510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE AND INSTRUMENT TEMPLATE

A. 510(k) Number:
K033612

B. Analyte:
Factor II (Prothrombin)

C. Type of Test:
Qualitative

D. Applicant:
Roche Diagnostics Corporation

E. Proprietary and Established Names:
Factor II (Prothrombin) G20210A Kit

F. Regulatory Information:

1. Regulation section:
   864.7280
2. Classification:
   Class II
3. Product Code:
   NPR
4. Panel:
   81 Hematology

G. Intended Use:

1. Intended use(s):
   The Factor II (Prothrombin) G20210A Kit is an *in vitro* diagnostic test for the detection and genotyping of a single point mutations (G to A at position 20210) of the human Factor II gene, from DNA isolated from human whole peripheral blood.
2. Indication(s) for use:
   The Factor II (Prothrombin) G20210A Kit is indicated as an aid to diagnosis in the evaluation of patients with suspected thrombophilia
3. Special condition for use statement(s):
   None.
4. Special instrument Requirements:
   The test is intended to be used on the LightCycler instrument. The sample preparation must be performed according to a workflow procedure described in the package insert.

H. Device Description

The Factor II (Prothrombin) G20210A Kit is an in vitro diagnostic test for the detection and genotyping of the Factor II (Prothrombin) G20210A mutation, from DNA isolated from human whole peripheral blood. The test incorporates fluorogenic target-specific hybridization to PCR-amplified Factor II gene DNA to detect the presence of the mutation. Melting curve analysis of the probe-target complex reveals the presence of wild type and mutant alleles by their different melting temperatures.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Factor V Leiden Kit
2. Predicate K number(s):
   K033607
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Specimen type	Blood	Same
Sample type	Purified DNA	Same
Instrumentation	LightCycler Version 1.2	Same
Thermal cycling program	(95, 55, 72^{o}C) X 45	Same
Precision	Tm1: 0.23-0.28% CV Tm2: 0.18-0.30% CV ΔTm: 1.09-1.35% CV	Tm1: 0.19-0.33% CV Tm2: 0.22-0.44% CV ΔTm: 1.23-1.53% CV
Sensitivity	50 allele copies/reaction	Same
Interferences	Heparin	Same
Comparator	Sequence	Same
Performance	98.0% sens., 99.6% spec. overall (nonclincal and clinical samples)	100% sens., 99.8% spec. overall (nonclinical and clinical samples)
Differences		
Item	**Device**	**Predicate**
Oligonucleotide probes and primers	Specific for Factor II (prothrombin) G20210A	Specific for Factor V Leiden
Melting curve	45-75 ^{o}C, 0.1 ^{o}C/sec ramp	40-70 ^{o}C, 0.1 ^{o}C/sec ramp
Melting temp.	49 ^{o}C, 55 ^{o}C	57, 65 ^{o}C
ΔTm	10 $\pm$1.5 ^{o}C	8$\pm$1.5 ^{o}C

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

Class II Special Controls Guidance Document: Factor V Leiden DNA mutation detection system

K. Test Principle:

Fluorogenic detection of PCR-amplified Factor II locus by melting curve analysis.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
    a. *Precision/Reproducibility:*
    Determined at three sites, with three instruments and three operators. Six replicates of each (control template and human DNA) were run at each site in ten separate runs with no more than 2 runs per day. Imprecision was calculated according to NCCLS EP-5A.

    Within-run:
    T_m1: 0.14-0.26% CV
    T_m2: 0.14-0.24% CV
    ΔT_m: 0.58-0.90% CV

    Total:
    T_m1: 0.19-0.33% CV
    T_m2: 0.22-0.44% CV
    ΔT_m: 1.23-1.53% CV

    Overall median: 0.36% CV

    Lot-to-lot: Eight heterozygous human samples were run in a single PCR using three lots of reagent. All lots performed within specifications.

    b. *Linearity/assay reportable range:*
    Crossing point <31
    c. *Traceability (controls, calibrators, or method):*
    Not applicable
    d. *Detection limit:*
    50 copies of locus
    e. *Analytical specificity:*
    5/5 heterozygous samples correctly called for human and plasmid control DNA
    f. *Assay cut-off:*
    Not applicable
2. Comparison studies:
    a. *Method comparison with predicate device:*
    Comparison is with DNA sequencing on Amersham MegaBASE 500 sequencer equipped with SW 3.0 and Cimarron Base Caller 3.12. Sequence method was validated and preferred sequencing direction determined by quality score of 50 samples. All remaining samples were sequenced in the preferred direction.
    Agreement between sequence and Factor II kit was 99.3% on 441 qualified retrospective repository samples. 19 repository samples could not be sequenced and were excluded from the study.
    b. *Matrix comparison:*

Blood samples from 50 volunteers were collected into EDTA, citrate and heparin anticoagulants. Student's t-test showed that samples collected in heparin had lower melting temperatures ($P<0.0001$) than those in EDTA or citrate. Heparin has been excluded as an allowable matrix for testing.

3. Clinical studies:
   a. *Clinical sensitivity:*
      One hundred twelve fresh samples from patients referred to hospital for thrombophilia testing were collected under IRB approval. Citrate (14) and EDTA (98) were used as anticoagulants. There was 100% (5/5) agreement between the sequence and the Factor II Kit results, with all 5 heterozygotes being correctly called by the Factor II kit.
   b. *Clinical specificity:*
      In the same 112 samples cited above, there was 100% specificity (107/107), with no wild-type samples being called as Factor II mutations by the Factor II Kit
   c. *Other clinical supportive data (when a and b are not applicable):*

4. Clinical cut-off:
   Not applicable
5. Expected values/Reference range:
   ΔTm's must be $10 \pm 1.5\ ^{o}C$ for wild type and mutant alleles. Melting point temperatures of $49^{o}C$ and $59^{o}C$ ($\pm 2.5\ ^{o}C$ for wild type and mutant, respectively.

M. Instrument Name:

LightCycler V 1.2, reviewed and cleared in K033607

N. System Descriptions:

1. Modes of Operation:
   Software driven, PCR, melting curve analysis
2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
Yes ___X_____ or No _____

3. Sample Identification:
   By position in carousel
4. Specimen Sampling and Handling:
   Sample applied manually or by MagnaPure instrumentation to capillaries inserted into carousel. No further handling.
5. Assay Types:
   Melting curve, any DNA change that can be distinguished from wild type by ΔTm
6. Reaction Types:

PCR, fluorogenic detection of melting curve

7. Calibration:
   N/A
8. Quality Control:
   Positive control material, user inspection of melting curves.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "L. Performance Characteristics" Section Of The SE Determination Decision Summary.

MagnaPure DNA purification instrument was reviewed and cleared in K033607, and may be used to purify sample and set up reactions.

P. Conclusion:

This device is found to be substantially equivalent to K033607 Factor V Leiden Kit.