standard deviations of calibrator A and calibrator B were used to determine the minimum detectable concentration for each run. The minimal detectable concentration was defined as MDD = $(2*SD_{ACal})*Conc_{BCal}/(\text{mean } RLU_{BCal} - \text{mean } RLU_{ACal})$. The analytical sensitivity is defined as the concentration at 2 standard deviations above the mean minimal detectable concentration. This represents the lowest measurable concentration distinguishable from zero. The mean minimal detectable concentration was 0.04 U/ml, standard deviation 0.017. The analytical sensitivity was the mean plus 2 standard deviations, or 0.07 U/ml. The applicant concludes that the analytical sensitivity was 0.07 U/ml at 95% confidence. The applicant also concludes that the data support the claim of the package insert of ≤ 0.5 U/ml.

To assess the comparability of the CA 15-3 assay on the Architect *i*2000 and $i2000_{sr}$ instruments, ten replicates of calibrator A and 2 replicates of calibrator B using 2 calibrator lots and 2 reagent lots were tested per assay run for 9 total runs. One Architect $i2000_{sr}$ and 2 *i*2000 instruments were utilized. The analytical sensitivity for the both instrument was 0.07 U/ml (mean CA 15-3 value for $i2000_{sr}$ was 0.041 ± 1 standard deviation of 0.016; mean CA 15-3 value for i2000 was 0.037 ± 1 standard deviation 0.018). The data indicate an equivalent analytical sensitivity for each Architect instrument family member.

e. Analytical specificity:

Interference from bilirubin, hemoglobin, protein, and triglycerides

To assess the potential for interference from bilirubin, hemoglobin, protein, and

triglycerides, NCCLS guideline EP7-P ("Interference testing in clinical chemistry, proposed guideline") was utilized to design an interference experiment. The endogenous CA 15-3 level of multiple samples was supplemented with additional CA 15-3 from a stock solution to give 5 different CA 15-3 concentrations ranging from approximately 50 U/ml to 400 U/ml. Aliquots of 5 of the samples were additionally supplemented with bilirubin, hemoglobin, protein, triglyceride, or nothing, 5 samples per interferent. Samples were tested in the proposed assay in duplicate. The interference was calculated as the percentage recovery of CA 15-3 relative to control without interfering substances. The results are as follows:

Interfering substance	Concentration of interferent	% recovery
Bilirubin	20 mg/dL	102%
Hemoglobin	500 mg/dL	98%
Protein	12 g/dL	101%
Triglycerides	3 g/dL	97%

Interference from chemotherapeutic agents

To assess the interference from 12 chemotherapeutic substances, a design similar to that above was utilized. Five samples with CA 15-3 supplemented from a stock solution to give 5 different concentrations ranging from 50 U/ml to 400 U/ml were additionally supplemented with β-estradiol, cisplatin, cyclophosphamide, doxorubicin, 5-fluorouracil, megesterol, methotrexate, mitomycin C, tamoxifen, testosterone, paclitaxel, vinblastine, or solvent without interferent. The percentage recovery of Ca 15-3 with interfering substance relative to the CA 15-3 concentration without interferent was calculated for the 5 samples tested with each interferent. The results are as follows:

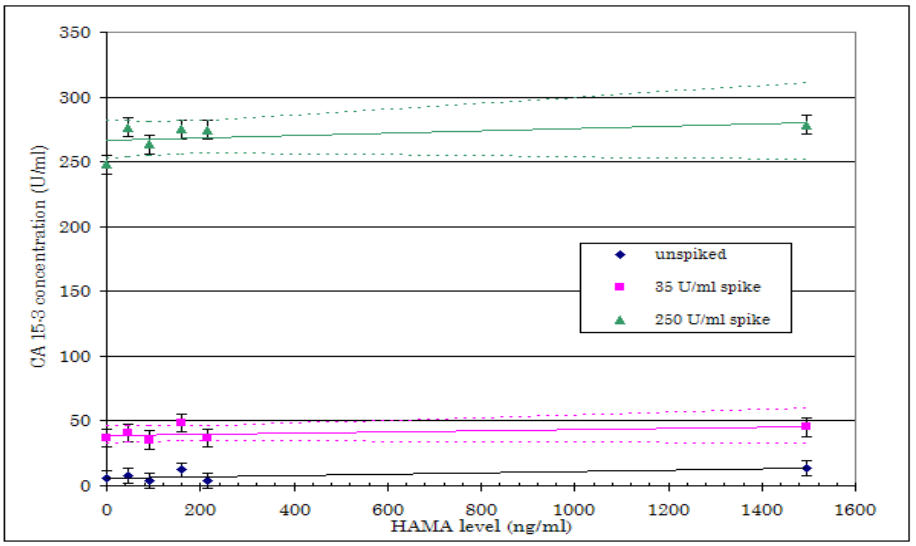
Interfering substance	Concentration of interferent	% recovery
β-estradiol	6.7 μg/mL	99%
Cisplatin	66.7 μg/mL	100%
Cyclophosphamide	330 μg/mL	102%
Doxorubicin	6.6 μg/mL	102%
5-fluorouracil	280 μg/mL	99%
Megestrol	39.6 μg/mL	100%
Methotrexate	13.2 μg/mL	97%
Mitomycin C	17.2 μg/mL	102%
Tamoxifen	5.0 μg/mL	104%

Testosterone	33 µg/mL	99%
Paclitaxel	3.5 ng/ml	98%
Vinblastine	1.3 µg/mL	100%

Interference from human anti-mouse antibody

To assess interference from human anti-mouse antibody (HAMA), each of 5 samples positive for HAMA and one normal sample were split into 3 aliquots. One aliquot was additionally supplemented with 35 U/ml CA 15-3, one aliquot was supplemented with 250 U/ml, and one aliquot was not supplemented. All aliquots were tested in duplicate in the same run using the proposed assay. Percent recovery was calculated for the HAMA containing samples compared with the control sample without HAMA. The calculation accounts for the endogenous CA 15-3 present in the samples. For samples containing 35 U/ml CA 15-3, the average %recovery was 106% (ranging from 101% to 117%). For samples containing 250 U/ml of CA 15-3, the average %recovery was 109% (ranging from 107% to 112%). There was 6% interference for samples containing 35 U/ml CA 15-3 and 9% interference for samples containing 250 U/ml CA 15-3. It is concluded that less than 12% interference was observed with samples containing elevated levels of HAMA.

No specification for acceptance was noted. Linear regression analysis of the final observed CA 15-3 level (y-axis) vs. the HAMA concentration (x-axis) was performed for each of 6 samples where CA 15-3 was added to samples containing endogenous CA 15-3 concentrations or for 6 samples without added CA 15-3. The best fit line and 95% confidence range for the regression line was also calculated. The following graph shows the results.



Note that the best fit line for samples without added CA 15-3 but containing varying HAMA concentrations has a slope that is flat (slope = 0.005 ± 0.003, $p > 0.05$ for hypothesis that slope equals 0). Note that the best fit line for samples with 35 U/ml of added CA 15-3 and varying HAMA concentrations has a slope that is flat (slope =

0.004 ± 0.004, $p > 0.05$ for hypothesis that slope equals 0). Note further that the 95% confidence interval of the regression line contains all samples with added HAMA and the sample without added HAMA.

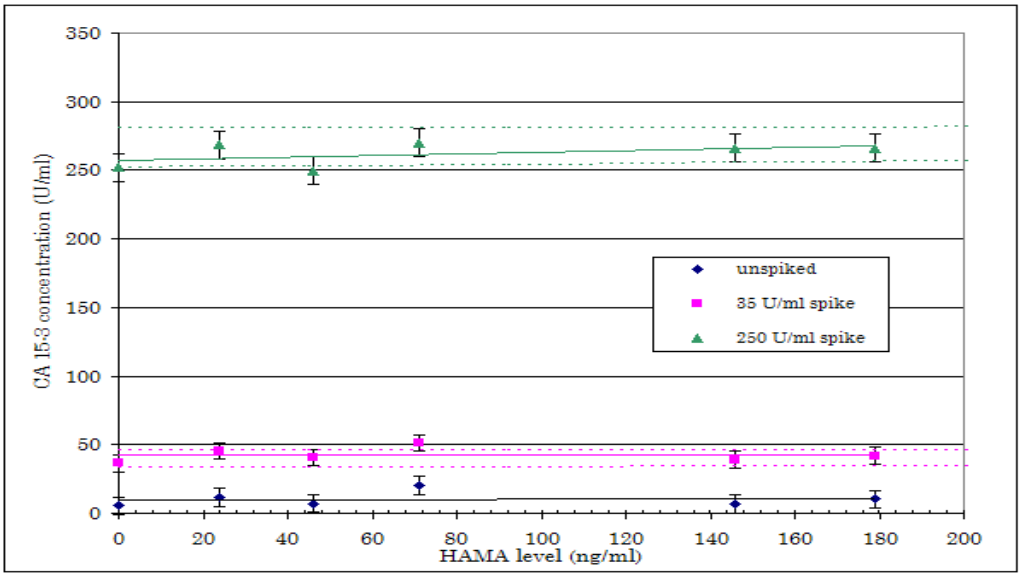
For samples containing 250 U/ml of added CA 15-3 and varying HAMA concentrations, the slope of the best fit line is flat (0.009 ± 0.009, $p > 0.05$ for hypothesis that slope equals 0).

Less than 12% interference was observed in the proposed assay with specimens containing elevated levels of HAMA.

Interference from rheumatoid factor

To assess interference from rheumatoid factor (RF), studies were performed similarly to interference from HAMA. Each of 5 samples positive for rheumatoid factor and one normal sample were split into 3 aliquots. One aliquot was additionally supplemented with 35 U/ml Ca 15-3, one aliquot was supplemented with 250 U/ml, and one aliquot was not supplemented. All aliquots were tested in duplicate in the same run using the proposed assay. Percent recovery was calculated for the RF containing samples compared with the control sample without RF. The calculation accounts for the endogenous CA 15-3 present in the samples. For samples containing 35 U/ml CA 15-3, the average %recovery was 104% (ranging from 99% to 109%). For samples containing 250 U/ml of CA 15-3, the average %recovery was 103% (ranging from 98% to 105%). It is concludes that there was 4% interference for samples containing 35 U/ml CA 15-3 and 3% interference for samples containing 250 U/ml CA 15-3. Generally that less than 12% interference was observed with samples containing elevated levels of RF.

No specification for acceptance was noted by the applicant. Linear regression analysis of the final observed CA 15-3 level (y-axis) vs. the RF concentration (x-axis) was performed for each of 6 samples where CA 15-3 was added to samples containing endogenous CA 15-3 concentrations or for 6 samples without added CA 15-3. The best fit line and 95% confidence range for the regression line was also calculated. The following graph shows the results.



Note that the best fit line for samples without added CA 15-3 but containing varying RF concentrations has a slope that is flat (slope = 0.004 ± 0.038, $p > 0.05$ for hypothesis that slope equals 0). Note also from the graph that the best fit line for samples with 35 U/ml of added CA 15-3 and varying RF concentrations has a slope that is flat (slope = 0.0007 ± 0.037, $p > 0.05$ for hypothesis that slope equals 0). Note further that the 95% confidence interval of the regression line contains all samples with added RF and the sample without added RF. The best fit line for samples with 250 U/ml of added CA 15-3 and varying RF concentrations has a slope that is flat (slope = 0.059 ± 0.055, $p > 0.05$ for hypothesis that slope equals 0). The 95% confidence interval of the regression line contains all samples with added RF and the sample without added RF.

f. Assay cut-off:
Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

To compare values of the proposed and predicate device and to assert accuracy by high correlation, 402 serum specimens were tested. Of the specimens, 250 were obtained from stage I to stage IV breast cancer patients. Seven of the tested samples had initial concentrations above the reportable range of either assay. These samples were diluted and re-tested in the appropriate assay. Passing-Bablok linear regression analysis was performed on all test results. The applicant shows a graphical representation of the results. The correlation coefficient was 0.98. The slope of the best fit line was 0.94 (95% confidence interval 0.92 to 0.97). The intercept of the best fit line was -0.3 U/ml (95% confidence interval -0.9 to 0).

Since the slope of the best fit line was not equivalent with 1.0, there is a statistically significant proportional bias of -6% (ranging from -3% to -8%). Since the confidence interval of the intercept of the best fit line includes 0, there appears to be no constant bias between assays. It is unclear what clinical effect would result from the statistical bias. The data would support a conclusion of substantial equivalence of assay result.

The following table shows the concordance of assay result using a cutoff of 31.3 U/ml CA 15-3 in each assay.

	Architect result		
AxSYM result	≤ 31.3 U/ml	> 31.3 U/ml	total
≤ 31.3 U/ml	280	0	280
> 31.3 U/ml	9	113	122
Total	289	113	402

The overall concordance was 97.8%. Agreement of assay results by chance was 58.6%. The kappa statistic comparing agreement vs. agreement by chance was 0.946 ± 1 standard error 0.050. For the hypothesis of perfect agreement (kappa = 1.0), the probability was greater than 0.05. The probability of rejecting the hypothesis of perfect agreement when it is true is less than 5%. Therefore, the observed agreement is equivalent with perfect agreement.

The Architect *i* 2000 and *i* 2000_{sr} are instrument systems in the Architect family of instruments. To confirm the equivalence of results of the CA 15-3 assay on both instruments, the following were tested on both instrument platform:

- Analytical sensitivity
- Precision
- Spike recovery
- Result comparison via correlation

The applicant notes that these characteristics were chosen as indicators of overall performance while other performance characteristics (such as specificity, dilution linearity, and stability) rely upon these other characteristics or are related to the reagent formulation used.

As part of a comparison of results on the *i*2000 and *i*2000_{sr} instrument systems, 149 specimens were tested on the *i*2000 and AxSYM instrument. A separate set of 249 specimens were tested on the *i*2000_{sr} and AxSYM instrument. Acceptance criterion was a slope of 1.0 ± 0.15 for either the *i*2000_{sr} or *i*2000 vs. the AxSYM instrument. Results are summarized as follows:

AxSYM vs.	Correlation coefficient	Least squares slope	Least square intercept	Passing-Bablok slope	Passing-Bablok intercept
*i*2000	0.972	0.982	-2.237	1.009	-1.74
i2000_{sr}	0.996	0.913	0.315	0.909	0.23

The 95% confidence interval of the slope for both instruments is between 0.85 and 1.15, therefore equivalence is demonstrated. The data indicate that both the Architect instruments are equivalent with the AxSYM instrument assay and do not necessarily indicate equivalence of each family member with the other member. The acceptance

criterion indicates that a bias in the slope up to 15% between Architect family members can be acceptable.

To assess equivalence of accuracy for the *i*2000 and *i*2000$_{sr}$ Architect family members, spiking recovery of 10 separate serum samples supplemented with 4 different concentrations of semi-purified CA 15-3 (45, 125, 365, and 565 U/ml) was performed. Each sample was assayed in duplicate determinations. The concentration of each augmented sample was compared with the expected concentration (sum of added CA 15-3 and endogenous CA 15-3) to calculate the percent recovery. The acceptance criterion of %recovery was 100% ± 15%. The overall mean %recovery using the *i*2000$_{sr}$ analyzer was 106%. The %recovery using the *i*2000 analyzer was 96%. The acceptance criterion was met using both analyzers, though no formal comparison of difference in mean %recovery between family members was performed.

To assess the ability of the assay to accurately recover added antigen, aliquots of 10 normal serum specimens with known endogenous CA 15-3 levels were supplemented with known concentrations and tested in duplicate in the assay. The percent recovery was calculated. For 45 U/ml added CA 15-3, the percent recovery was 90%. For 125 U/ml added CA 15-3, the percent recovery was 92%. For 365 U/ml added CA 15-3, the percent recovery was 96%. The sponsor concludes that the data support supports a claimed recovery of 100% ± 15%.

Analysis was not performed to assess the %recovery or the difference of observed and expected CA 15-3 (or expressed as relative differences). To assess the data for all 3 different CA 15-3 concentrations added to 10 samples, the relative difference was calculated. The mean relative difference (difference in observed and expected CA 15-3 concentration divided by the expected CA 15-3 concentration) was -7.2% for 30 samples (10 aliquots supplemented with either 45, 125, or 365 U/ml added CA 15-3). The maximum relative difference due to an assay imprecision (the %CV corresponding to the 99% confidence interval of the variance of the tested samples) was -10.7%. The probability that the mean relative difference was less than or equal to the maximum relative difference due to imprecision was 0.35. Since the mean relative difference was statistically equivalent or less than the maximum relative difference due to assay imprecision, the data supports a conclusion of accurate recovery of added CA 15-3.

b. Matrix comparison:

To assess the equivalence of blood collection tube types for serum and plasma as well as equivalence of plasma, anti-coagulated in 3 ways, with serum CA 15-3 concentrations, matched human serum and plasma were collected in the following tube types:

- Serum tube with no additive
- Serum separator tube
- EDTA anti-coagulated plasma
- Lithium heparin anti-coagulated plasma
- Sodium heparin anti-coagulated plasma

Twenty sample sets were tested unchanged in a preliminary assay. Four sets of 5 samples each were supplemented with additional CA 15-3 to give values of approximately 50 U/ml, 100 U/ml, 200 U/ml and 400 U/ml. All specimens were tested in duplicate using the Architect CA 15-3 assay (40 sample sets tested for each matrix). The mean concentration of each set of samples was compared to the mean value for the sample set for serum only by calculating the %recovery. The formula divides the mean value of the test sample of a set with the mean value of serum sample within a set. The percent recovery is as follows:

	SST	EDTA-plasma	Li heparin plasma	Na heparin plasma
Mean %recovery	99%	99%	98%	96%

The recovery for each specimen type was approximately 98%.

For specific analysis, it was necessary to calculate an expected mean value of the 40 samples for each sample matrices (serum separator tubes, EDTA anti-coagulated plasma, Lithium heparin anti-coagulated plasma, and sodium heparin anti-coagulated plasma). The mean expected value in serum would be the results of the 20 un-supplemented samples, 5 replicates of 50 U/ml, 5 replicates of 100 U/ml, 5 replicates of 200 U/ml, and 5 replicates of 400 U/ml (overall mean 100.02 U/ml). The expected difference would be the mean CA 15-3 concentration for each of the 40 samples with a given matrix minus the mean expected value for all 40 serum samples (100.02 U/ml). The observed mean difference was statistically compared with the expected mean difference using a paired t-test. The probabilities that the observed difference was equivalent with the expected difference were as follows:

	Serum only	SST	EDTA-plasma	Li heparin plasma	Na heparin plasma
Expected mean value	100.02	100.02	100.02	100.02	100.02
Observed mean value	101.51	101.92	100.34	99.82	98.63
Expected difference from 100.02 U/ml	1.49	1.90	0.32	-0.20	-1.39
Observed difference from observed serum value		0.41±0.88	-1.17±0.77	-1.69±0.96	-2.88±1.20
p-value of no difference from expected difference		0.642	0.278	0.131	0.222

The expected difference for serum only represents the random difference in observed CA 15-3 concentration from the expected concentration when adding various CA 15-3 concentrations to a portion of the samples. For each of the other specimen types, the observed difference is not different from the expected difference after allowing for error in the addition of CA 15-3 to a portion of the samples. This analysis would indicate that serum separator tubes and the 3 types of anti-coagulated plasma give equivalent results with serum CA 15-3 concentrations.

3. Clinical studies:
   a. *Clinical Sensitivity:*
      Not applicable
   b. *Clinical specificity:*
      Not applicable
   c. *Other clinical supportive data (when a. and b. are not applicable):*
      The distribution of CA 15-3 values was analyzed for 250 women treated for breast cancer. Of 250 women tested, 24% were stage I, 21% stage II, 22% stage III, and 33% stage IV. Eighty-six percent of breast cancer patients had CA 15-3 values below 31.3 U/ml regardless of stage. Of 250 samples tested in both the proposed and predicate assays, 98.4% were concordant (i.e. both less than or equal to 31.3 U/ml or both greater than 31.3 U/ml). Of the 250 subjects, 87% (218/250) had values in both assays less than or equal to 31.3 U/ml. Of 250 subjects, 11% (28/250) subjects had values in both assays greater than 31.3 U/ml.

      The following patient groups were tested in the proposed assay to determine the distribution of serum CA 15-3 values in various benign and malignant disease conditions:

Group	Number
Benign breast disease (fibrocystic or adenomal)	100
Benign ovarian disease	100
Benign pregnancy	50
Benign urogenital tract disease	49
Hypertension/chronic heart disease	100
Ovarian or cervical cancer	120
Colorectal cancer	50
Lung cancer	50

The following table summarizes the distribution in percent of CA 15-3 values in the various groups of subjects

Group	#	0-31.3 U/ml	31.4-60 U/ml	60.1-120 U/ml	>120 U/ml
Ovarian cancer	120	76.7%	13.3%	4.2%	5.8%
Colorectal cancer	50	96.0%	4.0%	0%	0%
Lung cancer	50	78%	14%	4%	4%
Breast disease	100	97.0%	3.0%	0%	0%
Ovarian disease	100	99.0%	1.0%	0%	0%
Urogenital disease	49	84%	16%	0%	0%

Group	#	0-31.3 U/ml	31.4-60 U/ml	60.1-120 U/ml	>120 U/ml
Pregnancy	50	100%	0%	0%	0%
Hypertension/chronic heart disease	100	94%	6%	0%	0%

To determine the ability of the proposed assay to aid in the monitoring of disease status in patients diagnosed with breast cancer, serial serum samples were obtained from a stored serum bank at M. D. Anderson Cancer Center. Testing of the specimens was performed by the applicant at their own site. Specimen testing utilized kit controls in each assay run. If the result for the kit control was out of the stated range, the assay must be repeated. All results from the failed assay are not used. There were no failed runs observed during the studies. A total of 377 serum specimens from 74 breast cancer patients were utilized. Patients were selected from stored sample banks based on the following criteria:

I. Inclusion
    a) Known diagnosis
    b) 0.5 ml minimum volume
    c) single freeze/shipped frozen
    d) normal specimen appearance
    e) informed consent

II. Exclusion
    a) Concurrent unrelated malignancy
    b) No known diagnosis
    c) Insufficient volume
    d) Multiple freeze-thaw/ stored or shipped at 4C
    e) Icteric, lipemic, hemolytic appearance of specimen or substantial particulates
    f) No consent

The inclusion criteria listed do not explicitly note if breast cancer patients were to be stage II and stage III cancer patients, as noted in the Intended Use. The average number of samples per patient was 5.1. The following table summarizes the distribution of serial specimens from patients.

Number of specimens/patient	Number of patients	Frequency (%)	Cumulative frequency (%)
3	6	8	8
4	20	27	35
5	20	27	62
6	17	23	85
7	11	15	100

It is assumed that the average number of follow-up clinical evaluations is 4. This assumption is based upon the idea that the first specimen in the serial sampling series is to determine the initial CA 15-3 value. Subsequent specimens in the sampling series are collected to calculate the change in CA 15-3 value and a clinical evaluation of the patient occurs at the second and subsequent serum samplings. The majority of patients evaluated (54%) had 3-4 clinical evaluations during cancer monitoring. Thirty-eight percent (38%) of subjects had 5-6 evaluations while 8% only had 2 clinical evaluations during cancer monitoring.

The average age of women in this group was 48 years (exact 95% confidence interval 45.3 – 48.9 years). Forty-seven percent (47%) of the women were post-menopausal at the time of diagnosis. Tumor stage information was available for 61 of the 74 women studied. Sixty-four percent (64%) of women with tumor stage information were evenly split between stage II and III. Twenty-three percent (23%) of women with tumor stage information were stage IV. Though specifically stated, it is assumed that the remaining 13% of women with tumor stage information were stage I.

The outcome measure for statistical analysis was clinical determination of progression of disease from time point *i* to succeeding time point *j*. Time point *i* is a clinical visit ranging from 1 to n-1, the number of clinical visits made by a patient after diagnosis and prior to death, loss to follow-up, or remission of disease. Time point j is the clinical visit ranging from *i*+1 to n. The variable w_{ij} is defined as progression status. Values of w_{ij} can have values 1, if progression is present from visit *i* to visit *j* or a value of 0 if there is no progression from visit *i* to visit *j*. Disease progression is determined by the patient physician based on ether or both of the following:

> Examination of clinical signs and symptoms, including results of lab tests currently part of standard care of cancer status
>
> Radiographic findings used in the assessment of cancer status (CAT scans, PET scans, MRI, x-ray images, or ultrasound images)

A monitoring value of the proposed device will be defined as v_{ij} with values

1 = the difference in CA 15-3 value from visit *i* to visit *j* greater than or equal to *d* where *d* is 2.5 times the coefficient of variation of the assay (for this study, d was 9.575%)

0 = the difference in CA 15-3 value from visit *i* to visit *j* less than *d*

The change in CA 15-3 value was chosen to ensure that a change in assay results was not due to random assay variation.

To find an association between w_{ij} and v_{ij}, a 2 x 2 contingency table was constructed. The table of actual results for 303 observed visit points (377 evaluable observations – 74 initial observations = 303 visit points) is as follows:

	progression	no progression	Total
≥ 9.575% increase in CA 15-3 from previous visit	50	84	134
< 9.575% increase in CA 15-3 from previous visit	16	153	169
total	66	237	303

Total concordance = 203/303= 0.669
Positive Concordance = 50/66= 0.757
Negative concordance = 153/237 = 0.645

The applicant notes that the data represent "panel" data. The applicant states that since each patient brings a unique set of visit pairs, the sets must be controlled. For this purpose, generalized linear models were used to obtain estimates of the concordance. The total concordance rate of 0.669 ranged from 0.613 to 0.721. The positive concordance rate of 0.757 ranged from 0.648 to 0.841. The negative concordance rate ranged from 0.582 to 0.704.

Since it is assumed that the majority (54%) of subjects had 3-4 clinical evaluations during clinical cancer monitoring and 3-4 values for the change in CA 15-3 value, then data in the table should not be viewed as completely independent events. In the majority of cases, the values in each cell contain data from 3-4 clinical evaluation events with associated changes in CA 15-3 values. Therefore, it is possible that concordance could occur by chance when no concordance actually occurs in a patient rather than the apparent agreement that occurred at each clinical evaluation visit. It is reasonable to analyze the concordance rate after modeling with general estimable equation fitting to account for the differing number of clinical evaluations and CA 15-3 tests in each patient.

The total concordance rate based upon the general estimable equation fitting is the average agreement of the percentage change in CA 15-3 value with status of progression/non-progression for all visits within each patient and all patients. The confidence interval for agreement (from 0.61 to 0.72) does not include the value 0.5 or 0.533, the rate of agreement by chance. This indicates that the probability of agreement is significantly higher than agreement by random chance.

A table showing the changes in CA 15-3 value (≥ 9.575% or < 9.575%) vs. disease status (progression or not progression) on a per patient basis is as follows:

	progression	no progression	Total
≥ 9.575% increase in CA 15-3 from previous visit	36	27	63
< 9.575% increase in CA 15-3 from previous visit	1	10	11
total	37	37	74

Total concordance = 0.613; 95% confidence interval 0.501 – 0.732
Positive Concordance = 0.973; 95% confidence interval 0.858 – 0.993
Negative concordance = 0.270; 95% confidence interval 0.138 – 0.441

The random chance agreement of change in CA 15-3 value with disease status was 0.500. The probability that the observed concordance was equal to random chance agreement was 0.002 (kappa = 0.243 ± 1 standard error 0.083). This indicates that the agreement is significantly higher than random chance agreement. The positive predictive value of a positive change in CA 15-3 value (i.e. ≥ 9.575%) was 0.571 (exact binomial 95% confidence interval 0.440 – 0.695) and was significantly higher (p = 0.009 for hypothesis of no difference) than the apparent prevalence of progression, 0.500, in the study. The negative predictive value of a change in CA 15-3 value < 9.575% was 0.900 (exact binomial 95% confidence interval 0.587 – 0.998).

For a patient or clinician, the risk of having progression when a positive test result is present (change in CA 15-3 value ≥ 9.575%) is approximately 57% while the risk of non-progression when a change in CA 15-3 value < 9.575% is approximately 91%. A clinician would have higher confidence of the lack of progression when the change in CA 15-3 was less than 10% (91%) than the confidence in the presence of progression when a change in CA 15-3 value is ≥ 10% (57%). The assay results should be utilized for clinical decision-making in light of this performance and other available information.

4. Clinical cut-off**:**
   No information provided to assess any cut-off.

5. Expected values/Reference range:
   To determine the distribution of Ca 15-3 values in apparently healthy normal individuals and suggest a cutoff of 31.3 U/ml, serum samples from 199 women (100 post-menopausal and 99 pre-menopausal) and 197 men who were apparently disease-free were tested in the assay. Subjects were included if self-declared healthy and the specimen was normal in appearance and contained a minimum volume of 0.5 ml. Samples were excluded if the subjects had either a concurrent illness (types not specified) and the specimen was not normal in appearance or contained insufficient volume. A cumulative distribution of CA 15-3 values was produced after single replicate test results were obtained from the assay. Of 199 females, 99.0% had CA 15-3 values of 31.3 U/ml or below (mean 12.9 U/ml, standard deviation 6.6 U/ml). These results are equivalent with results in the package insert for the predicate assay.

   In pre-menopausal women, 99.5% of subjects had CA 15-3 values of 31.3 U/ml or less. Among post menopausal women, 99.0% had CA 15-3 values of 31.3 U/ml or less.

Therefore, the 99th percentile value among all women is 31.3 U/ml. The 95% confidence interval of the 99th percentile ranged from 97.5 to 99.7th percentile. Among males, 98.7% had CA 15-3 values of 31.3 U/ml or less.

N. Instrument Name:

Abbott Diagnostics Architect *i* system – ARCHITECT *i* 2000 and ARCHITECT *i* 2000$_{SR}$. Both systems belong to the ARCHITECT family of instruments. The ARCHITECT *i* 2000$_{SR}$ is similar to the ARCHITECT *i* 2000 but has the following additional features a) STAT sampling hardware and software, b) Auto Retesting software and c) different composition and position of the RV loader.

O. System Descriptions:

Refer to K983212 for full description of instrument system clearance.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Q. Proposed Labeling:

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

R. Conclusion:

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