AutoGenomics

INFINITI® Factor II-V & MTHFR Panel

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

Manufactured by AutoGenomics,Inc. 1600 Faraday Avenue, Carlsbad, CA USA 92008

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
Schiffgraben 41, 30175 Hannover, Germany

AutoGenomics

INTENDED USE

The INFINITI Factor II-V & MTHFR Panel is an *in vitro* diagnostic device that consists of reagents and instrumentation which includes polymerase chain reaction (PCR) primers, hybridization matrices, a thermal cycler, an imager, and software for detection and genotyping of Factor II (Prothrombin) G20210A, Factor V Leiden G1691A, and MTHFR (A1298C and C677T) point mutations in deoxyribonucleic acid (DNA) obtained from human blood samples. The INFINITI Factor II-V & MTHFR Panel is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

The INFINITI Factor II-V & MTHFR Panel for detection and genotyping of Factor II, Factor V and MTHFR is indicated for use as an aid to diagnosis in the evaluation of patients with suspected thrombophilia.

BACKGROUND INFORMATION: Genetic risk factors are involved in the predisposition of individuals to venous thrombosis [1, 2]. The most common mutation associated with inherited thrombosis is Factor V Leiden G1691A mutation and results in resistance to activated protein C. This mutation has a relatively high prevalence in the general population (about 5% in Caucasians), and accounts for 85% to 95% of activated protein C resistant cases.[2] Factor V Leiden G1691A mutation renders an enhanced risk of venous thrombosis, with odds ratios (ORs) of 3 to 8 in heterozygotes and 30 to 140 in homozygotes [3].

The second most common mutation associated with hereditary thrombosis is the G20210A mutation in the prothrombin (Factor II) gene. This is associated with increased plasma prothrombin levels and is present in 1% to 2% of the general population [2] Heterozygote carriers of the prothrombin G20210A mutation have an estimated 3 to 8-fold increased risk for venous thrombosis. Homozygote carriers are very rare and information on the increased risk for thrombosis associated with the homozygous mutation is very limited.

Heterozygosity for both Factor V Leiden G1691A and Factor II (Prothrombin) G20210A mutations results in earlier onset of thrombosis and greater severity as compared to the presence of either mutation alone in the heterozygous state.

Increased plasma homocysteine levels is another risk factor for venous thrombosis, and is associated with homozygosity for a genetic variant in the methylene tetra-hydrofolate reductase (MTHFR) gene. The MTHFR C677T variant results in a thermolabile enzyme and decreased production of folate, a cofactor required for homocysteine remethylation. The MTHFR A1298C mutation is also associated with increased homocysteine and lowered plasma folate levels when present in combination with the C677T mutation.

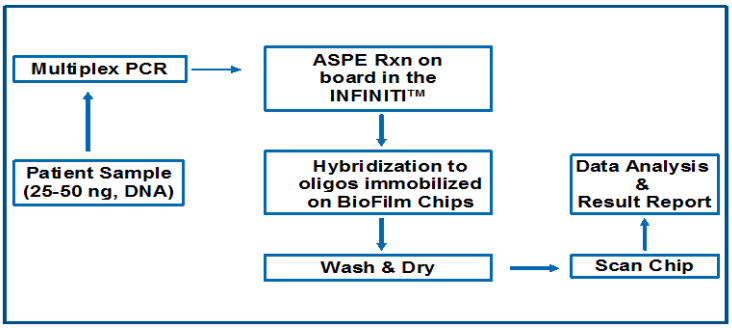
TEST PRINCIPLE/ASSAY OVERVIEW

The INFINITI Factor II-V & MTHFR Panel is designed to simultaneously detect mutations of Factor II (G20210A), Factor V (G1691A), and MTHFR (A1298C and C677T). The assay protocol is based on five major processes:

(a) DNA extraction from human blood samples.
(b) PCR amplification of purified DNA.
(c) Fluorescent label incorporation using analyte specific primer extension (ASPE).
(d) Hybridization of the ASPE primers to a microarray followed by washing.
(e) Scanning of the microarray.
(f) Signal detection and analysis (determination of the Factor II, Factor V and MTHFR genotypes).

Steps (c) through (f) are automated by the INFINITI Analyzer and INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI Factor II-V & MTHFR Panel is an *in vitro* diagnostic device which utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for the detection and genotyping of the Factor II (Prothrombin) G20210A, Factor V Leiden G1691A and MTHFR (A1298C and C677T) mutations from DNA obtained from human whole peripheral blood samples.

The INFINITI Factor II-V & MTHFR Panel is comprised of the BioFilmChip® Microarray, the Intellipac® Reagent Module, and the Analyzer with the Qmatic® Operating Software.

The **BioFilmChip Microarray** consists of a polyester film coated with proprietary multi-layer components designed for DNA analysis. The layers have been designed to provide a versatile surface to enhance test performance. The microarrays are designed to be assay specific. The INFINITI Factor II-V & MTHFR Panel uses a microarray chip (L-Chip) which contains unused Capture Probes which could potentially be used for certain specific assays. Therefore, multiple assays can be developed using the same microarray.

The **Intellipac Reagent Module** which acts as a communication link contains four reservoirs that house the test reagents and has an integrated memory chip. Reagent information such as lot number, expiration date, and volume usage are archived in the memory chip and appear on the worklist (run report).

The **INFINITI Analyzer** and the **INFINITI PLUS Analyzer** are instruments used for clinical multiplex systems intended to measure and sort multiple signals from a clinical sample. The Analyzers are designed to measure fluorescence signals of labeled DNA target hybridized to BioFilmChip microarrays. The Analyzers automate the MTHFR, Factor II and Factor V assays and integrates all the discrete processes of sample (PCR amplicon) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and the spots are read by the built-in confocal microscope. Results are analyzed and presented in numerical and graphical format.

The Analyzers have two main components: pipetting and optics modules. A variety of electronic components inside the instrument are used for its operation. These include multiple stepper motors, heating and cooling devices, a barcode reader, a photomultiplier tube, and a camera all connected to USB ports.

- Pipetting Module - The pipetting module performs all the operations related to dispensing and aspiration of reagent and processing the amplified sample to be dispensed on the microarray. When the sample has been processed and hybridized to the microarray, it is transferred to the optics module for measurement of fluorescence signal.
- Optics Module - The optics module is a lightproof assembly comprised of a 3-axis stage; camera, lasers, and a photo multiplier tube (PMT). It is the enclosed casement into which the microarray is transported

automatically prior to being processed on the stringency station. The optics' stage follows X-Y-Z motions that can be stepped at a very precise rate (2.0 micron per step). Using excitation wavelengths of a 760nm laser diode, the camera takes a 1.2x1.2mm picture for each registration spot of a fluorescent dye. Analyses of these pictures allow the location of three registration spots to be determined. With respect to the position of the three registration spots, coordinates of all the bio-spots can be located. While scanning, the stage moves along the Z-axis to focus the chip and the X and Y-axes to locate the individual spots on the microarray.

Instructions on how to use the Analyzers are provided in the Operator's Manuals.

The INFINITI Analyzer and the INFINITI PLUS Analyzer are CE-marked.

The Analyzers hardware is controlled by the **Qmatic® operating software**, which is installed with-in the on-board computer and utilizes a LCD screen display. The Analyzers modules are controlled by multitasking real time software. The Qmatic operating software has a schedule manager that is capable of controlling all operations of the Analyzer such as assay protocol, fluid handling, robotics, optical detection and result analysis. Results are available for review via the LCD screen. Management reports include results in numerical and graphical format. The operator can also print the displayed results in tabular form (printer not included with the Analyzers).

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with whole blood collected in EDTA. Do not freeze/thaw blood samples. Specimens should be assayed as soon as possible.
- Do not use Heparin with this procedure; Heparin might interfere with the PCR.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV or other infectious agents are absent.
  Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow normal precautions for handling laboratory reagents. Do not mix reagents from different containers or from different lots.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling

- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations.
- Safety data Sheets (SDS) are available upon request from AutoGenomics Customer Service.

AutoGenomics

Sample Preparation

- Refer to the safety instructions in the package insert provided with the DNA extraction kit used.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately**.**

INFINITI Analyzer or INFINITI PLUS Analyzer

- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

STORAGE / STABILITY

BioFilmChip Microarray: 24 months Refrigerated (2°C to 8°C)
Intellipac Reagent: 12 months Refrigerated (2°C to 8°C)
Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four weeks.
Amplification Mix: 18 months Frozen (-30°C to -15°C)
Note: Specific product expiration date is printed on the product label.

SPECIMEN COLLECTION AND STABILITY

- Peripheral blood drawn in an EDTA (purple-top) tube.
- Do not freeze/thaw blood samples. Specimens should be assayed as soon as possible.

MATERIALS PROVIDED (EACH PACKAGE IS SUFFICIENT FOR 48 TESTS)

- AutoGenomics Product Number 01-1010-02 Factor II-V & MTHFR Magazine – BioFilmChip® Microarray
  4 magazines per package; 12 tests per magazine
- AutoGenomics Product Number 01-2010-02 Factor II-V & MTHFR Intellipac® Reagent Module:
  2 modules per package; 24 tests per module. Each Intellipac module contains
  - 1.1ml ASPE master mix composed of:
    - d[AGT]TP Mix
    - Cy5 –dCTP
    - ASPE Primer Mix
    - Extension Buffer
  - 2.6ml Hybridization Buffer
    - SSC
    - Sodium Azide
    - Hybridization Positive Control
- AutoGenomics Product Number 01-3010-02 Factor II-V & MTHFR Amp Mix. Each package contains 4 x 250µl vials of AMP Mix containing:
  - d[AGT]TP Mix
  - dCTP
  - PCR Primer Mix
  - $MgCl_2$
  - PCR Buffer

- Product Number 12-0010-02: Wash buffer

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - The INFINITI Factor II-V & MTHFR Panel can detect Factor II (Prothrombin) G20210A, Factor V Leiden G1691A and MTHFR (A1298C and C677T) mutations using genomic DNA isolated from blood with sufficient purity, i.e., with the ratio of absorbance at 260nm to absorbance at 280nm of 1.7 to 2.0. Any DNA extraction method that meets this specification may be used. The INFINITI Factor II-V & MTHFR Panel has been tested with several commercially available kits and the user can contact AutoGenomics for further information.
- Distilled Water (DNAse and RNAse free)
- Titanium Taq DNA Polymerase (see AutoGenomics Product Catalog for recommended Titanium Taq DNA Polymerase and supplier)

EQUIPMENT

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml microcentrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Pipette Tips

- **FOR INFINITI Analyzer***:*
    - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
    - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
    - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate

- **FOR INFINITI PLUS Analyzer***:*
    - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
    - AutoGenomics Product Number 11-0100-00: 48-Well Plates
    - AutoGenomics Product Number 11-0110-00: 48 Well Plate Lid (reusable)

ASSAY PROCEDURE

DNA Extraction

Follow the instructions provided with the DNA extraction kit used.

DNA Controls

It is required to run known positive controls and a negative control should also be included in each test run.

Recommended Controls

It is recommended that positive controls (heterozygous and/or homozygous samples) are included in each test run. In addition, a negative control (i.e., wild type sample) and a no template control (i.e., molecular grade water) should also be included in each test run. Coriell DNA samples (www.coriell.org) are suitable positive controls for many of the detected genotypes.

PCR Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix tube for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.
- Filter tips and gloves must be used when handling specimens and controls.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

1. Prepare the PCR master mix.

Amplification mix	17.75 µl
Titanium Taq polymerase	0.25 µl
Total volume of PCR Master mix	18.0 µl

Note: Calculate the amount of each reagent needed based on the number of reactions.

2. Gently vortex the PCR master mix then dispense 18 µl of master mix into wells of the well plate.

3. Add 2 µl of sample DNA (25 ng/µl) to each well.

PCR master mix	18.0 µl
Sample DNA	2.0 µl
Total volume of amplification reaction	20.0 µl

4. Place the well plate, sealed with 8-well flat strip caps, in a thermocycler and immediately commence the amplification reaction using the following program:

Step No.	Temperature °C	Time	No. of Cycles
1	95	4 minutes	N/A
2	95 56 72	15 seconds 15 seconds 15 seconds	40x
3	4	Hold	1

Note: When an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was 1 hour and 9 minutes (± 5 min). If using other thermocycler models we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

Sample Loading

1) Carefully remove the 8-well flat strip caps to avoid splashing.

2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Catalog # 11-0030-00).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operations Manual) (Catalog # 11-0110-00, reusable).

3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
   - **FOR INFINITI Analyzer***:*
     Wash Buffer should be placed in the INFINITI bottle holders. The Wash Buffer goes in the left holder (near the magazine).
   - **FOR INFINITI PLUS Analyzer***:*
     Follow the INFINITI PLUS Analyzer Operator's Manual for checking and replacing Wash buffer.

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Part Number EM-34041)

QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI® or INFINITI® PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

LIMITATIONS

The results obtained from this method should be used and interpreted only in the context of the overall clinical diagnosis. AutoGenomics is not responsible for any clinical decisions that are taken.

The absence of the mutations detected by this Procedure is no guarantee that other mutations are not present. Presence of other mutations is possible and is not detected by this kit.

INTERPRETATION OF RESULTS

Results from the INFINITI Factor II-V & MTHFR Panel are reported to the user as a genotype "call", indicating which genotype was detected in the sample, i.e., Wild Type, Homozygous, or Heterozygous for Factor II (Prothrombin) G20210A, Factor V Leiden G1691A, MTHFR A1298C, and MTHFR C677T. In addition to the genotype "call", the ratio and the signal (RFU) are provided for each analyte.

If no results are displayed, and there is an error message located in the bottom right corner of the results page, then the assay should be repeated for that sample. A possible error message such as "Error #125 Non template control equivalent" indicates one or more of the analytes could not be genotyped. All errors are captured in the Error Log of the INFINITI. For more information on errors please refer to the Troubleshooting section of the INFINITI Analyzer Operator's Manual. For further clarification and resolution of the error please contact technical support at AutoGenomics.

DISPOSAL

Waste materials for the INFINITI Factor II-V & MTHFR Panel are common waste materials generated in clinical laboratories, and should be handled/disposed of in accordance with the policies/procedures in place in the clinical laboratory.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known genomic samples. Capture probe specificity was determined by hybridizing different oligos and demonstrating that only the correct oligo hybridizes to the known spot.

Analytical Sensitivity
Studies demonstrated that the INFINITI Factor II-V & MTHFR Panel can detect as low as 1ng DNA and as high as 300ng DNA. A minimum of 25ng DNA is recommended for this assay.

Percent Agreement
The results of the INFINITI Factor II-V & MTHFR Panel were compared a reference method. The results of the comparison studies are summarized below.

Genotype	Number tested	Number of correct calls 1st run	Number of incorrect calls 1st run	Number of invalid calls 1st run	Agreement 1sr run	Number of correct calls including repeat run	Number of incorrect calls on repeat run	Number of invalid calls on repeat run	Overall Agreement
Factor II									
WT	44	44	0	0	100%	44	0	0	100%
MUT	0					0			
HET	6	4	0	2	66.7%	6	0	0	100%
total	50	48	0	2	96.0%	50	0	0	100%
Factor V									
WT	37	34	1	2	91.9%	37	0	0	100%
MUT	2	2	0	0	100%	2	0	0	100%
HET	11	11	0	0	100%	11	0	0	100%
total	50	47	1	2	94.0%	50	0	0	100%
MTHFR C677T									
WT	22	20	2	0	90.9%	22	0	0	100%
MUT	8	8	0	0	100%	8	0	0	100%
HET	20	19	1	0	95.0%	20	0	0	100%
total	50	47	3	0	94.0%	50	0	0	100%
MTHFR A1298C									
WT	24	22	0	2	91.7%	24	0	0	100%
MUT	4	4	0	0	100%	4	0	0	100%
HET	21	18	1	2	85.7%	21	0	0	100%
total	49	44	1	4	89.8%	49	0	0	100%
TOTAL	199	186	5	8	93.5%	199	0	0	100%

Precision/Reproducibility – Assay
Chip-to-chip: Using the same sample and the same INFINITI Analyzer, the assay was run in five replicates using three BioFilmChip microarrays from one lot. The CVs using average triplicate spots for each mutation ranged from 9 – 12% for wild-type present calls. This was repeated two other times, each time using a different INFINITI Analyzer. All calls were 100% correct.

Lot-to-lot: Three lots of BioFilmChip microarrays were tested using the same instrument four times, each time using a different sample. Two-way ANOVA on the RFU readings did not detect lot-to-lot difference on three of the four test runs ($p > 0.05$), and detected lot-to-lot difference on one test run ($0.05 > p > 0.01$). Genotype calls were 100% correct.

Day-to-day: Known genomic sample was assayed 12 times on each of three days using one instrument. The RFU signal %CV ranged from 1.35 to 14.87 on day1, 0.77 to 19.72 on day 2 and 0.41 to 21.2 on day 3. Genotype calls were 100% correct.

Precision/Reproducibility - Instrument

- One DNA sample was analyzed using three different INFINITI Analyzers and one lot of BioFilmChips, five times (five runs).

  Intra-Instrument: The %CV using a single chip five times on a single instrument ranged from 0.9 to 28.3%CV. Genotype calls were 100% reproduced within each instrument.

  Inter-Instrument: The %CV using a single chip five times on each of three instruments ranged from 0.5% to 12%CV. All genotype calls were 100% correct and reproducible.

- Three instruments were tested on three different days using a Standard (non-assay) Microarray Chip. For each instrument tested, each capture probe spot on the Standard Microarray Chip was read 24 times, then averaged, and a %CV calculated for the spot. The following lists the ranges for the %CV for the three instruments tested.

Instrument	Ave %CV	%CV Range
1	4.03%	1.9-7.5%
2	3.99%	2.7-6.5%
3	3.24%	1.9-5.3%

Sample Carry-over

No carry-over was detected when a series of 300ng of a wild type sample was followed by 10ng of a positive sample, followed by 300ng of a heterozygous sample, followed by a "No Template Control" or water, was run six times.

Interference

Results of the interference studies demonstrate that there is no interference from 8mg/dL bilirubin, 70mg/dL cholesterol, and 1333v/dL heparin. No studies were conducted with oral anti-coagulants; therefore, no claims are made.

REFERENCES

1. Peterson LC, Hedner U, Wildgoose P. In: High KA, HR Roberts eds. Molecular basis of thrombosis and hemostasis. New York: Marcel Dekker,1995.

2. Grody W, Griffin J, Taylor A, Korf B, Heit, J. (2001) American College of Medical Genetics Consensus Statement on Factor V Leiden Mutation Testing, Genetics in Medicine, 3:2, 139-147.

3. Salomon O. et al; Single and Combined Prothrombotic Factors in Patients With Idiopathic Venous Thromboembolism; Arteriosclerosis, Thrombosis, and Vascular Biology. 1999;19:511-518) © 1999 American Heart Association, Inc.