



INFINITI[®] FMF Assay
Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use



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

INTENDED USE

The INFINITI FMF Assay is an *in vitro* diagnostic test for the detection and identification of the allelic variants in the *MEFV* gene. The INFINITI FMF Assay is designed to detect these mutations in whole blood samples.

The INFINITI FMF Assay is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

BACKGROUND INFORMATION

Familial Mediterranean fever (FMF) is an autosomal recessive, inflammatory disorder characterized by short, recurrent attacks of fever, accompanied by pain in the abdomen, chest or joints, and erysipelas-like erythema. The most severe complication is progressive amyloidosis, leading to end-stage renal failure.

FMF predominantly affects Turks, Arabs, Armenians and Sephardic Jews, with carrier rates reported as high as 1 in 5. FMF has also been observed, in lower frequencies, throughout the Mediterranean area.

FMF is caused by a number of mutations within the marenostin/pyrin-encoding gene *MEFV*. Five founder mutations, V726, M694V, M694I, M680I and E148Q, account for 74% of FMF chromosomes from typical cases (Armenians, Arabs, Jews, and Turks). The various combinations of *MEFV* mutations define severe to mild genotypes

Because of the non-specific clinical symptoms, molecular genetic analysis significantly improves early and correct diagnosis of FMF, and allows the beginning of lifelong prophylactic treatment of affected individuals with colchicine.

TEST PRINCIPLE/ASSAY OVERVIEW

The INFINITI[®] FMF Assay is designed to detect and identify allelic variants in the *MEFV* gene. The INFINITI[®] FMF Assay is designed to detect these mutations in whole blood samples.

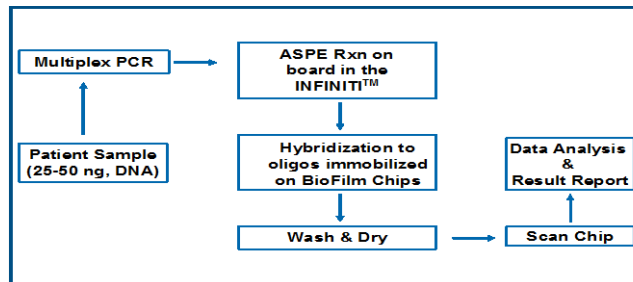
Gene	Polymorphism
<i>MEFV</i>	E148Q, R202Q, P369S, F479L, M680I (G>C), M680I (G>A), I692del, M694V, M694I, K695R, V726A, A744S, R761H

The assay protocol includes the following five major processes:

- a) Multiplex PCR amplification of DNA.
- b) Fluorescent label incorporation using analyte specific primer extension (ASPE).
- c) Hybridization of the ASPE primers to a microarray followed by washing.
- d) Scanning of the microarray.
- e) Signal detection and analysis.

Steps (b) through (e) are automated by the CE-marked INFINITI Analyzer or INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI FMF Assay utilizes AutoGenomics' proprietary film-based microarray technology combined with process automation, reagent management and software technology for multiplex detection of the allelic variants in the *MEFV* gene.

The INFINITI FMF Assay is comprised of the BioFilmChip[®] Microarray, the Intellipac[®] Reagent Module and the PCR Amplification Mix.

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 INFINITI FMF Assay uses a microarray chip which has capture probes spotted on the surface of the film. Two samples can be run on one microarray. Twelve (12) microarrays are housed in a magazine.

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 stored in the memory chip. The Intellipac Reagent Module communicates with the INFINITI Analyzer and provides the reagent information which appears on the assay report and printout. The Intellipac Reagent Management Module provides test reagent for 48 samples.

The **PCR Amplification Mix** consists of the reagents needed for the PCR amplification step of the assay. Each box of the PCR Amplification Mix provides 4 x 475µl vials of PCR Amplification.

The **INFINITI Analyzer** and the **INFINITI PLUS Analyzer** automates the INFINITI FMF Assay and integrates all the discrete processes of sample (PCR amplification product) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and read by the built-in confocal microscope. Results are analyzed and presented as *MEFV* genotype calls, indicating each allelic genotype detected as W (Wild type), H (Heterozygous), or M (Mutant).

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

The INFINITI Analyzer and INFINITI PLUS Analyzer are CE marked.

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.
- 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).

- Upon receipt of samples, visually inspect sample condition. Specifically, look for abnormal signs that indicate that sample integrity has been compromised (e.g., evaporation, decrease in volume, precipitation, spills, discoloration, sedimentation, separation, turbidity, etc.). If you observe or suspect any sample abnormality, do not perform any test.
- Samples should be handled with extreme caution to prevent contamination, spillage, sample mix-up. Sample containers should be labeled clearly to prevent mix-up.
- Store samples at the specified conditions.
- 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).
- 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.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves and 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

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 to 8°C)

Intellipac Reagent: 18 months Refrigerated (2 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

REAGENTS REQUIRED (SUFFICIENT FOR 96 TESTS) FROM AUTOGENOMICS

- Product Number 01-1120-02 INFINITI FMF BioFilmChip[®] Microarray Magazine
- Product Number 01-2120-02 INFINITI FMF Intellipac[®] Reagent Module
48 tests per module which contains 2 x 1.1 ml ASPE Master Mix:
 - dNTPs
 - Labeled-dCTP
 - Allele Specific Primers
 - Extension Reaction Buffer
- Product Number 01-3120-02 INFINITI FMF Amplification Mix
4 x 475µl vials of PCR reaction master mix containing
 - dNTPs
 - PCR Primer Mix
 - MgCl₂
 - PCR Reaction Buffer
- Product Number 12-0030-02 INFINITI Hybridization Buffer 2: 6 x 30ml bottles. The
- dization buffer consists of:
 - SSC
 - Sodium Azide Preservative 0.08%
 - EDTA
 - 10X Blocking Buffer
- Product Number 12-0020-02 INFINITI Wash Buffer 30 mL.

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - The INFINITI FMF Assay can detect the target FMF mutations using genomic DNA, isolated from whole blood samples with sufficient purity, i.e., with absorbance ratio $A_{260}/A_{280} \geq 1.60$ and a concentration of 30ng DNA/µl. Any DNA extraction method that meets this specification may be used. The INFINITI FMF Assay has been tested with several commercially available kits. The user can contact AutoGenomics for further information.
- Titanium Taq DNA Polymerase (Clontech, Catalog No: 639209)
- Distilled water (DNAse and RNAse free)

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 and Product Number 11-0110-00: 48 Well Plate Lid

DNA CONTROLS

Required known positive control(s) (heterozygous and/or homozygous samples) and a negative control (i.e., wild type sample) should also be included in each test run.

ASSAY PROCEDURE

DNA Extraction

Follow the instructions provided with the DNA extraction kit used.

PCR Reaction

Note:

- Keep Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix 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.
- Ensure that tubes are properly sealed to avoid evaporation or spillage.
- Make sure there is no abnormal evaporation of the PCR product. After PCR is complete, visually inspect for any volume change. All amplification reaction volumes should be about 15 μ l. Otherwise, do not proceed with the assay.

Note:

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

1. Thaw Amp mix on ice, centrifuge briefly, vortex 2 to 5 seconds and centrifuge briefly.

- Prepare the PCR master mix.

<u>Reagent</u>	<u>QTY per sample</u>
Amp mix	17.6 μ l
Titanium Taq polymerase	0.4 μ l
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Total volume of PCR Master Mix	18.0 μ l

Note: Calculate the amount of each reagent needed based on the number of reactions. Total number of samples should always be run in multiples of two. Samples and controls should be placed in consecutive order (i.e., well A1~C8 for a maximum of 24 tests per run). Do not skip wells or rows in the well plate.

- Gently vortex the PCR master mix then dispense 18 μ l of master mix into wells of the well plate.
- Add 2 μ l of sample DNA (30 ng/ μ l) to each well.

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

Note: This is a Duplex assay. When loading samples, always load the samples in multiples of twos and in consecutive order. For example, if loading 8 samples, load wells A1 to A8. **Do not** load the B wells.

- 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	94	3 min.	1
2	94	30 sec.	10x
	65 - 60 (-0.5°C/ cycle)	30 sec.	
	72	30 sec.	
3	94	30 sec.	30x
	60	30 sec.	
	72	30 sec.	
4	4	hold	1

Note: After each cycle in step 2 the temperature is decreased by 0.5°C per cycle. When an Eppendorf Mastercycler EP (aluminum block) was used with the ramp rate set at 100%, (heating 4°C/sec, cooling 3°C/sec), the total cycling time was 1 hour and 40 minutes. If using other thermocycler models we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

Sample Loading

- Carefully remove the 8-well flat strip caps to avoid splashing.
- 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 Operator's Manual) (Catalog # 11-0110-00, reusable).
- 3) Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
 - **FOR INFINITI Analyzer:**
The Wash Buffer bottle goes in the left INFINITI bottle holder (near the magazine) and Hybridization Buffer 2 in the right holder (near the Intellipac).
 - **FOR INFINITI PLUS Analyzer:**
The Hybridization Buffer 2 bottle should be placed in the right INFINITI bottle holder (near the Intellipac).

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 the INFINITI FMF Assay 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.

INTERPRETATION OF RESULTS

Results are analyzed and presented as *MEFV* genotype calls, indicating each allelic genotype detected as W (Wild type), H (Heterozygous), or M (Mutant). For example:

- An allelic genotype call for E148Q as W indicates the wild type analyte designed for the reference allele was detected as positive and the mutant analyte designed for the variant allele was detected as negative.
- An allelic genotype call for M680I as H (G/C) indicates both the wild type (G) and the mutant (C) analytes designed for the allele were detected as positive.
- An allelic genotype call for M680I as M (C) indicates the mutant (C) analyte was detected as positive and the wild type analyte (G) was detected as negative.
- An allelic genotype call for M680I as M (C/A) indicates both mutant analytes (C and A) were detected as positive.

When the assay is not completed, and no genotype call is made (No Call), the assay will need to be repeated. The report displays a message which indicates the reason why no genotype call was made. When an error occurs (e.g., "low DNA"), an Error Log is generated which identifies the problem. Please refer to the Trouble Shooting section of the INFINITI Analyzer Operator's Manual.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. There are three levels of specificity required in the assay

- PCR primers
- ASP – target binding region
- ASP – chip binding region

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 samples (e.g., Coriell). Capture probe specificity is determined by hybridizing different oligos and demonstrating that only the correct oligo hybridizes to the known spot.

Limit of Detection (LoD)

A limit of detection study was conducted to demonstrate the optimal detection range for the INFINITI FMF Assay. Two known DNA samples were diluted to 60ng, 30ng and 15ng. This was equivalent to 120, 60 and 30ng per test. The target/recommended DNA input is 60ng per test. Eight (8) replicates of each sample dilution were tested.

- One sample was Het for R202Q, and the other sample was Mut for E148Q/Het for R202Q.
- A total of 48 tests were run.
- 48 tests gave the correct calls. One test (120ng input) failed due to operator error.
- Overall, correct call rate was 97.9%

The study demonstrated that the optimal detection range for the INFINITI FMF Assay is 30 to 120ng per test.

Reproducibility

A study was performed to demonstrate lot-to-lot reproducibility of the INFINITI FMF Assay. Three lots of reagents were evaluated during the study. The study was performed using three known DNA samples. Eight replicates were run per sample for each lot evaluated for a total of 72 tests.

Results (genotype calls) were 100% consistent/reproducible throughout the samples tested. The following DNA samples were tested during the reproducibility studies:

Lot	Sample	Sample Genotype	Replicates Tested
1	1	R202Q Het	8
	2	E148Q Mut R202Q Het	8
	3	Wild Type	8
2	1	R202Q Het	8
	2	E148Q Mut R202Q Het	8
	3	Wild Type	8
3	1	R202Q Het	8
	2	E148Q Mut R202Q Het	8
	3	Wild Type	8

Sample Carry-over

Sample carry-over studies were performed using three (3) known DNA Coriell samples (R202Q Het; E148Q Mut/R202Q Het; Wild Type). Samples were run alternatively at different concentrations to determine if one sample will carry over to next. Results of the studies indicated no sample carry-over contamination.

Potential Interference from Drugs/Chemicals

Substance interference studies using whole blood specimens demonstrated no interference from the following potential interference substances.

Substances	Concentration
Bilirubin (conjugated)	60mg/dl
Bilirubin (unconjugated)	60mg/dl
Triglycerides	3000mg/dl
Human albumin	6g/dl

No studies were conducted with oral anti-coagulants, and no claims are being made.

Clinical Studies – Method Comparison

Clinical validation studies were conducted at three clinical laboratories to validate the INFINITI FMF Assay. Whole blood samples were tested using the INFINITI FMF Assay and results were compared to results obtained by sequencing. The total number of samples in the clinical studies was 141 samples.

INFINITI FMF Assay was repeated for samples which were discrepant based on the initial sequencing by the laboratories. Re-sequencing was performed to further resolve the discrepant samples.

Final results after resolution of discrepancies were 91.5%% (129/141) correct calls and 7.8% incorrect calls compared to the sequencing. There was one (0.7%) no call.

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