

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

K051054

B. Purpose for Submission:

Clearance of new device

C. Analyte:

Factor IX (Christmas Factor)

D. Type of Test:

ELISA

E. Applicant:

Affinity Biologicals™ Inc

F. Proprietary and Established Names:

VisuLize™ Factor IX Antigen Kit

G. Regulatory Information:

1. Regulation section:
21 CFR 864.7290
2. Classification:
Class II
3. Product Code:
GGP
4. Panel:
81

H. Intended Use:

1. Intended use(s):
The VisuLize™ Factor IX Antigen kit is an *in vitro* diagnostic Enzyme Immunoassay for the quantitative determination of Factor IX antigen in human plasma and Factor IX concentrates using the double antibody enzyme linked immuno-sorbent assay (ELISA).
2. Indication(s) for use:
3. Special condition for use statement(s):
4. Special instrument Requirements:

I. Device Description:

The VisuLize™ Factor IX Antigen Assay is a capture/detection double antibody sandwich assay. The kit is comprised of a 96 well microtiter plate coated with a goat polyclonal capture antibody to human Factor IX, a goat polyclonal anti-human Factor IX detection antibody conjugated to horseradish peroxidase, TMB substrate, Calibrator, Normal and Abnormal low controls, Sulphuric acid stopping solution, and associated diluents and buffers .

J. Substantial Equivalence Information:

1. Predicate device name(s):
Diagnostica Stago Asserachrom IX:AG
2. Predicate K number(s):
K854312
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Quantitative determination of Factor IX	same
Traceability of Calibrator	Calibrator is standardized against a secondary standard traceable to the WHO International Standard for Factor IX Activity 99/826	same
Sample Matrix	Plasma from blood collected into 3.2% buffered citrate	same
Differences		
Item	Device	Predicate
Expression of FIX level	IU/mL	% of normal
Incubation Times	70 mins	273 mins

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

NCCLS C28-A2, How to Define and Determine Reference Intervals in the Clinical Laboratory, Vol 15 No.4 (6/1/2000)

NCCLS EP5-T2, User Evaluation of Precision Performance of Clinical Chemistry Devices, Vol 19 No. 2 (2/1/1999)

NCCLS EP7-P, Interference Testing in Clinical Chemistry, Vol 6 No.13 (8/1/1996)

NCCLS EP17-P, Protocols for Determination of Limits of Detection and Limits of Quantitation, Vol 24 No.10 (2/1/1999)

CEN EN 13640:2002 (03/02) Stability Testing of in vitro Diagnostic Reagents

L. Test Principle:

When previously diluted plasma samples are applied to the microwell plate, any Factor IX in the sample will bind to the capture antibody coated on the microwell. Unbound material is then washed away, and the detection antibody, is added to the wells and incubated. The unbound detection antibody is washed away, and the TMB is added. After another incubation, the reaction is stopped and read at 450 nm. The intensity of the color produced is directly proportional to the concentration of Factor IX in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra- and Inter-assay precision was determined for 3 validation lots. The two controls supplied with each validation lot were tested across four plates from each lot (40 replicates per sample per plate). Intra-assay precision was also assessed by testing 3 plasma samples at different levels of Factor IX. 8 replicates of each sample were run over 10 days, by 2 operators for an n = 20. Method 1 reported a mean CV of 4.74% and Method 2 reported a mean CV of 4.70%.

Inter-assay intra-lot precision was determined by testing 8 replicates of 3 plasma samples over 10 days, by 2 operators, for a n=20. %CV were calculated according to NCCLS EP5-A. The mean inter-assay intra lot CV was 4.88%.

Precision between lots was determined by testing 10 different in-house controls on 3 Validation lots. The mean inter-assay inter-lot for Factor IX is 2.81%.

b. *Linearity/assay reportable range:*

Linearity was evaluated by analyzing calibration curves prepared from the Calibrator Plasma. Dilutions were tested in duplicated by 2 operators on 5 plates per operator in three validation lots. Each of the 3 validation lots demonstrated good linearity ($r^2 \geq 0.990$).

c. *Traceability (controls, calibrators, or method):*

The calibrator plasma is standardized against an internal secondary standard that is traceable to the WHO International Standard for Factor IX activity (99/826). Value assignment for each lot of

calibrator plasma is determined by testing 21 vials at four dilutions each by two operators and calculation the mean value of the determinations.

Control A is a normal control prepared from a frozen pool of citrated plasma from healthy donors. Control, B is an abnormal low control prepared from a frozen pool of Factor IX deficient plasma and normal plasma with a known Factor IX level. The normal plasma is added to the deficient plasma to bring the Factor IX value to within the target range. The calibrator plasma is used to assign values to the control plasma. Ranges are assigned to the control plasmas by testing 21 vials of each at 2 dilutions by 2 operators and calculating the mean \pm 2 stand deviations.

d. Detection limit:

2 different methods were used to determine the lower limit of the detection range. In method 1- the calibrator plasma was serially diluted 1/100 to 1/51200 ((point curve). The lower limit of detection was determined to be the lowest amount of Factor IX that could be distinguished from the blank by a difference of 3 std dev.

With method 2, 3 samples containing \sim 0.005 IU/mL Factor IX were prepared by mixing normal plasma with a known Factor IX activity level with Factor IX deficient plasma . % replicated per sample was analyzed. The limit of detection was calculated according to NCCLS EP-17-P.

Results from both methods indicate that the lower limit of detection is 0.005 IU/mL.

The upper limit of detection would be two times the value of the calibrator in the kit.

e. Analytical specificity:

Assay interference was determined by determining the effect of acetaminophen, acetylsalicylic acid, caffeine, ethanol, ibuprofen, nicotine, ϵ -aminocaproic acid, bilirubin, L-ascorbic acid, bicarbonate, hemoglobin, lipemia, albumin, heparin, and thymol, on the recovery of Factor IX in samples containing Factor IX. The testing was conducted according to NCCLS EP7-P. Results demonstrated no reproducible interference. Results demonstrated that high amounts of heterophilic antibodies such as lupus anticoagulant or rheumatoid factor cannot be excluded, and is indicated in the labeling.

f. Assay cut-off:

2. Comparison studies:a. *Method comparison with predicate device:*

A 3 site (One internal, two external) clinical study was performed. Samples used were commercially purchased and previously assayed by an independent activity assay. All samples were run in duplicate according to the product insert instructions for each kit. At the internal site each sample was tested in duplicate on 3 validation lots of the VisuLize™ Factor IX Antigen and in the predicate.

Internal Testing –Lot 1 vs lot 2 $y=0.99921x + 0.0031$, $r=0.9901$

Lot 2 vs lot 3 $y=0.9614x - 0.0013$, $r=0.9892$

Lot 1 vs lot 3 $y=1.0138x + 0.0164$, $r=0.9833$

	Internal Site	Site 1	Site 2
n	134	114	109
Pearson Product Moment correlation coef (r)	0.987	0.976	0.982
p-value (single factor ANOVA)	0.528	0.458	0.228
Regression		$y= 0.8591 +0.0385$	$y=1.1066+0.0259$

b. *Matrix comparison:*3. Clinical studies:a. *Clinical sensitivity:*b. *Clinical specificity:*c. *Other clinical supportive data (when a and b are not applicable):*4. Clinical cut-off:5. Expected values/Reference range:

The reference range for Factor IX in normal plasma as reported in the literature is 50-150% (0.5-1. IU/mL)

101 citrated plasma samples from healthy individuals with no known history of hemophilia B were test on the three validation lots of the VisuLize™ Factor IX Antigen Assay. Results demonstrated good agreement between the three validation lots with a mean correlation coefficient (r) of 0.864, and a p-value of 0.282.

	Lot 1	Lot 2	Lot 3	Combined Lots
Mean (IU/mL)	0.9997	0.9930	0.9683	0.987
STD Dev	0.1523	0.1489	0.1410	0.148
95% CI (IU/mL)	0.70-1.30	0.70 – 1.29	0.69-1.25	0.69 -1.28

N. Conclusion:

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