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

A. 510(k) Number:
K033734

B. Analyte:
Analyte used to validate instrumentation is Factor V Leiden DNA mutation

C. Type of Test:
Qualitative

D. Applicant:
Roche Diagnostics Corporation

E. Proprietary and Established Names:
LightCycler Instrument Version 1.2

F. Regulatory Information:

1. Regulation section:
   862.2170
2. Classification:
   Analyzer, Chemistry, Micro, for clinical use, Class I
3. Product Code:
   JJF
4. Panel:
   75 Clinical Chemistry

G. Intended Use:

1. Intended use(s):
   The LightCycler Instrument is a fully automated amplification and detection system for nucleic acids using fluorescence detection. The LightCycler is intended to be used by laboratory professionals trained in laboratory techniques and on the use of the Analyzer
2. Indication(s) for use:
   Not specific
3. Special condition for use statement(s):
   Professional use only
4. Special instrument Requirements:
   None

H. Device Description
The LightCycler Version 1.2 is a flexible, benchtop analyzer that automates the amplification and detection steps of the Polymerase Chain Reaction (PCR) through

programmable thermal cycling steps. The instrument uses heated air to control the activity of DNA polymerase that makes copies of sample DNA fragments. The LightCycler also calculates approximate DNA concentration of the amplified sample when compared to a standard, and generates melting curves of amplified DNA by measuring the dissociation of a fluorogenic probe sequence from the amplified DNA fragments.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   COBAS TaqMan Analyzer
2. Predicate K number(s):
   K012966
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended use	The LightCycler Instrument is a fully automated amplification and detection system for nucleic acids using fluorescence detection. The LightCycler is intended to be used by laboratory professionals trained in laboratory techniques and on the use of the Analyzer.	The COBAS TaqMan Analyzer is a fully automated amplification and detection system for nucleic acids using 5' nuclease technology. The COBAS TaqMan Analyzer is intended to be used by laboratory professionals trained in laboratory techniques and on the use of the Analyzer
Primary operational components	Integrated thermocycler and microvolume fluorimeter for walkaway PCR amplification and detection	Same
Detection procedure	Optical detection of stimulated fluorescence	Same
Specimen type	Purified nucleic acids	Same
Specimen preparation	Performed off-line	Same
Temperature range	40-98^{o} C	Same
User Interface	PC with instrument-specific software (LightCycler version 3.5 of higher)	PC with instrument-specific software (Amplilink Software version 3.0 or higher)
Differences		
Item	**Device**	**Predicate**
Heating method thermal cycling	Hot air cycling with glass capillaries	Peltier device with sample block

Number of thermal cyclers	One	Four
Sample positions	32	96
Sample size	10-20 uL in glass capillaries	100 uL in 200 uL K-tubes
Number of optical detection channels	Three with fixed wavelengths (530 nm, 640 nm, 710 nm)	Four with wavelength ranges 510-710 nm
Detection chemistry	Paired hybridization probes using fluorescence resonance energy transfer (FRET)	5' nuclease hydrolysis probes using FRET (TaqMan technology)
Detection timing	Detection occurs at defined intervals during PCR cycle and can be viewed in real-time	Detection occurs only at end of each PCR cycle and be viewed at completion of run
Absolute temperature accuracy	$\pm$ 0.4^{o}C	$\pm$ 1.5^{o}C

J. Standard/Guidance Document Referenced (if applicable):
EC Declaration of Conformity, TUV Certificate, IEC CB Test Certificate, and USL and CSL statement (standards); FDA "*Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices*".

K. Test Principle:
Fluorogenic detection of PCR-amplified DNA fragments by melting curve analysis.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:
(Incorporated by reference to K033607, Factor V Leiden Kit)
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:
(Incorporated by reference to K033607 Factor V Leiden Kit)
Crossing point <31

c. Traceability (controls, calibrators, or method):
N/A

d. Detection limit:
(Incorporated by reference to K033607 Factor V Leiden Kit) 50 copies of locus

e. Analytical specificity:
(Incorporated by reference to K033607 Factor V Leiden Kit) 5/5 heterozygous samples correctly called for human and plasmid control DNA

f. Assay cut-off:
N/A

2. Comparison studies:

a. Method comparison with predicate device:
(Incorporated by reference to K033607 Factor V Leiden Kit)
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:
(Incorporated by reference to K033607 Factor V Leiden Kit) 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:
(Incorporated by reference to K033607 Factor V Leiden Kit) 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:
(Incorporated by reference to K033607 Factor V Leiden Kit) 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:
(Incorporated by reference to K033607 Factor V Leiden Kit) ΔTm's must be 10 ± 1.5°C for wild type and mutant alleles. Melting point temperatures of 49°C and 59°C (± 2.5°C for wild type and mutant, respectively.

M. Instrument Name:
LightCycler V 1.2

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:
Not applicable
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.
All nonclinical and clinical data is incorporated by reference to K033607, Factor V Leiden Kit.

P. Conclusion:

This device is substantially equivalent to the COBAS TaqMan analyzer by similarity of device configuration and intended use, and demonstration of performance against a reference method (DNA sequencing) using reagents for the detection of Factor V Leiden mutations.