

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

A. 510(k) Number:

K143470

B. Purpose for Submission:

New device

C. Measurand:

Fibrinogen

D. Type of Test:

Quantitative immunoturbidimetric assay for fibrinogen

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme Fibrinogen Assay

Diazyme Fibrinogen Calibrator Set

Diazyme Fibrinogen Control Set

G. Regulatory Information:

1. Regulation section:

21CFR 864.7340, Fibrinogen determination system

2. Classification:

Class II

3. Product code:

GIS, Test, fibrinogen

GFX, Fibrinogen standard

GIL, Plasma, Fibrinogen Control

4. Panel:

81 Hematology

H. Intended Use:

1. Intended use(s):

The Dazyme Fibrinogen Assay is for the quantitative determination of fibrinogen levels in citrated human plasma. For in vitro diagnostic use only.

The Dazyme Fibrinogen Calibrator Set is intended for use in the calibration of the Dazyme Fibrinogen Assay. For in vitro diagnostic use only.

The Dazyme Fibrinogen Control Set is intended for use as quality controls for the Dazyme Fibrinogen Assay. For in vitro diagnostic use only.

The performance of this device has not been established in neonate and pediatric patient populations.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For in vitro diagnostic use only

4. Special instrument requirements:

The Dazyme Fibrinogen Assay is to be used with the Roche Modular P analyzer.

I. Device Description:

Dazyme Fibrinogen Assay System consists of the following:

1. The Dazyme Fibrinogen Assay contains reagent R1 and reagent R2. Reagent 1 contains buffer, saline, and sodium azide. Reagent 2 contains anti-human fibrinogen Ig fraction titrated to optimal concentrations dependent on sera lot and sodium azide.
2. Dazyme Fibrinogen Calibrator Set contains three levels of serum based calibrator. The calibrators are in lyophilized form containing purified human fibrinogen antigen, defibrinated human serum and sodium azide.

3. Diazyme Fibrinogen Control Set contains two levels of serum based control. The controls are in lyophilized form containing purified human fibrinogen antigen, defibrinated human serum and sodium azide.

The Diazyme Fibrinogen Assay is run on Roche Modular P analyzer. The patient's venous blood sample is first collected by a phlebotomist and then submitted to the clinical laboratory for determination of fibrinogen concentration using Diazyme Fibrinogen Assay. The Diazyme Fibrinogen Modular P application parameters are programmed into the Modular P analyzer. The reagents, calibrators and controls are loaded into the analyzer. Reagent and sample pipettes automatically aspirate and dispense specified amounts of reagent or calibrators, controls, and patient sample into reaction cells. The change in absorbance is measured at specified wavelengths.

The Roche Modular P analyzer calculates the fibrinogen concentration of a patient sample by interpolation of the obtained signal to a stored 4-point calibration curve.

J. Substantial Equivalence Information:

1. Predicate device name(s):

K-ASSAY Fibrinogen

2. Predicate 510(k) number(s):

K993482

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	The Diazyme Fibrinogen Assay is for the quantitative determination of fibrinogen levels in citrated human plasma. For in vitro diagnostic use only.	The K-ASSAY Fibrinogen Assay is intended to be used to determine the fibrinogen levels in citrated human plasma. For in vitro diagnostic use only.
Test Principle	Immunoturbidimetric	Same
Type of Test	Quantitative	Same
Specimen Type	Plasma	Same
Assay Range	100 - 900 mg/dL	Same
Reagent 2	Goat anti-human fibrinogen antiserum	Same
Calibrator Set	Lyophilized	Same
Control Set	Lyophilized	Same

Differences		
Item	Device	Predicate
Reagent 1	Phosphate Buffered Saline	Tris Buffered Saline
Calibrator Set	Three levels in 1 x 1.0 mL vials	Single-level in 1 x 1.0 mL vials
Control Set	Serum based Two levels in 1 x 1.0 mL vials	Plasma based Two levels in 5 x 1.0 mL vials

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

CLSI-EP5: Evaluation of Precision Performance of Quantitative Measurement Methods

CLSI-EP6: Evaluation of the Linearity of Quantitative Analytical Methods

CLSI-EP7: Interference Testing in Clinical Chemistry

CLSI-EP9: Measurement Procedure Comparison and Bias Estimation using Patient Samples

CLSI-EP17: Protocols for Determination of Limits of Detection and Limits of Quantitation

CLSI-EP28: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratories

L. Test Principle:

The Diazyme Fibrinogen Assay is based on an immunoturbidimetric test principle. Fibrinogen in plasma binds to specific anti-fibrinogen antibody and forms immune complexes. The degree of turbidity caused by immune complexes can be measured optically (340 nm), and is proportional to the quantity of fibrinogen in the sample. The Roche Modular P analyzer calculates the fibrinogen concentration by interpolation of the obtained signal of a 4-point calibration curve prepared from calibrators of known concentrations.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Precision Study (Reagent, Calibrator and Control):

The precision of the Diazyme Fibrinogen Assay, Calibrator and Control were evaluated according to CLSI EP5-A2 guideline on the Roche Modular P analyzer.

For reagent repeatability study, three lots of the reagents were used. For each lot of reagents, the same set of six levels of human citrated plasma specimens containing 100-115 mg/dL, 200-215 mg/dL, 300-340 mg/dL, 400-460 mg/dL, 700-720 mg/dL, and 800-860mg/dL of fibrinogen, respectively, were tested. Each sample run was

tested in duplicate, 2 runs per day for 20 working days according to CLSI EP5-A2. Within-run, between-run, between-lot, between-day, and total precision within site were calculated. The pre-established acceptance criterion, $CV \leq 8\%$, was met.

Reagent Repeatability Studies (3 lots)							
Sample	N	Mean mg/dL	Within-Run (SD, %CV)	Between-Run (SD,	Between-Lot	Between-Day	Total (SD, %CV)
Plasma 1	240	453.4	8.0, 1.8%	9.4, 2.1%	16.2, 3.6%	10.6, 2.3%	23.0, 5.1%
Plasma 2	240	333.4	5.8, 1.7%	5.2, 1.6%	10.7, 3.2%	7.4, 2.2%	15.1, 4.5%
Plasma 3	240	208.6	3.0, 1.4%	3.2, 1.5%	6.6, 3.2%	4.9, 2.4%	9.3, 4.5%
Plasma 4	240	107.3	1.6, 1.5%	1.7, 1.6%	3.6, 3.4%	2.8, 2.6%	5.2, 4.8%
Plasma 5	240	706.8	11.9, 1.7%	13.9, 2.0%	27.1, 3.8%	20.1, 2.8%	38.4, 5.4%
Plasma 6	240	823.9	16.3, 2.0%	12.7, 1.5%	30.3, 3.7%	22.3, 2.7%	42.9, 5.2%

For production calibrator and control repeatability studies, three lots of the calibrator (3 levels) and one lots of control (2 levels) were tested according to CLSI EP5-A2 guideline. Within-run, between-run, between-lot, between-day, and total precision within site were calculated. The pre-established acceptance criterion, $CV \leq 8\%$, was met.

Calibrator Repeatability Studies (3 lots)							
Sample	N	Mean mg/dL	Within-Run (SD, %CV)	Between-Run (SD, %CV)	Between-Lot (SD,%CV)	Between-Day (SD,%CV)	Total (SD, %CV)
Level 1	360	262.0	5.7, 2.2%	0, 0%	13.3, 5.1%	12.1, 4.6%	18.8, 7.2%
Level 2	360	578.0	17.9, 3.1%	0, 0%	27.9, 4.8%	22.5, 3.9%	40.1, 6.9%
Level 3	360	1130.1	21.2, 1.9%	0, 0%	44.9, 4.0%	40.4, 3.6%	64.0, 5.7%

Control Repeatability Studies (1 lot of Control and 2 lots of Reagent)							
Sample	N	Mean mg/dL	Within-Run (SD, %CV)	Between-Run (SD, %CV)	Between-Lot (SD,%CV)	Between-Day (SD,%CV)	Total (SD, %CV)
Level 1	160	173.2	2.3, 1.3%	1.4, 0.8%	4.1, 2.4%	3.1, 1.8%	4.1, 2.4%
Level 2	160	607.5	8.2, 1.4%	6.5, 1.1%	15.1, 2.5%	11.1, 1.8%	15.2, 2.5%

Reproducibility Study (Reagent, Calibrator and Control):

A multi-site precision study was performed at two external sites and one internal site on the Modular P analyzer. Six citrated plasma samples, containing one sample close to the very low end of the analytical measuring range (AMR) and one sample close to the very high end of the AMR, were tested in duplicates per run, two runs per day for five nonconsecutive working days. One lot of reagents was tested across three testing sites by three operators on three Modular P analyzers. Additionally, three lots of calibrators and three lots of controls were tested in duplicates per run, two runs per day for five nonconsecutive working days with one lot of reagents at three sites by three operators on three Modular P analyzers. Within-run, between-run, between-lot, between-day, between-site, and total precision were calculated. The pre-established

acceptance criteria, $CV \leq 8\%$ for within-run, between-run, between-lot, between day, within site and between-site and $CV \leq 10\%$ for total, were met.

Reproducibility Within-run Study (3 Sites)									
Sample	Lots	N	Mean mg/dL	Site 1		Site 2		Site 3	
				SD	%CV	SD	%CV	SD	%CV
Plasma 1	1	20	441.1	11.4	2.8%	9.33	2.5%	3.7	0.8%
Plasma 2	1	20	323.9	6.6	2.2%	5.29	1.9%	5.3	1.6%
Plasma 3	1	20	205.4	3.6	1.8%	1.9	1.0%	2.7	1.3%
Plasma 4	1	20	107.4	0.8	0.8%	1.55	1.6%	1.2	1.2%
Plasma 5	1	20	709.5	22.9	3.2%	26.47	3.9%	7.9	1.1%
Plasma 6	1	20	822.7	9.2	1.1%	11.0	1.3%	18.7	2.3%
Calibrator 1	3	60	250.7	3.3	1.3%	4.1	1.7%	3.9	1.5%
Calibrator 2	3	60	502.1	12.6	2.5%	6.6	1.3%	12.8	2.5%
Calibrator 3	3	60	1077.3	17.3	1.7%	16.7	1.6%	17.2	1.5%
Control 1	3	60	179.4	2.1	1.2%	2.5	1.4%	4.6	2.5%
Control 2	3	60	609.6	14.6	2.4%	15.3	2.5%	8.6	1.4%

Reproducibility Between-run Study (3 Sites)									
Sample	Lots	N	Mean mg/dL	Site 1		Site 2		Site 3	
				SD	%CV	SD	%CV	SD	%CV
Plasma 1	1	20	441.1	4.9	1.2%	10.86	2.9%	13.56	3.1%
Plasma 2	1	20	323.9	0.0	0.0%	5.27	1.9%	4.17	1.3%
Plasma 3	1	20	205.4	0.0	0.0%	3.07	1.7%	0.00	0.0%
Plasma 4	1	20	107.4	0.7	0.7%	0.82	0.9%	1.40	1.3%
Plasma 5	1	20	709.5	33.4	4.7%	0.0	0.0%	17.63	2.5%
Plasma 6	1	20	822.7	10.7	1.3%	11.2	1.4%	13.56	3.1%
Calibrator 1	3	60	250.7	3.2	1.3%	9.9	4.1%	1.2	0.5%
Calibrator 2	3	60	502.1	4.7	1.0%	11.6	2.3%	14.1	2.8%
Calibrator 3	3	60	1077.3	19.1	1.8%	19.9	1.9%	0	0%
Control 1	3	60	179.4	2.9	1.6%	2.6	1.5%	0	0%
Control 2	3	60	609.6	9.4	1.6%	13.6	2.3%	14.2	2.3%

Reproducibility Between-day Study (3 Sites)									
Sample	Lots	N	Mean mg/dL	Site 1		Site 2		Site 3	
				SD	%CV	SD	%CV	SD	%CV
Plasma 1	1	20	441.1	9.5	2.4%	SD	%CV	0.00	0.0%
Plasma 2	1	20	323.9	5.0	1.7%	8.21	2.2%	7.79	2.4%
Plasma 3	1	20	205.4	3.3	1.7%	2.52	0.9%	6.08	3.0%
Plasma 4	1	20	107.4	1.0	1.0%	0.6	0.3%	3.25	3.0%
Plasma 5	1	20	709.5	24.1	3.4%	1.55	1.6%	4.36	0.6%
Plasma 6	1	20	822.7	0.0	0.0%	28.76	4.2%	0.00	0.0%
Calibrator 1	3	60	250.7	7.3	3.0%	2.5	1.0%	10.7	4.1%
Calibrator 2	3	60	502.1	14.2	2.8%	17.0	3.4%	8.6	1.7%
Calibrator 3	3	60	1077.3	15.0	1.4%	35.2	3.3%	21.7	1.9%
Control 1	3	60	179.4	4.2	2.4%	5.0	2.8%	5.6	3.0%
Control 2	3	60	609.6	19.4	3.2%	21.9	3.6%	0.00	0.0%

Reproducibility Within-site (3 Sites)									
Sample	Lots	N	Mean mg/dL	Site 1		Site 2		Site 3	
				SD	%CV	SD	%CV	SD	%CV
Plasma 1	1	20	408.5	10.2	3%	8.8	2%	27.5	7%
Plasma 2	1	20	303.1	6.2	2%	2.3	1%	21.1	7%
Plasma 3	1	20	195.5	2.9	2%	1.8	1%	9.6	5%
Plasma 4	1	20	100.0	1.2	1%	1.1	1%	5.0	5%
Plasma 5	1	20	701.5	22.4	3%	23.8	3%	29.9	4%
Plasma 6	1	20	818.4	13.6	2%	7.7	1%	9.1	1%
Calibrator 1	3	60	250.7	3.8	2%	6.0	2%	11.7	5%
Calibrator 2	3	60	502.1	11.0	2%	10.9	2%	14.0	3%
Calibrator 3	3	60	1077.3	17.1	2%	14.9	1%	47.0	4%
Control 1	3	60	179.4	3.2	2%	1.9	1%	6.1	3%
Control 2	3	60	609.6	13.2	2%	12.6	2%	16.4	3%

Reproducibility Sites Combined and Total												
Sample	N	Mean mg/dL	With-run		Between-run		Between-day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma 1	60	408.5	10.2	3%	8.8	2%	27.5	7%	29.9	7.3%	42.8	10 %
Plasma 2	60	303.1	6.2	2%	2.3	1%	21.1	7%	21.6	7.1%	30.9	10 %
Plasma 3	60	195.5	2.9	2%	1.8	1%	9.6	5%	9.9	5.1%	14.2	7%
Plasma 4	60	100.0	1.2	1%	1.1	1%	5.0	5%	5.1	5.1%	7.4	7%
Plasma 5	60	701.5	22.4	3%	23.8	3%	29.9	4%	43.9	6.2%	62.2	9%
Plasma 6	60	818.4	13.6	2%	7.7	1%	9.1	1%	18.0	2.2%	25.5	3%
Calibrator1	180	250.7	3.8	2%	6.0	2%	11.7	5%	13.6	5.4%	19.3	8%
Calibrator2	180	502.1	11.0	2%	10.9	2%	14.0	3%	20.8	4.1%	29.5	6%
Calibrator3	180	1077.3	17.1	2%	14.9	1%	47.0	4%	51.8	4.8%	73.5	7%
Control 1	180	179.4	3.2	2%	1.9	1%	6.1	3%	7.1	4.0%	10.0	6%
Control 2	180	609.6	13.2	2%	12.6	2%	16.4	3%	24.4	4.0%	34.6	6%

b. Linearity/assay reportable range:

A linearity study was conducted following CLSI EP6-A for Dazyme Fibrinogen Assay on the Modular P analyzer. A human serum sample was spiked with fibrinogen stock solution to a final concentration of 1032.8 mg/dL Fibrinogen. The final concentration of the high sample pool was determined using Dazyme Fibrinogen assay with triplicate analysis. The prepared high sample pool was diluted with saline to create the linearity samples.

The linearity samples were tested with Dazyme Fibrinogen Assay in triplicate and the results are provided below.

Sample Recovery Results							
Level	Recovery Triplicate mg/dL			Recover Mean mg/dL	Expected Value mg/dL	Error mg/dL	% Recovery
0	0	0.7	0.0	0.4	0.0	-0.37	NMA
1	108.1	110.1	103.3	109.1	103.3	-5.82	105.63%
2	198.7	197.9	206.6	197.1	206.6	9.43	95.43%
3	301.1	296.2	309.9	296.3	309.9	13.55	95.63%
4	380.1	385.8	413.1	385.9	413.1	27.27	93.40%
5	533.7	521.3	516.4	527.1	516.4	-10.68	102.07%
6	622.1	622.9	619.7	620.7	619.7	-1.00	100.16%
7	740.1	716.6	723.0	728.0	723.0	-5.02	100.69%
8	859	844.6	826.3	847.0	826.3	-20.70	102.51%
9	940.1	954.4	929.6	952.9	929.6	-23.35	102.51%
10	1025.6	1035. 4	1032.8	1032.8	1032.8	0.00	100.00%

The results showed that the best fitting model for Diazyme Fibrinogen Assay is linear up to 1032.8 mg/dL and the recovery mean values for each level were within 10% of the expected values. The AMR of Diazyme Fibrinogen assay is claimed to be 100–900 mg/dL.

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

Traceability:

Master Calibrator (reference calibrator) Preparation and Value Assignment

- i. The reference calibrators were prepared by spiking fibrinogen to diluted human serum to target concentrations. The value point of the three levels of the calibrators is set across the reportable range. The highest calibrator is set to close to the upper measuring range. Since the calibration curve is non-linear, manufacturing targets for each level is designed for best curve fit.

The initial value of the fibrinogen at each level was assayed by the predicate method, Kamiya Biomedical K-Assay Fibrinogen. The initial assigned value of the master calibrators was finalized after verification testing with WHO fibrinogen reference materials NIBSC code 09/242. The WHO fibrinogen reference material was used to prepare the stock solution (1500 mg/dL) and three diluted fibrinogen solutions (375, 500, and 750 mg/dL). After calibration with the Diazyme Fibrinogen Calibrator Set, the reference material dilutions were tested with the Diazyme Fibrinogen Assay in duplicate on the Modular P analyzer. The result showed that the WHO reference material dilutions recovered within $\pm 10\%$ of the target values. Therefore, Diazyme Fibrinogen Calibrators of the Diazyme Fibrinogen Assay are traceable to WHO reference material NIBSC code 09/242.

Master Calibrator Value Assignment Data						
Sample	Expected mg/dL	Dilution Factor	Replicate 1 mg/dL	Replicate 2 mg/dL	Mean mg/dL	% Recovery
09/242	375	4	365.0	362.1	363.6	96.9%
09/242	500	3	524.1	512.4	518.3	103.7%
09/242	750	2	773.5	771.9	772.7	103.0%
09/242	1500	No dilution				

Production Calibrator Preparation and Value Assignment

- ii. Production calibrator and value assignment is prepared from a calculated amount of human fibrinogen stock solution is added into a diluted serum base to target concentrations. The value point of the three levels of the calibrators is set across the reportable range. The highest calibrator is set close to the upper measuring range. Since the calibration curve is non-linear, manufacturing targets for each level is designed for best curve fit. The production calibrators are value assigned by use of master calibrator and Diazyme reagent with multiple testing runs in three days.

The mean value of each level was used to calibrate Diazyme Fibrinogen Assay.

Production Control Preparation and Value Assignment

Production controls are prepared by spiking fibrinogen stock solution to a diluted human serum to the target fibrinogen concentrations. For the level 1 (normal) control the manufacturing target is approximately 180 mg/dL. For level 2 (abnormal) control the manufacturing target is approximately 600 mg/dL. The Diazyme Fibrinogen Assay and calibrators are used in replicate analysis to determine the mean value of the newly prepared controls. Testing was performed with one Modular P analyzer in two runs in duplicate for three days. The final value is the mean of replicate values and the expected range is calculated as $\pm 15\%$ from the mean value for the respective controls.

Stability:

Reagent Shelf-life Stability:

Three lots of Diazyme Fibrinogen Assay reagents were used to determine shelf-life stability. The reagents from each lot were kept in incubators at 37°C and 25°C for the accelerated stability study and 2-8°C for the real-time stability study. At the indicated time, the Diazyme Fibrinogen Assay reagents were removed from the incubators and evaluated by testing six citrated human plasma samples containing fibrinogen covering the claimed AMR after each calibration. The acceptance criterion was sample value recovery within 10% deviation from the results at day 0.

Reagent Accelerated Stability (37°C/3 lots)												
Day	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	Res	Rec	Res	Rec	Res	Rec	Res	Rec	Res	Rec	Res	Rec
0	452.	99.7	327.9	98.4	206.3	98.9	105.9	98.7	703.8	99.6	823.3	99.9
3	456.	100.6	323.6	97.1	200.3	96.0	102.9	95.9	731.2	103.5	869.5	105.5
7	442.	97.5	323.3	97.0	203.4	97.5	103.5	96.5	683.5	96.7	796.8	96.7
11	436.	96.4	321.5	96.5	205.8	98.6	105.3	98.1	670.9	94.9	798.3	96.9
14	442.	97.5	327.2	98.2	207.2	99.3	106.7	99.4	676.2	95.7	801.1	97.2
15	426.	94.1	315.6	94.7	200.9	96.2	102.1	95.1	684.1	96.8	815.3	99.0

Res: Result (mg/dL)

Rec: Recovery (%)

Reagent Accelerated Stability (25°C/3 lots)												
Months	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	Res	Rec	Res	Rec	Res	Rec	Res	Rec	Res	Rec	Res	Rec
0	426.	94.1	319.0	95.7	197.8	94.8	103.5	96.5	709.3	100.4	831.7	100.9
1	445.	98.2	329.4	98.8	208.1	99.7	107.1	99.8	686.0	97.1	795.7	96.6
3	415.	91.6	303.8	91.1	192.1	92.0	98.4	91.7	666.7	94.3	782.3	94.9

Res: Result (mg/dL)

Rec: Recovery (%)

The results demonstrated that the reagents were stable for 14 days when stored at 37°C and stable for over 3 months when stored at 25°C. According to an Arrhenius equation the reagent shelf-life is calculated to 18 months when kept at 2-8°C. The real time stability at 2-8°C is on-going and final results will be reported.

Reagent On-Board Stability on Roche Modular P:

Three lots of reagents were evaluated for on-board stability on the Roche Modular P analyzer. The reagents were kept in the Roche Modular reagent bottle in the 2-8 °C chamber of the instrument. In this study, six citrated human plasma samples were tested after each calibration on the indicated days. The corresponding testing days and results are listed in the table below. The acceptance criterion was sample value recovery within 10% deviation from day 0.

Reagent On-Board Stability (2-8°C/3 lots)												
Day	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	Res	Rec	Res	Rec	Res	Rec	Res	Rec	Res	Rec	Res	Rec
0	426.	94.1	319.0	95.7	197.8	94.8	103.5	96.5	709.3	100.4	831.7	100.9
5	452.	99.7	327.1	98.1	200.3	96.0	101.8	94.9	755.6	106.9	872.6	105.9
10	440.	97.2	327.0	98.1	208.4	99.8	106.6	99.3	676.5	95.7	803.2	97.5
15	448.	98.9	325.5	97.6	205.9	98.7	105.2	98.0	688.8	97.5	821.4	99.7
21	445.	98.2	329.4	98.8	208.1	99.7	107.1	99.8	686.0	97.1	795.7	96.6
24	436.	96.2	323.6	97.1	204.8	98.1	104.7	97.5	677.6	95.9	796.0	96.6
25	439.	96.9	319.2	95.8	201.6	96.6	102.1	95.1	688.6	97.4	799.3	97.0

Res: Result (mg/dL)

Rec: Recovery (%)

Based on the results above, the reagent is stable for 20 days on board the Roche Modular P analyzer.

Calibrator and Control Stability Test:

Accelerated Stability at 37°C

Three lots of the Diazyme Fibrinogen Calibrators and two lots of the Controls were kept in a 37°C incubator. At the indicated time, the calibrators and Controls were removed, reconstituted, and tested as samples with Diazyme Fibrinogen Assay reagent and master calibrators stored at 2-8°C on the Roche Modular P analyzer. The acceptance criterion was calibrator and control value recovery within 10% deviation from day 0.

Calibrator and Controls Accelerated Stability Study (37°C, 3 lots)										
Day	Calibrator						Control			
	Level 1		Level 2		Level 3		Level 1		Level 2	
	Res	Rec	Res	Rec	Rec	Rec	Res	Rec	Rec	Rec
0	253.	100.0	556.9	100.0	1098.	100.0	176.2	100.0	675.6	100.0
3	259.	102.7	562.1	100.9	1177.	107.1	184.0	104.4	686.6	101.6
7	260.	103.0	531.9	95.5	1062.	96.6	186.1	105.7	650.5	96.3
11	260.	103.3	528.7	94.9	1101.	100.2	186.4	105.8	669.2	99.1
14	262.	103.6	531.5	95.4	1121.	102.0	189.3	107.5	645.6	95.6
15	263.	104.2	533.6	95.8	1155.	105.2	184.1	104.5	658.7	97.5

Res: Result (mg/dL)

Rec: Recovery (%)

Results demonstrated that the calibrators and controls were stable for 14 days when stored at 37°C. According to an Arrhenius equation the reagent shelf-life is calculated to 18 months when kept at 2-8°C.

Real Time Stability (2-8°C)

The real time stability studies at 2-8°C for calibrators and controls are on-going and the final results will be reported. The acceptance criterion is calibrator and control value recovery within 10% deviation from day 0.

Reconstitution Stability Assay at 2-8°C

Three lots of the Diazyme Fibrinogen Calibrators and two lots of Controls were reconstituted and kept in a 2-8°C incubator. At the indicated time, the calibrators and Controls were removed and tested as samples with Diazyme Fibrinogen reagent (one lot) and master calibrators stored at 2-8°C.

Calibrator and Control Reconstitution Stability Study (2-8°C, 3 lots)										
Day	Calibrator						Control			
	Level 1		Level 2		Level 3		Level 1		Level 2	
	Res	Rec	Res	Rec	Rec	Rec	Res	Rec	Rec	Rec
0	255.	100.0	556.9	100.0	1115.	100.0	182.8	100.0	675.5	100.0
3	263.	103.0	570.9	102.5	1185.	106.3	188.5	103.1	680.8	100.8
5	266.	104.3	550.8	98.9	1159.	104.0	188.8	103.2	675.2	100.0
7	259.	101.6	524.0	94.1	1015.	91.0	185.7	101.5	625.9	92.6
8	251.	98.4	526.7	94.6	1008.	90.4	187.2	102.4	622.9	92.2

Res: Result (mg/dL)

Rec: Recovery (%)

The results listed in the table above demonstrated that the calibrators and controls were stable for 7 days after reconstitution and stored at 2-8°C.

Sample stability (2-8°C real-time stability)

Six citrated plasma samples covering the whole AMR were stored at 2-8°C for one and two days and used for a real-time patient sample stability study. At the indicated time points, samples were tested with Diazyme Fibrinogen Assay on the Roche Modular P analyzer. The results are listed in the table below.

Patient Sample Real-time Stability Data						
Sample ID	0 Days		1 Days		2 Days	
	Results mg/dL	Recovery %	Results mg/dL	Recovery %	Results mg/dL	Recovery %
Plasma 1	420.0	100%	428.6	102%	453.40	108%
Plasma 2	294.8	100%	311.0	105%	319.60	108%
Plasma 3	204.7	100%	205.2	100%	206.6	101%
Plasma 4	103.5	100%	107.7	104%	107.4	104%
Plasma 5	722.3	100%	699.5	97%	717.00	99%
Plasma 6	826.3	100%	818.6	99%	846.50	102%

After storage of 1 day and 2 days, all six samples recovered within 10% deviation from day 0. The pre-determined acceptance criterion was met.

Patient Sample Freeze-Thaw Stability

Six fresh citrated plasma samples covering the whole AMR were tested before and after one and two freeze-thaw cycles with one lot Diazyme Fibrinogen Assay reagent on the Roche Modular P analyzer. The acceptance criterion was the recovery after freeze within 10% deviation from before freezing. Results demonstrated that all six patient samples were not affected by one freeze-thaw cycle.

Patient Sample Freeze-Thaw Stability Data								
Sample ID	Before Freeze mg/dL	Recovery	Freeze/Thaw Cycle 1 mg/dL	Recovery	Pass/Fail	Freeze/Thaw Cycle 2 mg/dL	Recovery	Pass/Fail
Sample 1	444.5	100%	445.8	100%	Pass	471.9	106%	Pass
Sample 2	321.7	100%	316.1	98%	Pass	330.6	103%	Pass
Sample 3	199.0	100%	203.0	102%	Pass	211.1	106%	Pass
Sample 4	102.9	100%	102.8	100%	Pass	105.2	102%	Pass
Sample 5	683.7	100%	689.4	101%	Pass	728.8	107%	Pass
Sample 6	819.8	100%	802.1	98%	Pass	826.4	101%	Pass

d. *Detection limit:*

The Limit of Blank (LoB) and Limit of Detection (LoD) of the Diazyme Fibrinogen Assay were determined by testing on Roche Modular P analyzer with three lots of reagents and three lots of calibrators according to CLSI EP17-A2. True blank is 7.5% bovine serum albumin (BSA) solution in saline with 60 replicates for LoB determination. To determine LoD, five low level positive plasma samples were diluted with 7.5% BSA in 0.9% saline to a targeted low sample range using 12 replicates.

$$\text{LoD} = \text{LoB} + (1.645 \times \text{SD of LoD low sample})$$

The LoQ of the Diazyme Fibrinogen Assay was determined by following CLSI EP17-A2. Five citrated plasma samples were diluted with 7.5% BSA to concentrations of 1 to 20 times of assumed LoB. The diluted citrated plasma samples were tested with three lots of the Diazyme Fibrinogen Assay reagents on the Roche Modular P analyzer. The results are summarized below.

LoB, LoD and LoQ (3 lots reagents)		
LoB	LoD	LoQ
2.15 mg/dL	5.08 mg/dL	12.9 mg/dL

e. *Analytical specificity:*

- i. To determine the level of interference from substances present in citrated plasma, the Diazyme Fibrinogen Assay was used to test three citrated plasma samples, which contain low (100 mg/dL), medium (300 mg/dL), and high (800 mg/dL) fibrinogen concentrations spiked with various concentrations of substances according to CLSI EP7-A2 guideline. Each spiked sample was tested in triplicate on the Roche Modular P analyzer using one lot of Diazyme Fibrinogen Assay reagent and one lot of calibrator. The acceptance criterion was the difference of mean fibrinogen concentration between the spiked sample and the un-spiked sample $< \pm 10\%$.

The common endogenous and exogenous interfering substances and their interference results are listed below and showed no significant interference ($< \pm 10\%$) up to the indicated concentration for all three tested citrated plasma samples at 100 mg/dL, 300 mg/dL and 800 mg/dL, respectively. The

additional interference studies for anticoagulant medications were also performed.

Endogenous Substances	
Interferent	Concentration
Ascorbic Acid	176 mg/dL
Bilirubin	40 mg/dL
Bilirubin Conjugated	40 mg/dL
Hemoglobin	1000 mg/dL
Rheumatoid Factor	220 IU/ml
Triglycerides	1000 mg/dL

Exogenous Substances	
Interferent	Concentration
Acetylsalicylic Acid	2.78 mM
Na ₂ -Cefoxitin	1554 µM
Ibuprofen	2438 µM
Unfractionated Heparin	3000 U/L
Low Molecular Weight Heparin	3000 U/L

Additional Interference	
Interference	Concentration
Warfarin	65 µM
Dabigatran	3.7 µg/mL
Hirudin	25 µg/mL
Rivaroxaban	7.0 µg/mL
Fibrin Degradation Product	0.5 mg/mL
Argatroban	20 µg/mL

ii. High Dose Hook Effect

To test for High Dose “Hook Effect” associated with the fibrinogen assay, an 11-level linearity set was prepared with the highest level containing 1426.0 mg/dL fibrinogen. The linearity set prepared was first tested with Diazyme Fibrinogen Assay on the Roche Modular P in triplicate with the normal dilution ratio of 1:20. The value recovery data is shown below.

Hook Effect, 1:20 Dilution							
Level	rep 1 mg/dL	rep 2 mg/dL	rep 3 mg/dL	Average mg/dL	Expected mg/dL	Error mg/dL	% Error
1	25.6	22.8	21.8	23.4	22.8	0.58	-2.50%
2	52.5	49.4	51.5	51.1	47.5	3.60	-7.04%
3	98.1	102.6	99.9	100.2	95.1	5.14	-5.13%
4	187.6	190.6	191.5	189.9	190.1	-0.23	0.12%
5	269.8	289.7	278.9	279.5	285.2	-5.72	2.05%
6	365.2	356.0	354.4	358.5	380.3	-21.72	6.06%
7	471.3	451.7	451.0	458.0	475.3	-17.32	3.78%
8	700.1	695.2	696.1	697.1	713.0	-15.84	2.27%
9	929.5	932.6	937.4	933.2	950.6	-17.47	1.87%
10	1152.3	1171.0	1156.7	1160.0	1188.3	-28.30	2.44%
11	1393.2	1320.7	1376.7	1363.5	1426.0	-62.42	4.58%

Due to the solubility limit of fibrinogen antigen, extremely high concentrations of the analyte could not be stably prepared. The same linearity set prepared above was tested again with a 1:5 sample dilution. The test results showed that the sample containing theoretical fibrinogen concentration of 4990 mg/dL was recovered as 1715.1 mg/dL. This value was still greater than the upper end of the AMR of 900 mg/dL. Therefore, the Diazyme Fibrinogen Assay demonstrated no hook effect at least at 4990 mg/dL Fibrinogen concentration.

Hook Effect, 1:5 Dilution							
Level	rep 1 mg/dL	rep 2 mg/dL	rep 3 mg/dL	Average mg/dL	Expected mg/dL	Error mg/dL	% Error
12	84.5	88.1	87.9	86.8	79.9	7.0	-8.04%
13	196.8	182.4	170.4	183.2	166.4	16.8	-9.19%
14	315.8	328.9	324.4	323.0	332.7	-9.7	3.00%
15	671.0	666.2	687.2	674.8	665.5	9.4	-1.39%
16	979.9	1062.9	1046.4	1029.7	998.2	31.6	-3.07%
17	1195.1	1254.4	1339.9	1263.1	1330.9	-67.8	5.36%
18	1525.9	1453.5	1442.0	1473.8	1663.6	-189.8	12.88%
19	1789.9	1791.5	1826.6	1802.7	2495.4	-692.8	38.43%
20	1855.5	1866.7	1884.8	1869.0	3327.2	-1458.2	78.02%
21	1835.9	1856.8	1827.2	1840.0	4159.0	-2319.1	126.04%
22	1718.0	1707.0	1720.2	1715.1	4990.8	-3275.8	191.00%

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

To demonstrate accuracy, one lot of Diazyme Fibrinogen Assay reagent was tested on the Roche Modular P analyzer with one lot of the Diazyme Fibrinogen calibrator on

individual citrated plasma samples from the intended target population. One lot of Kamiya K-Assay Fibrinogen reagent was used as the predicate and tested on the Hitachi 917 on the same citrated plasma samples. CLSI EP9-A3 was used as a guideline for the method comparison studies. All samples were tested in singlet with both methods. Sample demographic information was included as provided by the collection center. All samples were collected in 3.2% citrate tubes.

A total of 176 unique samples were tested across the three sites and the acceptance criteria were: Slope = 1.0 ± 0.1 ; $r^2 \geq 0.95$; y-intercept < LoQ. The comparison results obtained by both methods are tabulated below:

Regression Analysis (Regular Linear)				
Parameter	Site 1	Site 2	Site 3	Total
N	59	59	58	176
Slope	0.972	0.983	1.004	0.986
95% CI	0.953 – 0.992	0.965 – 1.000	0.984 – 1.023	0.975 – 0.997
Intercept	7.20	1.54	-0.41	2.72
95% CI	-1.03 – 15.43	-5.40 – 8.48	-8.18 – 7.37	-1.70 – 7.13
Standard Error of Estimate	15.69	13.74	15.75	15.31
Correlation Coefficient(R)	0.9972	0.9978	0.9973	0.9973
R ²	0.9944	0.9956	0.9946	0.9946
Sample Range (Diasyme)	119.6 – 825.6	107.8 – 869.9	107.5 – 870.0	107.5 – 870.0

Regression Analysis (Deming Regression)				
Parameter	Site 1	Site 2	Site 3	Total
n	59	59	58	176
Slope	0.975	0.985	1.006	0.989
95% CI	0.956 – 0.994	0.968 – 1.002	0.987 – 1.026	0.978 – 0.999
Intercept	6.22	0.81	-1.31	1.80
95% CI	-2.01 – 14.45	-6.14 – 7.75	-9.09 – 6.48	-2.68 – 6.22
Standard Error of Estimate	15.70	13.75	15.76	15.32
Correlation Coefficient(R)	0.9972	0.9978	0.9973	0.9973
R ²	0.9944	0.9956	0.9946	0.9946
Sample Range (Diasyme)	119.6 – 825.6	107.8 – 869.9	107.5 – 870.0	107.5 – 870.0

The method comparison study results met acceptance criteria.

b. Matrix comparison:

Not applicable

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):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

To establish the reference interval for the Dazyme Fibrinogen Assay, citrated plasma samples from 120 apparently healthy adults were tested in singlet using the Dazyme Fibrinogen Assay on the Roche Modular P analyzer according to CLSI C28-A3 guideline. The established reference interval is from 130.7 to 397.5 mg/dL.

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 completed and supports a substantial equivalence decision.