AutoGenomics

INFINITI® CFTR-31 Assay

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

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INTENDED USE

The INFINITI CFTR-31 Assay is an *in vitro* diagnostic device used to simultaneously detect and identify variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA samples isolated from human peripheral whole blood specimens.

The INFINITI CFTR-31 Assay is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

Together with available clinical and laboratory information, the INFINITI CFTR-31 Assay is intended for use in cystic fibrosis carrier screening for adults of reproductive age and as an aid in newborn screening for cystic fibrosis, and also as a diagnostic test to confirm cystic fibrosis in newborns and children.

The INFINITI CFTR-31 Assay is not indicated for use in fetal diagnostic or pre-implantation testing.

BACKGROUND INFORMATION

Cystic fibrosis (CF), an autosomal recessive disorder, is a common inherited defect with mutations associated with a chloride channel/regulator (cystic fibrosis transmembrane conductance regulator or CFTR). Affected children may present at birth with meconium ileus, or in infancy with malnutrition and pneumonia.

More than 1,000 mutations have been found in the CFTR gene; however, most of these mutations are very rare. The most common mutation is a deletion of phenylalanine at position 508 of the CFTR protein, and this accounts for about 70% of CFTR alleles in European Caucasians. About 50% of affected Caucasians have two copies of this gene mutation. Incidences in Caucasians, African American and Asians are approximately 1:2,500, 1:15,000 and 1:30,000 individuals respectively. [(1)]

TEST PRINCIPLE/ASSAY OVERVIEW

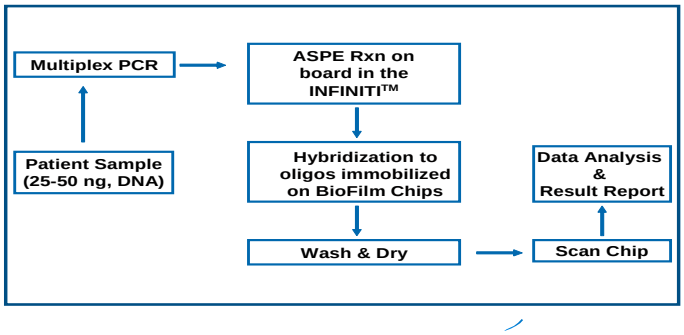
The INFINITI CFTR-31 Assay is designed to detect the presence of the following CFTR gene mutations:

G85E	DelF508	3659delC
R117H	1717-1G>A	3849+10kbC>T
I148T	G542X	R1162X
R334W	G551D	W1282X
R347P	R553X	N1303K
621+1 G>T	R560T	IVS8-5T/7T/9T
711+1 G>T	1898+1G>A	I506V
A455E	2184delA	I507V
1078delT	2789+5G>A	F508C
DelI507	3120+1G>A	

The INFINITI CFTR-31 Assay protocol is based on five major processes:

a) DNA extraction from human blood sample.
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) Signal detection and analysis.

Steps (c) through (e) are automated by the INFINITI Analyzer and the INFINITI PLUS Analyzer. A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI CFTR-31 Assay 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 cystic fibrosis transmembrane conductance regulatory (CFTR) gene in genomic deoxyribonucleic acid (DNA) obtained from EDTA-anticoagulated whole blood samples.

The INFINITI CFTR-31 Assay is comprised of the BioFilmChip® Microarray, the Intellipac® Reagent Module, Amplification Mix, and the INFINITI 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 CFTR-31 Assay uses a microarray chip (Q-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 **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 250µl vials of PCR Amplification Mix.

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 CFTR assay 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 as genotype calls.

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

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

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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.

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 and the 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

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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 (SUFFICIENT FOR 48 TESTS)

- AutoGenomics Product Number 01-1060-02 INFINITI CFTR-31 BioFilmChip® Microarray Magazine
  4 magazines per package; 12 microarray chips per magazine;
- AutoGenomics Product Number 01-2060-02 INFINITI CFTR-31 Intellipac® Reagent Management Module 2 modules per package; 24 tests per module. Each Intellipac module contains:
  - 1.1ml ASPE Master Mix:
    - dNTPs
    - Labeled -dCTP
    - Allele Specific Primers
    - Extension reaction buffer
  - 2.6ml Hybridization Buffer
    - SSC
    - Hybridization Positive Control
    - Sodium Azide Preservative 0.08%
- AutoGenomics Product Number 01-3060-02 INFINITI CFTR-31 Amp Mix. Each package contains 4 x 250µl vials of AMP Mix containing:
  - dNTPs
  - Multiplex primers mix
  - $MgCl_2$
  - PCR reaction buffer
- Product Number 12-0010-02: Wash buffer

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - The INFINITI CFTR-31 Assay can detect the CFTR gene using genomic DNA isolated from blood with sufficient purity, i.e., with the ratio of absorbance at 260nm to absorbance at 280nm of ≥ 1.60. Any DNA extraction method that meets this specification may be used. The INFINITI CFTR-31 Assay has been tested with several commercially available kits. 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.

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 testing. 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	94	2 Minutes	n/a
2	94 50 72	20 Seconds 30 Seconds 30 Seconds	40x
3	72 4	2 min. Hold	n/a

Note: When an Eppendorf Mastercycler EP was used with the ramp rate set at 75%, the total cycling time was 1 hour and 37 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 the INFINITI CFTR-31 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

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The INFINITI CFTR-31 Assay is designed to detect and genotype the CFTR gene. The assay results are provided as a genotype "call", indicating which genotype was detected in the sample. A "no call" means no results available, i.e., no genotype call was made. This could be due to instrument, quality of DNA sample, operator error. When an error message occurs, refer to the Trouble Shooting section of the INFINITI Analyzer Operator's Manual.

All results with a "no call" require a repeat assay.

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 correct oligo hybridizes to the known spot.

Limit of Detection (analytical sensitivity)

Studies demonstrated that the INFINITI CFTR-31 Assay can detect as low as 3ng DNA and as high as 300ng DNA. A minimum of 25ng DNA is recommended for this assay.

Assay Precision and Reproducibility

A three-site inter-laboratory reproducibility study was conducted to demonstrate the reproducibility of the INFINITI CFTR-31 Assay. The study involved three lots of the INFINITI CFTR-31 Assay. The sites ran identical samples comprised of five genomic DNA samples and three whole blood samples. The sites were blinded to sample identity. At each site, each sample was run in triplicate per day/operator for six days. Three operators were required for each site. Three instruments were used in the study.

Instrument-to-Instrument Reproducibility

Instrument	# samples tested	# genotype calls	Correct call rate* (1st time run)	Correct call rate** (Final)
1	132	3,336	99.97%	100%
2	132	3,336	99.0%	99.6%
3	132	3,336	98.6%	99.7%

Lot-to-Lot Reproducibility

Lot	# samples tested	# genotype calls	Correct call rate* (1st time run)	Correct call rate* (Final)
A	132	3,336	99.3	99.8%
B	132	3,336	99.0	99.6%
C	132	3,336	99.2	99.9%

Inter-Laboratory Reproducibility

Site	# samples tested	# genotype calls	Correct call rate* (1st time run)	Correct call rate** (Final)
1	132	3,336	99.97%	100%
2	132	3,336	98.6%	99.7%
3	132	3,336	99.0%	99.6%
Total	396	10,008	99.2%	99.8%

* Correct call rate = # correct genotype calls / total # genotypes

** Final correct call rate based on one retest.

Drug Interference

The effect of potential interfering substances was evaluated. No interference was observed in samples spiked with bilirubin (8mg/dl and 0.08mg/ml), Cholesterol (70mg/dl and 7mg/dl), and Heparin (1333U/dl and 133/Udl). No studies were conducted with oral anti-coagulants, and no claims are being made.

Sample Carry-Over

There was no sample carry-over when varying DNA concentrations were assayed in sequence (one run).

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Method Comparison

The INFINITI CFTR-31 Assay was compared to a commercially available CFTR assay (Tag-It™). The study was conducted at three clinical laboratories; each laboratory used its own CFTR samples.

CF Mutation*	# of Samples	% Agreement First Time Run	% Agreement Final**
delta F 508	30	90%	100%
G542X	8	100%	100%
W1282X	12	83.33%	100%
G551D	12	83.3%	100%
621+1G>T	6	66.7%	100%
N1303K	5	100%	100%
R553X	2	100%	100%
delta I507	3	66.7%	100%
3849+10kbC>T	8	75%	100%
3120+1G>A	3	100%	100%
R117H	22	100%	100%
1717-1G>A	4	100%	100%
2789+5G>A	2	100%	100%
R347P	1	100%	100%
711+1G>T	1	100%	100%
R334W	2	50%	100%
R560T	2	100%	100%
R1162X	5	100%	100%
3659delC	2	100%	100%
A455E	2	100%	100%
G85E	2	100%	100%
2184delA	1	100%	100%
1898+1G>A	3	100%	100%
1078delT	0	n/a	100%
I148T	4	75%	100%
IVS8-7T	1	100%	100%
Total	143	91.6%	100%

* *Mutation determined by Tag-It (Reference comparator method)*

** After one repeat

BIBLIOGRAPHY

1. ACCE review of CF/Prenatal Clinical Validity
http://www.cdc.gov/genomics/gtesting/ACCE/FBR/CF/CFCliVal.htm