

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

A. 510(k) Number:

K042687

B. Purpose for Submission:

The purpose of this submission is to obtain clearance for a modification in the capture antibody used in the assay from a polyclonal antibody to a monoclonal antibody.

C. Measurand:

Prothrombin 1.2

D. Type of Test:

Quantitative sandwich enzyme immunoassay

E. Applicant:

Dade Behring Inc.

F. Proprietary and Established Names:

Enzygnost™ F1 + 2(monoclonal)

G. Regulatory Information:

1. Regulation section:

21 CFR 864.7320

2. Classification:

Class II

3. Product code:

MIF

4. Panel:

81 Hematology

H. Intended Use:

1. Intended use(s):

Enzygnost F 1 + 2 (monoclonal) is an enzyme immunoassay for the quantitative determination of human prothrombin F 1 + 2 in plasma.

2. Indication(s) for use:

Enzygnost F 1 + 2 (monoclonal) is an enzyme immunoassay for the quantitative determination of human prothrombin F 1 + 2 in plasma. Measurement of F 1 + 2 is used as an aid in the diagnosis, monitoring, and evaluation of acquired or hereditary blood coagulation disorders. It is indicated as an aid in assessing risk of thrombosis and in monitoring efficacy of anticoagulant therapy.

3. Special conditions for use statement(s):

4. Special instrument requirements:

I. Device Description:

The assay is an enzyme immunoassay based on the sandwich principle in a microtiter format utilizing a mouse monoclonal antibody as the capture antibody. The assay kit is comprised of an anti-F 1 + 2 microtitration plate coated with mouse monoclonal antibodies directed against human F 1 + 2, anti-human prothrombin, peroxidase-conjugated, monoclonal mouse antibodies, tris buffer, 4 human prothrombin-fragment F 1 + 2 standards, bilevel lyophilized human plasma controls, buffers, washing solution, chromogen and stopping solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Enzygnost™ F 1 + 2 micro test kit

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

K922934

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For the quantitative determination of the human prothrombin fragment F 1 + 2 in plasma as an aid in diagnosing, monitoring, and evaluating blood coagulation disorders involving changes in coagulation system activity.	same
Principle	Enzyme immunoassay based on the sandwich principle	same

Differences		
Item	Device	Predicate
Measuring Range	20-1200 pmol/L	0.04-10 nmol/L (40-10000 pmol/L)
Capture Antibody	Monoclonal	polyclonal

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

L. Test Principle:

The assay is an enzyme immunoassay based on the sandwich principle in a microtiter format utilizing a mouse monoclonal antibody as the capture antibody. During the first incubation, the F 1+ 2 antigen in the sample binds to F 1+ 2 antibodies attached to the surface of the microtitration plate. After washing, peroxidase-conjugated antibodies to human prothrombin are bound to a free F 1 + 2 determinants in a second reaction. The excess enzyme-conjugated antibodies are removed by washing. The enzymatic reaction between hydrogen peroxide and chromogen is terminated by the addition of dilute sulfuric acid. The color intensity, which is proportional to the concentration of F 1 + 2, is determined photometrically and quantified by means of a calibration curve based on the standards included in the kit.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was determined by testing eight replicates each of a normal control, a normal control diluted 1:2, a normal plasma pool, and a high plasma pool, over five days.

(n=40)	Normal Control		Dil 1:2		Normal Plasma		High Plasma	
	(%CV)		Normal control (%CV)		Pool 1 (%CV)		Pool (%CV)	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot1	Lot 2
Within run	3.9	5.5	4.7	4.7	3.6	4.6	3.9	4.7
Run to Run	11.2	4.4	5.1	7.1	7.5	6.3	9.1	8.6
Total	11.8	6.8	6.8	8.4	8.2	7.7	9.8	9.7

b. Linearity/assay reportable range:

20-1200pmol/L

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

Controls are prepared from a frozen plasma pool collected from normal blood donors. F1 + 2 concentration is determined from four replicates, to determine if the specification for F1 + 2 concentrations met. If so, the pool is filtered, vialled and lyophilized. Control values are assigned by testing at least two vials of control, on two separate runs and testing eight replicates. Results are calculated from a lot- specific reference curve. A range is assigned based on the grand mean of mean values of each run $\pm 20\%$.

Standards are prepared from a purified prothrombin concentrate. The concentration of a purified F2-antigen preparation is determined by quantitative amino acid analysis. Based on this concentration the master lot is calibrated against dilution of the purified F2 with antigen dilution buffer resulting in concentration of 20, 80, 400 and 1200 pmol/L

d. Detection limit:

e. Analytical specificity:

Interference testing was performed to determine the effect of hemolysis, bilirubin, and lipemia on assay performance. Normal and pathological base pools were used and spiked with increasing concentrations of the potential interferant. No interference was seen up to 60 mg/dL for bilirubin, up to 600 mg/dL for hemoglobin, and up to 3000 ng/dL for triglycerides.

Five normal samples were spiked with rheumatoid factor in concentration of 67 to 197 IU/mL. Enzygnost™ 1 + 2(monoclonal) testing was performed in quadruplicate determinations. No interference was seen.

f. Assay cut-off:

2. Comparison studies:

a. Method comparison with predicate device:

Specimens spanning the assay range (20.77 – 1169.55 pmol/L) from 190 patients were tested using the Enzygnost™ 1 + 2(monoclonal) vs. the Enzygnost™ 1 + 2 micro test kit. Samples were tested in duplicate. The regression analysis was $y = 0.265x - 29.378$, $r = 0.96$

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:

Specimens from 152 healthy subjects were studied using the Enzygnost™ 1 + 2(monoclonal) Test Kit. The 5th to 95th percentile was determined to be 62 to 197 pmol/L with a median value of 102 pmol/L.

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.