K063787

## 510(k) SUMMARY

MAR 13 2008

**TRADE NAME**:

InPlex™ CF Molecular Test

**COMMON NAME**:

*CFTR* Gene Mutation Detection System

**CLASSIFICATION NAME:**

Class II, 21 CFR 866.5900: Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) Gene Mutation Detection System

**PREDICATE DEVICE**:

Tag-It™ Cystic Fibrosis Kit, K043011

**DEVICE DESCRIPTION:**

InPlex™ CF Molecular Test amplifies specific regions of the *CFTR* gene in genomic DNA extracted from human whole peripheral blood. Each amplified DNA sample is subsequently mixed with Cleavase® enzyme and buffer then added to a loading port on an InPlex™ micro-fluidic card. An InPlex™ card contains eight sample-loading ports, each connected to 48 independent reaction chambers. Twenty-eight of these reaction chambers contain dried assay mixes specific for reporting the 23 ACMG/ACOG recommended *CFTR* mutations and variants. The remaining chambers consist of a "No Invader® Control", an Independent Quality Control, and several unused chambers.

After an InPlex™ card is loaded; the channels are mechanically sealed using a micro-fluidic card sealer, isolating each individual reaction chamber from all other chambers. The card is then incubated to allow individual Invader® reactions to occur. Following incubation, the card is read in a multi-well fluorometer and the raw signal data are imported into the InPlex™ CF Molecular Test Call Reporting Software for final result analysis.

**INDICATIONS FOR USE / INTENDED USE:**

InPlex™ CF Molecular Test is an *in vitro* diagnostic device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in genomic DNA samples isolated from human peripheral whole blood specimens. The panel includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG). The InPlex™ CF Molecular Test is a qualitative

genotyping test that provides information intended to be used for cystic fibrosis carrier screening as recommended by ACMG and the 2005 American College of Obstetricians and Gynecologists (ACOG) for adults of reproductive age, as an aid in newborn screening for cystic fibrosis, and in confirmatory diagnostic testing for cystic fibrosis in newborns and children.

The test is not indicated for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes and results should be used in conjunction with other available laboratory and clinical information.

## SUMMARY OF TECHNOLOGICAL CHARACTERISTICS:

| Characteristics | Tag-It™ Cystic Fibrosis Kit | InPlex™ CF Molecular Test |
|---|---|---|
| Intended Use | The Tag-It™ Cystic Fibrosis Kit is a device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in human blood specimens. The panel includes mutations and variants currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG), plus some of the worlds most common and North American-prevalent mutations. The Tag-It™ Cystic Fibrosis Kit is a qualitative genotyping test which provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children.<br><br>The kit is not indicated for use in fetal diagnostic or pre-implantation testing. This kit is also not indicated for stand-alone diagnostic purposes. | Same as the predicate device for the ACMG/ACOG panel, but with no further mutations beyond the ACMG/ACOG panel. |
| Gene mutation and variant screening | The Tag-It™ Cystic Fibrosis Kit simultaneously screens for the 23 cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations, plus 16 other mutations and 4 *variants (polymorphisms)* recommended by the American College of Medical Genetics (ACMG) and American College of Obstetricians and Gynecologists (ACOG) | The InPlex™ CF Molecular Test simultaneously screens for the 23 cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations and IVS8 *variants (polymorphisms)* recommended by the American College of Medical Genetics (ACMG) and American College of Obstetricians and Gynecologists (ACOG). |

| Characteristics | Tag-It™ Cystic Fibrosis Kit | InPlex™ CF Molecular Test |
|---|---|---|
| Technology: Genomic DNA sample preparation | Genomic DNA Sample Preparation from whole blood | Same as the predicate device |
| Technology: Assay reactions | (1) Multiplex PCR<br>(2) Multiplex Allele Specific Primer Extension (ASPE)<br>(3) PCR samples are treated with enzymes (Alkaline Phosphatase (SAP) to inactivate any remaining nucleotides (especially dCTP), and with Exonuclease I (EXO) to degrade any primers left over from the PCR reaction)<br>(4) Multiplex allele-specific primer extension (ASPE) for genotyping<br>(5) Hybridization using 5 µL ASPE with 45 µL bead | (1) Multiplex PCR<br>(2) Amplified samples are mixed with enzyme (Cleavase®) and buffer and added to micro-fluidic cards<br>(3) Invader® reactions occur whereby two oligonucleotides (a discriminatory Primary Probe and an Invader® oligonucleotide) hybridize in tandem to the target DNA to form an overlapping structure that is subsequently cleaved by an enzyme (Cleavase®). |
| Data Analysis | Assay result data is analyzed by a software program which provides a final genotype | Same as the predicate device |

## PERFORMANCE DATA:

### ANALYTICAL STUDIES

#### DNA Extraction Equivalency

Equivalency between four different DNA purification methodologies and their subsequent performance using the InPlex™ CF Molecular Test was evaluated using 7 CF positive genomic DNA samples isolated from whole peripheral blood. Internal processing involved the purification of the same 7 samples using four commonly used, commercially available DNA extraction kits. These samples were also genotyped by DNA sequencing to confirm results.

Genotype calls as generated by the InPlex™ CF Molecular Test were compared to DNA sequencing based genotyping for all extraction methods.

The seven CF positive genomic DNA samples extracted from human peripheral whole blood using four different DNA extraction kits generated a total of 644 calls with 28 positive and 616 normal (negative) calls. Each extraction method provided 161 data points. All calls were concordant with the DNA sequencing based genotyping, providing an overall agreement of 100% (99.5%, 1-sided lower 95% Confidence Limit).

**Fluorometer Equivalency**

Equivalency between three multi-well fluorometers meeting indicated specifications in generating signal data from the InPlex™ CF Molecular Test micro-fluidic card was determined using a panel of eight genomic DNA (gDNA) samples. The genomic DNA samples were either positive for a subset of the *CFTR* mutations listed as part of the ACMG recommended panel or normal. The eight samples were tested in triplicate providing a minimum of 1,656 data points (8 samples x 23 assays x 3 replicates x 3 plate reader data).

All InPlex™ CF Molecular Test genotype results for the characterized gDNA samples described were concordant with DNA sequencing genotype results and in 100% (99.8% 1-sided lower 95% Confidence Limit) agreement regardless of the fluorometer used.

**Incubator Equivalency**

Three thermal incubators were evaluated for equivalency in generating concordant genotype data using the InPlex™ CF Molecular Test. A panel of eight gDNA samples was tested in triplicate using each incubator. A total of 1,656 data points (8 samples x 23 assays x 3 replicates x 3 incubators - 552 data points per incubator) were generated in this study. The percent agreement to known genotypes was 99.88% (99.74%, 1-sided 95% Confidence Limit).

**Interfering Substances**

The performance of the InPlex™ CF Molecular Test was evaluated using eight, CF positive, genomic DNA samples isolated from whole peripheral blood in the absence (untreated) and presence (treated) of potential interfering substances. These substances included compounds that are endogenous to the blood sample matrix, compounds associated with blood collection and those that may result from sample preparation solutions. Depending on the nature of the substance, compounds were added either directly to the blood sample or to purified genomic DNA. Compounds added to the blood sample included bilirubin, triglycerides, and potassium EDTA (blood collection anti-coagulant). Compounds added to the purified genomic DNA sample included Qiagen® Buffer AW2 and hemoglobin. Following DNA extraction, eight whole peripheral blood samples were each tested as treated and untreated samples. The number of samples provided 1,104 data points (8 blood samples x 23 assays x 6 treatments).

The InPlex™ CF Molecular Test detected a total of 23 *CFTR* mutations. The genotype calls for the DNA samples containing an interfering substance were compared to the untreated samples for calculation of percent agreement. The results of this study showed that the InPlex™ CF Molecular Test achieved a 100% agreement (99.7%, 1-sided lower 95% Confidence Limit)

between the genotypes of the samples containing potential interfering substances and bi-directional sequencing.

**Limit of Detection**

An input genomic DNA concentration range was evaluated with the InPlex™ CF Molecular Test. A panel of eight gDNA samples were prepared and tested at eight concentrations ranging from 1ng/µL to 150ng/µL (total input DNA range of 5-750ng / reaction). The number of samples analyzed provided 1,472 data points (8 samples x 8 concentrations x 23 assays). One hundred and eighty four genotype calls were generated per DNA concentration tested (8 samples x 1 concentration x 23 assays).

Genotype call results for all eight characterized samples were assessed for percent agreement at each concentration. The lower limit of detection was defined as the lowest DNA concentration in which a 99% or greater concordance with DNA sequencing was observed.

Based on these results, a 5ng/µL DNA concentration (input DNA of 25ng) provided a percent agreement of 100% (98.4%, 1-sided lower 95% Confidence Limit) with DNA sequencing qualifying it as the lower limit of detection. At the highest DNA concentration tested, 150ng/µL (input DNA of 750ng), a 99.5% percent agreement was obtained (98.6%, 1 sided lower 95% Confidence Limit). The remaining DNA concentrations tested, 10, 20, 50, and 100ng/µl (input DNA of 50, 100, 250 and 500ng) all obtained a 100 percent agreement (98.4%, 1-sided lower 95% Confidence Limit) with expected results (see Table 3).

**Table 3. Percent Agreement for Each DNA Concentration**

| DNA Concentration (ng/µL ) | # of Concordant Calls | # of Low Signal Calls | # of Equivocal Calls | Total # of Calls | Percent Agreement | 1 sided lower 95% CI |
|---|---|---|---|---|---|---|
| 1 | 148 | 27 | 9 | 184 | 80.40% | 75.6% |
| 2 | 178 | 4 | 2 | 184 | 96.80% | 94.6% |
| 5 | 184 | 0 | 0 | 184 | 100.00% | 98.4% |
| 10 | 184 | 0 | 0 | 184 | 100.00% | 98.4% |
| 20 | 184 | 0 | 0 | 184 | 100.00% | 98.4% |
| 50 | 184 | 0 | 0 | 184 | 100.00% | 98.4% |
| 100 | 184 | 0 | 0 | 184 | 100.00% | 98.4% |
| 150 | 183 | 0 | 1 | 184 | 99.50% | 98.6% |

**Lot-to-Lot Equivalency**

Equivalency between three lots of InPlex™ CF Molecular Test kits was evaluated. A panel of 23 *CFTR* gDNA samples was tested in singlicate with each lot. Five hundred twenty-nine (529) genotype calls were generated for each lot tested (23 samples x 23 assays). All genotype calls were in 100% (99.4% 1-sided lower 95% Confidence Limit) agreement to pre-characterized gDNA genotypes for each lot tested.

## Accuracy and Repeat Rate

Accuracy and repeat rate of the InPlex™ CF Molecular Test was determined by comparing InPlex™ CF Molecular Test genotyping results from of a panel of unique genomic DNA samples to bi-directional DNA sequence analysis. The sample panel tested consisted of genomic DNA samples isolated from peripheral whole blood and cell lines. A total of 23 CFTR mutations (as well as the IVS8-5T/7T/9T variants) were tested in this study. Genotype calls were compared between the DNA sequencing results and the InPlex™ CF Molecular Test results for the calculation of overall agreement. In addition, positive and negative agreement for each mutation was calculated. The repeat rate was determined by the number of samples that generated an invalid genotype call for one or more mutations with the InPlex™ CF Molecular Test on the first attempt.

This study was performed on a total of 123 unique samples containing 144 positive *CFTR* calls and 2808 normal (negative) *CFTR* calls.

The results of this study showed that the InPlex™ CF Molecular Test achieved 99.96% (2951/2952) overall agreement (99.9%, 1-sided lower 95% Confidence Limit), 100% (144/144) positive agreement (97.9%, 1-sided lower 95% Confidence Limit), and 99.96% (2807/2808) negative agreement (99.9%, 1-sided lower 95% Confidence Limit). There was one "Invalid" call out of 123 tests, resulting in a repeat rate of 0.8%. This overall agreement of 99.96% is derived without repeat testing. No repeat testing was done to support this study.

Of the 123 unique samples in the initial study, four (4) samples (negative for R117H) were confirmed by sequencing as 7T/9T, but miscalled as 7T/7T by the InPlex™ CF Molecular Test. The four miscalled samples were tested in a reproducibility study once a day for 5 days in conjunction with two 7T/7T and two 7T/9T samples, which were called correctly in the initial study. Upon retesting all four (4) miscalled samples gave the correct 7T/9T result on each of the five consecutive days. The root cause of the miscalls was determined to potentially be due to the InPlex cards failing to be rotated during incubation resulting in temperature inconsistencies

(e.g., hot spots) near the heat source causing the 9T/7T ratio to fall below the cut-off for the 9T/7T call. The 7T/7T miscall was reproduced in one of the four samples experimentally.

**Freeze-Thaw Tolerance**

A study was performed to establish the tolerance of the InPlex™ CF Molecular Test to various freeze-thaw cycles. The InPlex™ CF Molecular Test kit were subjected to 2, 4, 6, 8, 10 and 12 freeze-thaw cycles (test points) followed by functional testing of the product with control samples. Each freeze-thaw cycle consisted of freezing at -20°C±3°C for at least 24 hours and thawing at room temperature for 30 minutes. For the multiple freeze-thaw cycles the cards were returned to -20°C±3 °C storage after 30 minutes at room temperature.

This study used a panel of 23 CFTR gDNA samples to evaluate the 23 CFTR mutations at each freeze thaw cycle. The samples generated 3,703 data points (23 samples x 23 assays x 7 test points). Each Freeze-Thaw cycle generated 529 data points (23 samples x 23 assays).

The InPlex™ CF Molecular Test achieved an overall percent agreement of 100% (99.92%, 1-sided lower 95% Confidence Limit) as compared to DNA sequencing for all freeze-thaw cycles tested. See Table 4 below.

**Table 4. Percent Agreement for Each Freeze-Thaw Period**

| Freeze-Thaw Cycle | Concordant Calls | Total # of Calls | Percent Agreement | 1-sided lower 95% CI |
|---|---|---|---|---|
| 0 | 529 | 529 | 100% | 99.4% |
| 2 | 529 | 529 | 100% | 99.4% |
| 4 | 529 | 529 | 100% | 99.4% |
| 6 | 529 | 529 | 100% | 99.4% |
| 8 | 529 | 529 | 100% | 99.4% |
| 10 | 529 | 529 | 100% | 99.4% |
| 12 | 529 | 529 | 100% | 99.4% |
| Total | 3,703 | 3,703 | 100% | 99.92% |

Eight or fewer freeze-thaw cycles for the InPlex™ Molecular Test are recommended to ensure the InPlex™ CF Molecular Test maintains the ability to generate accurate genotype calls.

**Real-Time Stability**

A study was performed with the objective to establish the real-time stability of the InPlex™ CF Molecular Test when stored under pre-defined storage conditions.

Three lots of the InPlex™ micro-fluidic cards and three lots of InPlex™ reagents were used to create three master lots of the InPlex™ CF Molecular Test used in this study. A panel of seven *CFTR* gDNA samples and a panel of eight control samples comprised of pooled amplicons that cover the mutations in the InPlex™ CF Molecular Test were used to assess the performance. The samples were tested in duplicate with each lot for each storage condition at each time point.

Three lots of the InPlex™ Reagents are being maintained at the recommended storage temperature of (-20°C +/-3°C). The InPlex™ micro-fluidic cards are stored at the recommended temperature (-20°C +/-3°C), substandard temperature (4°C – 8°C), and ship-stressed conditions (37°C±1°C for 48 hours, then room temperature (25 °C+/-3°C).

A total of 345 calls were evaluated (15 samples x 23 assays) for each lot tested. All lots met the passing criterion for the Real-Time Stability performance evaluation study with an observed percent agreement of 100% (99.1%, 1-sided lower 95% Confidence Limit) (see Table 5).

**Table 5: Percent Agreement for Each Lot at Each Storage Condition**

| Time point | Storage Condition | Master Lot | # of Concordant Calls | Total # of Calls | Percent Agreement | 1-sided lower 95% CI |
|---|---|---|---|---|---|---|
| T=0 | NA | C04AC | 345 | 345 | 100% | 99.1% |
| T=0 | NA | C05AA | 345 | 345 | 100% | 99.1% |
| T=0 | NA | C06AA | 345 | 345 | 100% | 99.1% |
| T=1 | -20°C±3°C | C04AC | 345 | 345 | 100% | 99.1% |
| | | C05AA | 345 | 345 | 100% | 99.1% |
| | | C06AA | 345 | 345 | 100% | 99.1% |
| T=1 | 4-8°C | C04AC | 345 | 345 | 100% | 99.1% |
| | | C05AA | 345 | 345 | 100% | 99.1% |
| | | C06AA | 345 | 345 | 100% | 99.1% |
| T=1 | 37°C±1°C for 48 hours, then hold at room temp | C04AC | 345 | 345 | 100% | 99.1% |
| | | C05AA | 345 | 345 | 100% | 99.1% |
| | | C06AA | 345 | 345 | 100% | 99.1% |

This study showed that multiple lots of the InPlex™ CF Molecular Test produce equivalent results after storage for one month under the conditions tested. This is an ongoing study for which the results from future time points will be used to extend shelf-life dating.

**Reproducibility Study**

Samples from pre-characterized commercially available reference materials were used to assess the reproducibility of the InPlex™ CF Molecular Test. The study was conducted in two main phases, a proficiency phase, and a performance phase. The purpose of the proficiency phase was to ensure that each site had the required expertise in the fundamental methodologies so that meaningful assay reproducibility measures could be calculated from the data generated during the performance phase. The proficiency phase was carried out in two steps, one for the training on the product methodology, and one for the confirmation of proficiency. Proficiency testing was performed using eight (8) pre-characterized, gDNA samples. Technicians at each of three investigative sites ran the same DNA samples in duplicate. Data generated during this phase of the study was to demonstrate technician proficiency only and not used to demonstrate the clinical performance of the test.

The reproducibility testing for the performance phase was conducted using 23 samples containing mutations representing the ACMG recommended panel. Each site ran the same samples tested in duplicate by each technician, with tests being run on each of five (5) non-consecutive days. There were two technicians at each test site.

Percent agreement ranged from 99.962% to 100% at each of the three sites and 99.987% to 99.994% across all sites. Out of 31,740 calls generated (23 [samples] x 23 [assays] x 2 [replicates] x 2 [operators] x 5 [days] x 3 [sites]), the within operator agreement for two calls at one site yielded an equivocal result for both replicates of a single mutation. The overall accuracy rate was 31,738/31,740 = 99.994% (99.986, 1-sided lower 95% Confidence Limit).

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

MAR 13 2008

Third Wave Technologies, Inc.
c/o Andrew A. Lukowiak, Ph.D.
Associate Vice President of Product Development
502 S. Rosa Road.
Madison, WI 53719-1256

Re: k063787
Trade/Device Name: InPlex™ CF Molecular Test
Regulation Number: 21 CFR 866.5900
Regulation Name: Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation detection system
Regulatory Class: Class II
Product Code: NUA
Dated: March 7, 2008
Received: March 10, 2008

Dear Dr. Lukowiak:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The

FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (240) 276-0450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at 240-276-3474. For questions regarding the reporting of device adverse events (Medical Device Reporting (MDR)), please contact the Division of Surveillance Systems at 240-276-3464. You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Robert L. Becker, Jr., M.D., Ph.D.
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

## INDICATIONS FOR USE STATEMENT

**510(k) Number (if known): K063787**

**Device Name: InPlex™ CF Molecular Test**

**Indications For Use:**

InPlex™ CF Molecular Test is an *in vitro* diagnostic device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in genomic DNA samples isolated from human peripheral whole blood specimens. The panel includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG), plus other more common and North American-prevalent mutations. The InPlex™ CF Molecular Test is a qualitative genotyping test that provides information intended to be used for cystic fibrosis carrier screening as recommended by ACMG and the 2005 American College of Obstetricians and Gynecologists (ACOG) for adults of reproductive age, as an aid in newborn screening for cystic fibrosis, and in confirmatory diagnostic testing for cystic fibrosis in newborns and children.

The test is not indicated for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes and results should be used in conjunction with other available laboratory and clinical information.

**(PLEASE DO NOT WRITE BELOW THIS LINE - CONTINUE ON ANOTHER PAGE IF NEEDED)**

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**Concurrence of CDRH, Office of Device Evaluation (ODE)**

Prescription Use X OR Over-The-Counter Use

(Per 21 CFR 801.109) (Optional Format 1-2-96)

maria m chan

**Division Sign-Off**

**Office of In Vitro Diagnostic Device Evaluation and Safety**

510(k) K063787