510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I   **Background Information:**

A   **510(k) Number**

K233946

B   **Applicant**

Siemens Healthcare Diagnostics Products Ltd

C   **Proprietary and Established Names**

IMMULITE 2000 BR-MA

D   **Regulatory Information**

Product Code(s)	Classification	Regulation Section	Panel
MOI	Class II	21 CFR 866.6010 - Tumor-Associated Antigen Immunological Test System	IM - Immunology

II   **Submission/Device Overview:**

A   **Purpose for Submission:**

Modification of the previously cleared device to mitigate biotin interference.

B   **Measurand:**

Cancer Antigen 15-3 (CA 15-3)

C   **Type of Test:**

Quantitative, chemiluminescent immunometric assay

III   **Intended Use/Indications for Use:**

A   **Intended Use(s):**
See Indications for Use below.

B   Indication(s) for Use:

For in vitro diagnostic use with the IMMULITE 2000 Systems Analyzers – for the quantitative measurement of CA15-3 antigen in human serum and plasma, as an aid in the detection of recurrence in previously treated stage II and stage III breast cancer patients, and in the management of metastatic breast cancer patients by monitoring disease progression or response to treatment. Serial testing for patient CA15-3 values should be used in conjunction with other clinical methods used for detecting early recurrence in stage II and stage III disease and for monitoring response to treatment in patients with metastatic breast cancer.

C   Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For In Vitro Diagnostic Use Only

The package insert of the device contains the warning: "CA15-3 antigen levels in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity. Results reported by the laboratory to the physician must include the identity of the assay used to measure CA15-3 antigen levels. Values obtained with different assays cannot be used interchangeably. Before changing assays, the laboratory must confirm baseline values for patients being serially monitored."

D   Special Instrument Requirements:

IMMULITE 2000 Systems Analyzer (K970227)

IV   Device/System Characteristics:

A   Device Description:

IMMULITE 2000 BR-MA consists of the reagent pack. Each reagent pack is for 200 tests and contains:

- BR-MA Bead Pack (1 pack, 200 beads/pack): coated with anti-CA15-3 murine monoclonal antibody
- BR-MA Reagent Wedge:
    - Well 1 (11.5 mL): serum-based buffer, with preservative
    - Well 2 (11.5 mL): alkaline phosphatase (bovine calf intestine) conjugated to murine monoclonal anti-CA15-3 antibody in buffer, with preservative

- BR-MA Adjustors (Low and High; 2 vials, 3 mL/vial): lyophilized CA15-3 in a nonhuman serum matrix, with preservative; Reconstitute each vial with 3.0 mL distilled or deionized water.

Materials Provided Separately:

- Multi-Diluent 2
- Chemiluminescent Substrate Module

- Probe Wash
- Probe Cleaning Kit
- Disposable Reaction Tubes, Sample Diluent Test Tubes, Sample Diluent Tube Caps

The manufacturer recommends the use of commercially available quality control materials or sample pools with at least two levels (low and high) of CA15-3.

To mitigate the risk of biotin interference, the IMMULITE 2000 BR-MA reagent pack has been modified from the previously cleared assay, which was susceptible to interference from biotin.

B Principle of Operation:

The IMMULITE 2000 BR-MA is a two-step sequential solid-phase chemiluminescent immunometric assay. There are two incubation cycles of 30 minutes each. During the initial 30-minute cycle, the patient sample is incubated with a biotinylated antibody coated bead and buffer (reagent wedge well 1). The biotinylated antibody on the bead captures the antigen in the patient sample. On completion of the first 30-minute cycle, unbound sample/buffer mixture is removed via a centrifugal wash. During the second 30-minute cycle, alkaline phosphatase antibody conjugate in buffer (reagent wedge well 2) is added to complete the bead pair immunocomplex sandwich consisting of capture Ab-antigen-detection Ab. On completion of the second 30-minute cycle, unbound conjugate is removed by centrifugal wash. The amount of alkaline phosphatase bound is directly proportional to the analyte in the patient sample. Following the two 30-minute incubation periods, IMMULITE chemiluminescent substrate is added for a further 5-minute incubation period to generate the luminogenic reaction. The chemiluminescent substrate undergoes hydrolysis in the presence of the alkaline phosphatase to yield an unstable intermediate, which then emits photons. The sustained emissions are measured by the luminometer. The resulting relative light units (RLU) are proportional to the concentration of CA 15-3 in the sample, which is expressed as U/mL. The CA15-3 concentration in the serum is determined from a reagent lot specific calibration curve that is correlated to the individual instrument using supplied high and low adjustors.

V Substantial Equivalence Information:

A Predicate Device Name(s):

IMMULITE 2000 BR-MA

B Predicate 510(k) Number(s):

K013984

C Comparison with Predicate(s):

Device & Predicate Device(s):	K233946	K013984 (Predicate)
Device Trade Name	IMMULITE 2000 BR-MA	IMMULITE 2000 BR-MA
General Device Characteristic Similarities		
Intended Use/ Indications for Use	For *in vitro* diagnostic use with the IMMULITE 2000 Systems Analyzers — for the quantitative measurement of CA15-3 antigen in human serum and plasma, as an aid in the detection of recurrence in previously treated stage II and stage III breast cancer patients, and in the management of metastatic breast cancer patients by monitoring disease progression or response to treatment. Serial testing for patient CA15-3 values should be used in conjunction with other clinical methods used for detecting early recurrence in stage II and stage III disease and for monitoring response to treatment in patients with metastatic breast cancer.	Same
Analyte	Cancer Antigen (CA) 15-3	Same
Measurement	Quantitative	Same
Sample Type	Human serum; heparinized and EDTA plasma	Same
Measuring Range	1–300 U/mL	Same
Assay technology	Chemiluminescent immunoassay	Same
Instrument	IMMULITE 2000 System Analyzers	Same
Sample Volume	5 mL	Same
Calibrators (Adjustors)	Two levels of lyophilized CA15-3 in a non-human serum matrix	Same
Detection Antibody	Alkaline phosphatase (bovine calf intestine) conjugated to murine monoclonal anti-CA15-3 antibody	Same
Capture Antibody	Anti-CA15-3 murine monoclonal antibody	Same
General Device Characteristic Differences		
Assay Modification	Modification of solid phase	No modification
Biotin Interference	Up to 3500 ng/mL	Up to 100 ng/mL

Detection Limit	Limit of Blank = 0.21 U/mL Limit of Detection = 0.30 U/mL Limit of Quantitation = 1 U/mL	Analytical Sensitivity: 1.0 U/mL

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures, Approved Guideline – Third Edition

- CLSI EP06-A2, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition

- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition

- CLSI EP09c, Measurement Procedure Comparison and Bias Estimation Using Patient Samples – Third Edition

- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, Approved Guideline – Third Edition

- CLSI EP34, Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking– First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The studies were performed following CLSI EP05-A3.

Within-laboratory precision

Five native pooled serum samples were assayed in two replicates per sample per run, two runs per day for 20 days using one lot of modified IMMULITE 2000 BR-MA assay on one IMMULITE 2000 analyzer (n=80 per sample). Samples 1 and 2 were prepared by mixing native serum samples from two individual patients while samples 3-5 were prepared by mixing native pooled serum samples into an individual native serum specimen. The data was analyzed using ANOVA for standard deviation (SD), % coefficient of variation (%CV) to evaluate within-run, between-run, between-day, and within-laboratory attributes for each sample. The results are summarized in the following table:

Sample	Mean (U/mL)	Within-Run SD	Within-Run %CV	Between-Run SD	Between-Run %CV	Between-Day SD	Between-Day %CV	Within-Lab SD	Within-Lab %CV
1	21.0	1.12	5.3	1.08	5.2	0.00	0.0	1.56	7.4
2	38.7	1.85	4.8	2.02	5.2	0.00	0.0	2.74	7.1
3	79.0	4.35	5.5	3.70	4.7	0.00	0.0	5.71	7.2
4	175	8.86	5.1	8.65	4.9	1.53	0.9	12.48	7.1
5	234	16.0	6.8	6.37	2.7	0.00	0.0	17.22	7.4

Lot-to-lot imprecision

The study was performed at a single site with three assay lots. Samples 1-5, described above, were tested on one IMMULITE 2000 analyzer for five days, with five replicates per sample per run per day (N = 75 per sample). The data was analyzed using ANOVA for SD, %CV to evaluate within-run (repeatability), between-day, between-lot, reproducibility (total precision) attributes for each sample. The results are summarized in the following table:

Sample	Mean (U/mL)	Within-Run SD	Within-Run %CV	Between-Day SD	Between-Day %CV	Between-Lot SD	Between-Lot %CV	Total SD	Total %CV
1	20.7	0.88	4.3	0.65	3.1	0.00	0.0	1.09	5.3
2	39.9	1.72	4.3	0.51	1.3	0.00	0.0	1.80	4.5
3	77.6	3.96	5.1	2.50	3.2	0.00	0.0	4.69	6.0
4	176.1	9.34	5.3	0.00	0.0	0.00	0.0	9.34	5.3
5	234.2	12.98	5.5	0.00	0.0	3.6	1.5	13.45	5.7

2. Linearity:

The linearity study was conducted following CLSI EP06-Ed2. A panel of 10 dilution levels was prepared by mixing a high CA 15-3 pooled serum native sample with a low CA 15-3 native single serum sample to cover the claimed analytical measuring range of the IMMULITE 2000 BR-MA assay. Four replicates per sample were tested using three reagent lots on one IMMULITE 2000 analyzer. The data was analyzed using weighted least squares linear regression. Percent deviations from linearity were calculated as differences between the observed values (mean values) and the predicted values divided by the predicted values. The table below summarizes the linearity data analysis:

Kit Lot	Dilution Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2	% Deviation from Linearity
1	0.72 – 385.86	1.02 (0.97; 1.07)	-0.08 (-0.35; 0.19)	1.00	1.67 – 8.04 %
2	0.85 – 404.92	0.99 (0.96; 1.02)	-0.01 (-0.16; 0.14)	1.00	2.30 – 7.17%
3	0.80 – 385.36	0.99 (0.96; 1.03)	-0.04 (-0.23; 0.15)	1.00	1.51 – 8.45%

The data support the linearity interval from 0.72 to 404.92 ng/mL with the deviations from linearity within ±10%. The study results support the linearity of the claimed analytical measuring interval (AMI): 1 – 300 U/mL.

Automatic dilution versus manual dilution

To verify the auto dilution function on IMMULITE 2000 analyzer, one high sample (> 300 U/mL) was diluted using the onboard dilution function and compared with dilution of the same sample that was manually diluted by the same dilution factor. The high native serum pool was diluted, using assay diluent 10x, 20x and 40x. The study was conducted on three lots of modified reagent packs on one IMMULITE 2000 analyzer with five replicates run per diluted sample. The dose by onboard dilution was compared to the dose obtained by manual dilution and % bias calculated and compared to the acceptance criteria. The study results support the automatic dilution function of IMMULITE 200 analyzer for the modified IMMULITE 2000 BR-MA.

High Dose Hook Effect

The high-dose hook effect of the modified IMMULITE 2000 BR-MA assay was determined by testing dilutions of a sample with a concentration of approximately 140,000 U/mL CA 15-3. All samples were tested in duplicate using three modified reagent lots on one IMMULITE 2000 analyzer. The results showed no high dose hook effect up to 80,000 U/mL.

3. Analytical Specificity/Interference:

Cross-reactivity

The cross-reactivity of the modified IMMULITE 2000 BR-MA assay with Alpha-fetoprotein (AFP), Cancer Antigen 125 (CA 125), Cancer Antigen 19-9 (CA19-9), and Carcinoembryonic Antigen (CEA) was evaluated by testing two patient serum sample pools at low level (between 4.7–5.6 U/mL) and one at a mid-level close to the cut-off (between 30–36 U/mL). Each substance was spiked into test samples, and control samples were prepared by spiking solvent into the samples. Samples were run in replicates of five using one modified reagent lot on one IMMULITE 2000 Analyzer. The cross-reactivity was calculated by comparing measurements of the test and control samples. No significant cross reactivity (≤ ±10% difference of test from control) for the modified IMMULITE 2000 BR-MA assay was observed up to the following concentrations of the potential cross-reacting substances tested:

Cross-reacting Substance	Concentration
AFP	5,000 IU/mL
CA 125	10,000 U/mL
CA 19-9	2,000 U/mL
CEA	5,000 ng/mL

Interference

The interference of the modified IMMULITE 2000 BR-MA assay was performed following recommendations of CLSI EP07, 3rd Edition.

Potential endogenous and exogenous interfering substances were tested for their ability to interfere with the modified IMMULITE 2000 BR-MA assay using two serum samples with CA 15-3 concentration around 30 U/mL and 120 U/mL. For each interfering substance, test samples were spiked with the test substance and results were compared to control samples spiked with an equal volume of solvent. Samples were measured in three replicates except for biotin samples which were run in five replicates. The study was performed using one modified reagent lot run on one IMMULITE 2000 analyzer.

Human Anti-Mouse Antibodies (HAMA) interference was evaluated by testing two patient serum samples at ~8 U/mL and ~34 U/mL. The samples were spiked with commercially available HAMA serum. The spiked sample and the corresponding serum sample without HAMA were tested in five replicates using one modified reagent lot on one IMMULITE 2000 analyzer.

The interference was calculated by comparing the mean measurements of the test and control samples. No significant interference ($\leq \pm 10\%$ difference of test from control) were observed for the modified IMMULITE 2000 BR-MA assay up to the following concentrations for each endogenous and exogenous substances tested:

Substance	Concentration
Endogenous:	
HAMA	792 µg/mL
Hemoglobin	381 mg/dL
Bilirubin (Conjugated)	200 mg/L
Bilirubin (Unconjugated)	200 mg/L
Intralipid	3,000 mg/dL
Biotin	3,500 ng/mL
Exogenous:	
5-Fluorouracil	1,000 µg/mL
Cisplatin	100 µg/mL
Cyclophosphamide	1,000 µg/mL
Doxorubicin Hydrochloride	100 µg/mL
Mitomycin-C	100 µg/mL
Vincristine	1 µg/mL

Alkaline phosphatase interference was not evaluated in this review but the package insert states that "(t)he drug asfotase alfa (STRENSIQ), a recombinant form of alkaline phosphatase, is expected to interfere with in vitro diagnostic assays utilizing an alkaline phosphatase detection system. Test samples from patients taking asfotase alfa with a non-alkaline phosphatase methodology."

4. Assay Reportable Range:

   1 – 300 U/mL

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability

The assay is traceable to an internal standard; no international reference standard is available for CA 15-3 currently.

Stability

The stability of the modified IMMULITE 2000 BR-MA assay is evaluated according to the recommendation of CLSI EP25-A.

Shelf-life (unopened): A study was performed to establish the real-time shelf-life stability of the modified IMMULITE 2000 BR-MA. Three lots of modified reagents were stored in product storage conditions (2–8°C). Lots were tested using three commercial control samples, three standards, and three native pooled serum samples, each representing low, mid, and high CA 15-3 concentrations in replicates of five at the following time-points 0, 1, 2, 31, 45, 60, 91, 179, 225, 301, and 325 days on one IMMULITE 2000 analyzer. The data of each sample at each testing time point were compared to the data of initial value tested at baseline. The data supports the reagent stability of 301 days at 2–8°C. The stability study is ongoing.

Open in-use stability: The modified IMMULITE 2000 BR-MA assay was evaluated for in-use stability. One lot of reagent pack was opened, tested at time 0 and stored at 2–8°C. Three commercial control samples, three standards, and three native pooled serum samples, each representing low, mid, and high CA 15-3 concentrations, were tested in replicates of five at time-points 0, 31, 38, 60, 66, 90, and 98 days on one IMMULITE 2000 analyzer. The data of each sample at each testing time point were compared to the data of initial value tested at baseline. The data supports the open reagent pack stability of the modified IMMULITE 2000 BR-MA up to 90 days stored at 2–8°C after initial use.

6. Detection Limit:

   Limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were established for modified IMMULITE 2000 BR-MA assay following the recommendations of CLSI EP17-A2.

   The LoB was determined from the measurement of four analyte-free serum samples tested in five replicates per sample over three days using three modified reagent lots on one IMMULITE 2000 analyzer (60 measurement replicates per lot). The LoB was calculated using the classical non-parametric approach by establishing the 95% rank. The claimed LoB is 0.21 U/mL which is the highest LoB value observed across three reagent lots.

   The LoD was determined from the measurement of eight low-level serum samples in five replicates, two runs per day over five days using three lots of modified reagents on one IMMULITE 2000 analyzer (400 measurements, per lot). The LoD was calculated as the LoB + 1.645 x SD of the replicates for the low-level samples. The claimed LoD is 0.30 U/mL which is the highest LoD value observed across lots.

   The LoQ was determined based on the LoD study described above using the precision profile. LoQ was defined as the level meet the %CV of the within-lab imprecision of 20%. The claimed LoQ is 1 U/mL.

7. Assay Cut-Off:

The assay cut-off was established in K013984.

Decision Point	Interpretation
< 38 U/mL	Normal
> 38 U/mL	Elevated

B Comparison Studies:

1. Method Comparison with Predicate Device:

A total of 315 native serum samples were tested in singlicate over eight days using three lots of the modified IMMULITE 2000 BR-MA assay and one lot of the predicate device (K013984) on one IMMULITE 2000 analyzer. The samples included 144 samples from patients with breast cancer, 50 samples from patients with non-breast cancer malignant disease, 61 samples from patients with non-cancer diseases, 49 samples from healthy females (24 pre-menopausal, 25 post-menopausal), and additional 11 contrived samples (pooled breast cancer samples) to cover the upper end of the measuring range. Each contrived sample was prepared by pooling two individual native breast cancer samples. Only the samples (N=274) within the measuring ranges of both the modified device and the predicate were analyzed by Passing-Bablok regression, and the results are summarized below:

Lot #	N	Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r)
1	274	3.76 – 297.70	0.98 (0.96; 0.99)	0.71 (0.22; 1.10)	0.989
2	274	3.76 – 297.70	0.99 (0.97; 1.01)	0.16 (-0.22; 0.64)	0.992
3	274	3.76 – 297.70	1.04 (1.02; 1.05)	-0.79 (-1.29; -0.42)	0.990

2. Matrix Comparison:

Matrix comparison study was performed to demonstrate that serum separator tube (SST), and lithium heparin plasma and EDTA plasma matrices yield comparable values as serum with the modified IMMULITE 2000 BR-MA assay. The 40 matched sample sets covering the analytical measuring interval (AMI) of the assay were tested in singleton using one modified reagent lot on IMMULITE 2000 analyzer. Passing-Bablok regression analyses were performed by comparing the results of samples from SST and different plasma samples (y) to the results of corresponding serum samples (x). The results are summarized in the tables below:

	Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r)
SST vs serum	6.96 – 265.94	1.05 (0.98; 1.10)	-0.44 (-1.14; 1.00)	0.997
Li-heparin plasma vs serum	6.96 – 265.94	0.96 (0.92; 0.99)	0.09 (-0.81; 1.11)	0.998
EDTA plasma vs serum	6.96 – 265.94	1.03 (0.94; 1.08)	-1.02 (-2.08; 0.85)	0.998

	SST vs. Serum	Lithium Heparin-Plasma vs. Serum	EDTA-Plasma vs. Serum
Bias for modified IMMULITE 2000 BR-MA			
At cut-off 38 U/mL	3.84%	-3.76%	0.32%

C Clinical Studies:

Clinical studies were conducted in K013984.

D Clinical Cut-Off:

Clinical cut-offs were established in K013984.

E Expected Values/Reference Range:

To verify if the reference range of the modified IMMULITE 2000 BR-MA assay is the same as that established in K013984, a total of 69 samples (31 pre-menopausal and 38 post-menopausal female) from apparently healthy female individuals were tested per recommendations in CLSI EP28-A3c. The age cut-off of 50 years was used to define pre- and post-menopausal status of the donor. The samples were from subjects representing four major ethnic groups (41 Caucasian, 13 Hispanics, 10 African American, and 5 Asian). The samples were run in single replicate on three lots of each of the modified reagent kits and one reference lot of unmodified reagent lot on one IMMULITE 2000 analyzer. The unmodified assay's reference range found that ≥ 90% of normal female samples fell within the range of the normal range described in K013984 as 6.4–58 U/mL. In each of the three modified lots, 65 of the 69 samples (94%) fell within this range, supporting a claim that there is no change of reference range established previously in K013984.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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