

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k081769

**B. Purpose for Submission:**

To obtain clearance for a new device.

**C. Measurand:**

Antithrombin

**D. Type of Test:**

Quantitative, Chromogenic

**E. Applicant:**

Siemens Healthcare Diagnostics Inc.

**F. Proprietary and Established Names:**

INNOVANCE™ Antithrombin

**G. Regulatory Information:**

1. Regulation section:  
21 CFR 864.7060; Antithrombin III assay
2. Classification:  
Class II
3. Product code:  
JBQ; Antithrombin III quantitation
4. Panel:  
81 Hematology

**H. Intended Use:**

1. Intended use(s):  
INNOVANCE™ Antithrombin is a chromogenic assay for the automated quantification of functionally active antithrombin in human citrated plasma and can be used as an aid in the diagnosis of antithrombin deficiency.
2. Indication(s) for use:  
Same as Intended Use
3. Special conditions for use statement(s):  
For Prescription Use only.
4. Special instrument requirements:  
BCS®/BCS® XP System

**I. Device Description:**

INNOVANCE™ Antithrombin is a chromogenic assay kit containing three reagents used for the determination of antithrombin activity. The reagents are in liquid form and are composed of human Factor Xa, bovine serum albumin, heparin, hirudin, aprotinin and preservatives, chromogenic substrate and buffer.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Dade Behring Berichrom™ Antithrombin III (A)
2. Predicate K number(s):  
k933125
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Automatic quantification of functionally active antithrombin in citrated plasma.	Same
Sample type	Human citrated plasma	Same
Platform	Automated coagulation analyzers with reading capability at a wavelength of 405 nm	Same

Differences		
Item	Device	Predicate
Intended Use	INNOVANCE™ Antithrombin is a chromogenic assay for the automatic quantification of functionally active antithrombin in human citrated plasma and can be used as an aid in the diagnosis of antithrombin deficiency.	For the quantitative determination of the functional activity of antithrombin III (ATIII) in plasma using automated analyzers for increased consumption and for monitoring substitution therapy.
Test Methodology	Chromogenic substrate, human Factor Xa	Kinetic test, thrombin-specific substrate
Reagents	Liquid	Lyophilized

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

Not applicable.

**L. Test Principle:**

The INNOVANCE™ Antithrombin assay utilizes a chromogenic measuring principle. An excess of factor Xa is added to citrated plasma. In the presence of heparin, a portion of the enzyme is complexed and inactivated by the antithrombin present in the sample. Excess, uninhibited factor Xa then cleaves a specific chromogenic substrate, causing the release of a dye. The rate of the substrate cleavage is determined by the increase in the absorbance value at 405 nm.

antithrombin + heparin  $\longrightarrow$  [antithrombin • heparin]  
 [antithrombin • heparin] + FXa (excess)  $\longrightarrow$  [antithrombin • heparin • FXa] + FXa (residual)

FXa (residual)

chromogenic FXa substrate  $\longrightarrow$  tripeptide + dye

The release of dye is inversely proportional to the inhibiting activity of the antithrombin in the plasma sample, i.e. the smaller the concentration of functionally active antithrombin, the higher the absorbance signal per time unit.

## M. Performance Characteristics (if/when applicable):

### 1. Analytical performance:

#### a. *Precision/Reproducibility:*

Precision studies were conducted with the BCS<sup>®</sup> System, as described in the CLSI Guideline EP5-A2, using Control Plasma N (control plasma in the normal range) and Control Plasma P (control plasma in the pathological range) as well as a pathological plasma pool (human plasma pool in the decision range and prepared from a minimum of 20 different individuals). Plasmas were tested in duplicate for 20 days, two runs per day (with at least a 2 hour time interval between the two runs). Analysis of variance was performed on the results.

Acceptance criteria: Within device/within laboratory CV  $\leq 10\%$ ; Repeatability CV for controls within normal range and controls in low pathological range were  $\leq 5\%$  and  $\leq 7\%$ , respectively. Between-run CV for controls within normal range and controls in the low pathological range were  $\leq 5\%$  and  $\leq 10\%$ , respectively.

Acceptance criteria were met for all three levels tested.

Sample	Precision (N = 80)		
	Mean [% of the Norm]	Repeatability CV [%]	Within-device/lab CV [%]
Control Plasma N	95.8	2.8	3.7
Control Plasma P	31.4	2.6	4.5
Pathological Plasma Pool	61.7	1.9	3.5

#### b. *Linearity/assay reportable range:*

Linearity for INNOVANCE<sup>™</sup> Antithrombin on the BCS<sup>®</sup> System was found to be from 6.0% to 127.9% within 2.9% measured maximum deviation in this interval. Verification of dilution linearity on the BCS<sup>®</sup> System was performed according to CLSI guideline EP6-A. A high pool (Control Plasma N reconstituted with 700  $\mu$ L of purified water) and a low pool (INNOVANCE<sup>™</sup> Antithrombin Buffer) were mixed according to a dilution scheme with equally spaced concentrations to establish a linear range of 9 concentration levels across the anticipated measuring range of 0 % to 150 % Antithrombin. Four replicates were used at each concentration level. The mean result of each level was compared to expected result for the same concentration level.

Statistical calculation of the relative difference between predicted values from the linear regression analysis versus those from the best fitting polynomial in the diagnostically relevant range vary from 0.48 to 2.86%, which was within the pre-defined criterion for nonlinear error of  $\leq 8\%$ .

#### c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Real Time Stability and Open Vial Stability - Three lots of INNOVANCE<sup>™</sup>

Antithrombin Reagent, INNOVANCE™ Antithrombin Substrate and INNOVANCE™ Antithrombin Buffer were tested (vials with the respective volumes of the large vial size kit OPFH 055 and of the small vial size kit OPFH 035) using Control Plasma N (CPN), Control Plasma P (CPP) and a sample in the decision range of 75% antithrombin activity of the norm. Testing of the three lots was performed over a period of 12 months and at least one month after the end of expected shelf life (day 0; 6 months, 12 months and 13 months). Vials of reagent, substrate and buffer of each of the three lots were opened and stored as claimed in the package insert. All measurements were performed in triplicate.

Acceptance criteria: All test results (mean of triplicates) must fall within the assigned ranges of the controls and of the sample with an antithrombin activity of 75% of the norm. Stability data support a shelf life of 12 months and Open Vial stability of 8 hours at +15° to +25°C and 4 weeks at +2° to +8°C.

*d. Detection limit:*

The determination of LoB for the BCS® System was performed using one blank and four low-level samples. Studies were conducted by two operators over four different days, using three different INNOVANCE Antithrombin reagent lots and one analyzer consistent with the CLSI guideline EP17-A.

Limit of blank (LoB) was derived from measuring INNOVANCE™ Antithrombin Buffer in 60 replications. Non-parametric analysis methods were used for all reagent lots. LoB was defined as 5.1%, the maximum value obtained in the three individual runs.

Limit of detection (LoD) for INNOVANCE™ Antithrombin assay on the BCS® coagulation system was determined with four low-level samples, three reagent lots and one analyzer. For the evaluation of LoD the four low level samples were measured in 15 replications each. The limit of detection (LoD) was defined as 6.0 %, the maximum value obtained in the three individual runs. The acceptance criterion  $LoD < 7\%$  was met.

*e. Analytical specificity:*

All interference studies were conducted using a normal plasma pool and a pathological plasma pool spiked with increasing concentrations of the potential interfering substance. Nine concentrations ranging from 0% to 100% of the potential interferent were measured on the BCS® / BCS® XP System for each pool. Four replicates were measured for each concentration. Successive concentrations were tested until the maximum acceptable deviation from the reference samples was observed in two consecutive concentrations. The acceptance criterion was 10% deviation from the original value obtained with a neat sample for each pool. Results obtained with interferent levels that exceeded the acceptance criterion were considered unacceptable.

Interference studies were performed for hemoglobin, bilirubin and triglycerides using the INNOVANCE™ Antithrombin reagent on the BCS® System. Acceptable results were obtained for interferent levels up to 60 mg/dL for bilirubin, 100 mg/dL for hemoglobin, and 211 mg/dL for triglycerides. Interference studies were performed for direct Factor Xa inhibitors, e.g., Tissue Factor Pathway Inhibitor (TFPI) and Fondaparinux Sodium. In addition, studies to evaluate the potential interference caused by direct thrombin inhibitors, general serine protease inhibitor and indirect Factor Xa inhibitor were conducted.

*f. Assay cut-off:*

Not applicable. Discrimination point between normal and deficient subjects in around the lower limit of the reference range (~80% of the norm).

2. Comparison studies:

*a. Method comparison with predicate device:*

The method comparison studies were conducted at three different sites, one internal and one external evaluation using the BCS® System. The studies were conducted using different populations obtained from patients at risk for thrombotic events with suspected or known thrombophilia. Fresh and frozen, male and female patient samples were analyzed by both the proposed and predicate devices in single determination. The study also included samples from normal patients and diluted samples to ensure samples were tested over the entire measuring range. A total of 284 specimens with antithrombin activity ranging from 4.8 to 131.3% of the norm were tested during different days with two lots of INNOVANCE™ Antithrombin reagent on the BCS® System and compared to the Dade Behring Berichrom™ Antithrombin III (A) reagent on the BCS® System. Results were analyzed using Passing-Bablok regression. Acceptance criteria: Coefficient of regression  $r > 0.9$ ; Slope  $> 0.9$  and  $< 1.1$ .

INNOVANCE™ Antithrombin vs. Berichrom Antithrombin AT III (A)

Study Site	N	Slope	Intercept (%)	r
Munich	109	1.000	0.800	0.982
Internal	175	1.051	1.443	0.934
Pooled MC (Munich & Internal)	284	1.040	0.340	0.944
Giessen	147	1.054	0.469	0.987

*b. Matrix comparison:*

Comparability Study between Fresh and Frozen Samples:

Comparison between fresh and frozen specimens was done externally in Munich. The following samples were measured fresh and after storage at  $< -60^{\circ}\text{C}$  for at least 7 days:

- 26 samples from patients with acquired AT deficiency
- 42 samples with normal AT deficiency
- 39 diluted samples

- 33 samples from patients under Heparin therapy

The results of the comparison are depicted in the table and graph below. Results of the regression analysis demonstrated excellent concordance between fresh and frozen samples.

Methods	n	r	r <sup>2</sup>	slope	Intercept [% of normal]
fresh vs. frozen	140	0.994	0.989	1.007	- 0.480

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):

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Fresh samples from 150 apparently healthy blood donors were measured on the BCS® System with two lots of INNOVANCE™ Antithrombin reagent. The donors ranged in age from 18 to 61 years and were comprised of 43 females and 107 males. The time between collection of blood samples and measurement did not exceed 4 hours. The mean of the reference range was 99.4 and the central 95% range (2.5<sup>th</sup> percentile and 97.5<sup>th</sup> percentile) was 82.9 – 118.2.

**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.