510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k043011

B. Purpose for Submission:
New device

C. Measurand:
CFTR (cystic fibrosis transmembrane conductance regulator) gene from human blood specimens

D. Type of Test:
Multiplex PCR followed by multiplex allele specific primer extension for genotyping, hybridized to multiplexed fluorescing microparticles, detected by flow cytometry

E. Applicant:
Tm Bioscience Corporation

F. Proprietary and Established Names:
Tag-It™ Cystic Fibrosis Kit

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5900, CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection system
2. Classification:
   Class II
3. Product code:
   NUA, System, test, CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   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.

   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.

2. Indication(s) for use:
   Same as intended use.

3. Special conditions for use statement(s):
   For prescription use only.

   Since the Tag-It™ Cystic Fibrosis Kit detects a limited number of mutations (out of more than 1300 mutations identified in the CFTR gene), it should not be used alone to diagnose cystic fibrosis. Assay results should be interpreted only in the context of the overall testing algorithm and clinical status of the patient.

   Carrier screening for CF using molecular testing has been recommended by the NIH and the ACMG/ACOG for reproductive couples of all ethnicities, individuals with a family history of CF, and pregnant women. When used in CF carrier detection, the Tag-It™ Cystic Fibrosis Kit may identify: (i) positive/positive, positive/negative, and negative/negative couples; (ii) individuals who have a family history of CF; (iii) otherwise healthy males who carry mutations or variants associated with infertility. Interpretation of results will depend on the patient demographics, and must take into consideration that some mutations are more common in certain populations.

   Clinical variability of the disease based on the genotype-phenotype correlation variation for different mutations may result in the need for genetic counseling. Genetic counseling will enable individuals and couples to receive accurate information about risks and prognostic factors.

4. Special instrument requirements:
   Luminex 100 IS (Integrated System); other names used: Luminex® 100 xMAP™ System.

I. Device Description:

The Tag-It™ Cystic Fibrosis Kit includes the following components:

- Multiplex PCR Primer Mix including dNTPs designed to simultaneously produce 16 amplimers of the CFTR gene
- Multiplex ASPE Primer Mix including dNTPs (86 primers designed to hybridize to either wild-type or mutant alleles with proprietary sequences at their 5' ends designed to specifically hybridize to complementary sequences coupled to the bead component of the kit)
- Coupled Bead Suspension (86 spectrally distinguishable populations of 5.0 micron polystyrene beads internally dyed with red and infrared fluorochromes coupled to proprietary DNA sequences designed to specifically hybridize to complementary sequences on the ASPE primers)
- 10X Wash Buffer
- Tag-It™ Data Analysis Software (TDAS CF-I)

J. Substantial Equivalence Information:

1. Predicate device name(s):
   None
2. Predicate 510(k) number(s):

None

3. Comparison with predicate:
   Not applicable

K. Standard/Guidance Document Referenced (if applicable):

- American College of Medical Genetics (ACMG)/American College of Obstetricians and Gynecologists
  - 2001, 2002, 2004 ACMG Technical Standards and Guidelines for CFTR Mutation Testing
  - ACMG 2004 Standards and Guidelines for Clinical Genetics Laboratories
- 2004 Cystic Fibrosis Foundation (CFF) / Center for Disease Control (CDC) Recommendations on Newborn Screening for Cystic Fibrosis
- CLSI Guidances
  - MM01-A: Molecular Diagnostic Methods for Genetic Diseases
  - EP5-A: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline.
  - EP12-A: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline.
- FDA Guidances
  - CDRH Draft Guidance for Industry and FDA Reviewers titled: *Multiplex Tests for Heritable DNA Markers, Mutations and Expression Patterns,* Feb 27, 2003.
  - CDRH Draft Guidance for Industry and FDA Reviewers titled: *Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests,* Mar 12, 2003.
  - CDRH Guidance for Industry and FDA Reviewers titled: *Guidance for the content of premarket submissions for software contained in medical devices,* May 29, 1998.
  - CDRH Guidance for Industry and FDA Staff titled: *General Principles of Software Validation*, Jan 11, 2002.

L. Test Principle:

The Tag-It™ Cystic Fibrosis Kit tests for 39 mutations and 4 polymorphisms in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. These mutations include those currently recommended for testing by the ACMG/ACOG (denoted with an asterisk in the list below) plus 16 mutations shown to be associated with CF phenotypes in Caucasian Americans, Hispanic Americans and African Americans:

ΔF508*	1717-1G>A*	W1282X*	2307insA
ΔI507*	R560T*	N1303K*	Y1092X
G542X*	R553X*	394delTT	M1101K
G85E*	G551D*	Y122X	S1255X
R117H*	1898+1G>A*	R347H	3876delA
621+1G>T*	2184delA*	V520F	3905insT
711+1G>T*	2789+5G>A*	A559T	*5/7/9T*
1078delT	3120+1G>A*	S549N	*F508C*
R334W*	R1162X*	S549R	*I507V*

R347P*	3659delC*	1898+5G>T	*I506V*
A455E*	3849+10kbC>T*	2183AA>G	

After sample preparation of genomic DNA (which is not a part of the Tag-It™ assay), a multiplex PCR reaction is carried out under optimized conditions. Multiplex allele-specific primer extension (ASPE) is then used for genotyping. In this step, each allele (wild-type or mutant) is detected by a primer with a unique DNA sequence (tag) at its 5' end. Each mutant locus has two allele-specific primers (ASPs), or three for a tri-allelic locus. For each ASP, the 3' end of the primer is a perfect match for its allele, but will have a 3' mismatch on any other allele. A DNA polymerase is used that will only extend the primer when there is a perfect match on the 3' end, so that the primer is only extended if its target allele is present in the sample. Biotin-dCTP is incorporated into the extending chain if extension occurs. After ASPE, the reaction is added directly to microwells containing bead-immobilized oligonucleotides (anti-tags) which are the complements of the DNA tags on the allele specific primers. The beads which contain the anti-tags are spectrally distinguishable from each other. A fluorescent reporter molecule (streptavidin-phycoerythrin) is bound to the biotin on the extended primers. Each tagged primer hybridizes only to its unique anti-tag complement; therefore, each colored bead represents a specific allele, through the bead/anti-tag/tagged primer association. The beads are then analyzed by the Luminex® xMAP™ machine. The xMAP™ instrument contains two lasers: one identifies the color-coded bead, and the other identifies the presence or absence of extended allele specific primer through the phycoerythrin reporter. Thus, the genotype of that locus is identified by the presence of phycoerythrin signal attached to one or both ASPs.

All mutations and polymorphisms are genotyped in a single multiplex reaction. The data generated by the xMAP™ instrument is analyzed by the Tag-It™ Data Analysis Software (TDAS CF-I) to provide a final genotype for the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
   The Tag-It™ Cystic Fibrosis Kit has a reproducibility of >99.99%. This performance characteristic was established by testing 3 lots of the kit at each of 3 sites (1 internal, 2 external sites, at least 2 operators at each site), over multiple days, using 4 Luminex® systems. The samples tested were genomic samples obtained from the Coriell Cell Repository (Camden, NJ) which were sequenced at all loci probed by the Tag-It™ assay. For each Tag-It™ run, samples were assayed in quadruplicate. Table 1 below summarizes the genotypes of the samples tested, the number of replicates tested, the calls confirmed by sequencing and the missed calls. Of the 828 replicates tested, there were 7 replicates requiring re-runs due to a sample failure resulting from inadequate signal. TDAS CF-I generates a Sample Failure if one of the probed loci has signals below a predefined threshold. In this situation, TDAS CF-I does not provide genotyping calls for any of the mutations/variants probed in that sample. After these 7 replicates were re-run, all calls were made correctly.

   Each of the 828 replicates tested were assayed at 43 loci by the Tag-It™ Cystic

Fibrosis Kit, generating a total of 35,604 calls. Of these, 35,602 (i.e. reproducibility >99.99%) were confirmed by sequencing. The 2 missed calls are highlighted in the table below. In one case, the expected call for the R560T locus was HET but TDAS CF-I generated a NO CALL the first time that one of the 36 replicates of that sample was run. TDAS CF-I generates a NO CALL when the allelic ratio does not fall within pre-defined ranges for either a WT, HET, or Mu D call. When this replicate was re-run, the expected call for this locus (i.e. R560T HET) was made, consistent with the calls made for all other replicates of that sample. In the second case, the expected call for ΔF508 was HET whereas TDAS generated a Mu D call the first time that one of the 36 replicates of that sample was run. When this replicate was re-run, the expected call for (i.e. ΔF508 HET) was made, consistent with the calls made for all other replicates of that sample.

Table 1: Reproducibility of the Tag-It™ Cystic Fibrosis Kit (>99.99%: site-to-site, lot-to-lot, operator to operator)

Purified Genomic Samples: Mutations and Variants detected by bi directional, dideoxy terminal DNA sequencing (all other probed loci were WT)	Number of Sample Replicates Assayed by the Tag-It™ Cystic Fibrosis Kit (3 kit lots used at each of the 3 sites)			Number of Confirmed Tag-It™ Calls out of a total of 516 per sample (43 calls per sample x 4 replicates x 3 lots) per site			
Sample Genotype	**Site A**	**Site B**	**Site C**	**Site A**	**Site B**	**Site C**	**Missed Calls**
ΔF508/ΔF508/9T	12	12	12	516	516	516	0
R553X/ΔF508/7T/9T	12	12	12	516	516	516	0
3659delC/ΔF508/7T/9T	12	12	12	516	516	516	0
R560T/ΔF508/7T/9T	12	12	12	515	516	516	1[a]
N1303K/G1349D /7T/9T	12	12	12	516	516	516	0
R334W/7T	12	12	12	516	516	516	0
3120+1G>A/621+1G>T /7T/9T	12	12	12	516	516	516	0
G542X/7T/9T	12	12	12	516	516	516	0
G542X/G542X /9T	12	12	12	516	516	516	0
M1101K/M1101K /7T	12	12	12	516	516	516	0
711+1G->T(T)/621+1G->T /7T/9T	12	12	12	516	516	516	0
G551D/7T/9T	12	12	12	516	516	516	0
W1282X/5T/7T	12	12	12	516	516	516	0
ΔI507/7T	12	12	12	516	516	516	0
621+1G->T/ΔF508/9T	12	12	12	516	516	516	0
G85E/621+1G->T /7T/9T	12	12	12	516	516	516	0
A455E/ΔF508/9T	12	12	12	516	516	516	0
2789+5G->A/2789+5G->A /7T	12	12	12	516	516	516	0

Purified Genomic Samples: Mutations and Variants detected by bi directional, dideoxy terminal DNA sequencing (all other probed loci were WT)	Number of Sample Replicates Assayed by the Tag-It™ Cystic Fibrosis Kit (3 kit lots used at each of the 3 sites)			Number of Confirmed Tag-It™ Calls out of a total of 516 per sample (43 calls per sample x 4 replicates x 3 lots) per site			
3849+10C->T/3849+10C->T /7T	12	12	12	516	516	516	0
1717-1G->T(A)/7T	12	12	12	516	516	516	0
R1162X/7T	12	12	12	516	516	516	0
R347P/G551D /7T	12	12	12	516	516	516	0
R117H/ΔF508/5T/9T	12	12	12	516	516	515	1[b]

a) One replicate at Site A had a No Call for R560T. The expected call (R560T HET) was made after re-run.
b) One replicate at Site C had a Mu D call for ΔF508. The expected call (ΔF508 HET) was made after re-run.

The Tag-It™ Cystic Fibrosis Kit detects all mutant/variant/wild-type alleles of the 43 loci assayed with a precision of >99.99%. This performance characteristic has been defined based on an analysis of all genotyping calls (WT, HET, Mu D, variant detected) that can be made at each locus probed by the Tag-It™ Cystic Fibrosis Kit. Table 2 below summarizes data from a study designed according to the CLSI EP5-A guideline. In that study, variation in allelic ratio (AR) values was assessed from results derived from day-to-day (a total of 5 days), user-to-user (a total of 5 users), lot-to-lot (a total of 3 lots of Tag-It™ reagents), reagent-to-reagent (a total of 3 lots of each of the 4 ancillary reagents used to perform the assay), and machine-to-machine (including 3 thermocyclers and 3 Luminex® systems) analyses. AR variation was evaluated both within and between Tag-It™ runs using replicates of a Coriell genomic sample and sequenced synthetic controls (see below) representing all genotyping calls that can be made for all 43 loci that are included on the Tag-It™ panel. Depending on the experiment, between 60 and 240 calls (WT, HET, Mu D, and variant detected) were compared per locus. The repeatability of calls is shown in Table 6 below. Overall, results of this study show that all genotyping calls that can be made by the Tag-It™ Cystic Fibrosis Kit can be made correctly and reproducibly across days, users, reagent lots (Tag-It™ and ancillary) and machines (Luminex® systems and thermocyclers).

Table 2: Precision of the Tag-It™ Cystic Fibrosis Kit (>99.9% repeatability between days, users, reagents, machines)

43 loci tested	% correct calls made (WT, HET, Mu D, variant detected) for each locus on the Tag-It™ panel[a]					
	Day to Day	User to User	Lot to Lot (Tag-It™ reagents)	Reagent to Reagent (3 ancillary)[c]	Thermocycler to Thermocycler	Luminex to Luminex
	5 days	5 users	3 lots	3 lots of each	3 machines	3 machines
G85E	100%	99.6%[b]	100%	100%	100%	100%
394delTT	100%	100%	100%	100%	100%	100%
R117H	100%	100%	100%	100%	100%	100%
Y122X	100%	100%	100%	100%	100%	100%
621+1G>T	100%	100%	100%	100%	100%	100%
711+1G>T	100%	100%	100%	100%	100%	100%

43 loci tested	% correct calls made (WT, HET, Mu D, variant detected) for each locus on the Tag-It™ panel[a]					
	Day to Day	User to User	Lot to Lot (Tag-It™ reagents)	Reagent to Reagent (3 ancillary)[c]	Thermocycler to Thermocycler	Luminex to Luminex
	5 days	5 users	3 lots	3 lots of each	3 machines	3 machines
1078delT	100%	100%	100%	100%	100%	100%
R334W	100%	100%	100%	100%	100%	100%
R347P/R347H	100%	100%	100%	100%	100%	100%
A455E	100%	100%	100%	100%	100%	100%
dI507/ dF508	100%	100%	100%	100%	100%	100%
V520F	100%	100%	100%	100%	100%	100%
1717-1G>A	100%	100%	100%	100%	100%	100%
G542X	100%	100%	100%	100%	100%	100%
S549N	100%	100%	100%	100%	100%	100%
S549R (T>G)	100%	100%	100%	100%	100%	100%
G551D	100%	100%	100%	100%	100%	100%
R553X	100%	100%	100%	100%	100%	100%
A559T	100%	100%	100%	100%	100%	100%
R560T	100%	100%	100%	100%	100%	100%
1898+1G>A	100%	100%	100%	100%	100%	100%
1898+5G>T	100%	100%	100%	100%	100%	100%
2183AA>G	100%	100%	100%	100%	100%	100%
2184delA	100%	100%	100%	100%	100%	100%
2307insA	100%	100%	100%	100%	100%	100%
2789+5G>A	100%	100%	100%	100%	100%	100%
3120+1G>A	100%	100%	100%	100%	100%	100%
Y1092X	100%	100%	100%	100%	100%	100%
M1101K	100%	99.6%[b]	100%	100%	100%	100%
R1162X	100%	100%	100%	100%	100%	100%
3659delC	100%	100%	100%	100%	100%	100%
S1255X	100%	100%	100%	100%	100%	100%
3849+10kbC>T	100%	100%	100%	100%	100%	100%
3876delA	100%	100%	100%	100%	100%	100%
3905insT	100%	100%	100%	100%	100%	100%
W1282X	100%	100%	100%	100%	100%	100%
N1303K	100%	100%	100%	100%	100%	100%
5T/7T/9T	100%	100%	100%	100%	100%	100%
I506V/I507V/ F508C	100%	100%	100%	100%	100%	100%

a) Depending on the experiment, between 60 and 240 calls (WT, HET, Mu D, and variant detected) were compared per locus using sequenced genomic and synthetic samples

b) On one occasion (out of 240) for each of M1101K and G85E, there was a "No Call" resulting from low signal which caused ARs to fall outside of the pre-defined ranges for making either a WT, HET or Mu D genotyping call.

c) 3 separate experiments, each with the same lot of the Tag-It™ Cystic Fibrosis Kit but with 3 different lots of Taq, Tsp, and SAP, respectively.

b. Linearity/assay reportable range:
Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
Stability studies on the Tag-It™ Cystic Fibrosis Kit support a shelf-life of 1 year when kit reagents are stored at -25°C to -15°C. Inadvertent temporary exposure (up to 6 days) at temperatures exceeding the recommended storage temperature (up to

37°C) and repeated freeze-thaw cycles (up to 3) will not compromise the integrity of the Tag-It™ Cystic Fibrosis Kit.

d. Detection limit:
Although the recommendations of the insert are that 25 ng of extracted DNA be used for the PCR reaction described above, it has been established with purified genomic samples that, when the Tag-It™ assay is run as per the methods described, samples with input values ranging between 1 ng and 200 ng provide the correct genotyping calls. It is recommended that the DNA sample be extracted from whole blood (EDTA or citrate) using methodologies which provide purified genomic DNA with an ultraviolet light absorbance ratio at 260/280 greater than 1.7.

e. Analytical specificity:
Interfering Substances
The Tag-It™ Cystic Fibrosis Kit can be used to detect mutations in the CFTR gene using genomic DNA isolated from blood with a sufficient purity, i.e., with the ratio of absorbance at 260 nm vs. that at 280 nm being 1.7-2.0. Interference studies were not conducted since input into the assay is purified genomic DNA and there is sufficient data in the literature to support a recommendation that the DNA sample be extracted from whole blood (EDTA or citrate) using methodologies which provide genomic DNA with an ultraviolet light absorbance ratio at 260/280 which is greater than 1.7.

f. Assay cut-off:
Not applicable.

2. Comparison studies:
   a. Method comparison with predicate device:
   All genotyping calls made by the Tag-It™ Cystic Fibrosis Kit have been compared to those made by bi-directional dideoxy terminal DNA sequencing in clinical samples or, in cases of rare alleles for which clinical samples were not available, in replicates of purified genomic samples obtained from the Coriell Institute (Camden, NJ) and synthetic oligonucleotide controls. Concordance of calls for the 39 disease causing mutations and 4 variants included on the Tag-It™ panel are provided in Table 3 below as percent agreements. All results summarized in Table 3 below are from studies in which the input DNA was equivalent to the concentration recommended in this insert (25 ng). Synthetic controls were prepared by adding the DNA containing the mutation at a copy number equivalent to a natural sample blended in a genomic DNA matrix to represent all possible calls (WT, HET, Mu D, variant detected) for each mutation and variant probed for by the Tag-It™ Cystic Fibrosis Mutation Detection Kit. Not included in the table below are clinical samples for which DNA sequencing was unable to provide an unambiguous genotyping call for the particular locus in question.

Table 3: Samples (clinical, genomic replicates and synthetic controls) in which Tag-It™ calls were compared to sequencing calls

Mutations / Variants	Number of Samples in which the wild-type allele was identified				Number of Samples in which the mutant/variant allele was identified				Percent Agreement
	Total	Clinical	Genomic replicates	Synthetic controls	**Total**	Clinical	Genomic replicates	Synthetic controls	All Samples
G85E	**695**	137	540	18	**83**	1	36	46	100%
394delTT	**973**	139	828	6	**48**	0	0	48	100%
R117H	**953**	125	792	36	**77**	13	36	28	100%
Y122X	**973**	139	828	6	**30**	0	0	30	100%
621+1G>T	**834**	132	684	18	**195**	7	144	44	100%
711+1G>T	**940**	130	792	18	**84**	2	36	46	100%
1078delT	**936**	102	828	6	**59**	1	0	58	100%
R334W	**926**	98	792	36	**69**	5	36	28	100%
R347P	**898**	100	792	6	**75**	3	36	36	100%
R347H	**937**	103	828	6	**40**	0	0	40	100%
A455E	**953**	137	792	24	**77**	1	36	40	100%
ΔI507	**953**	137	792	24	**50**	2	36	12	100%
ΔF508	**666**	66	576	24	**337**	73	252	12	100%
V520F	**1009**	139	828	42	**24**	0	0	24	100%
1717-1G>A	**969**	129	792	48	**63**	9	36	18	100%
G542X	**932**	128	756	48	**100**	10	72	18	100%
S549N	**972**	138	828	6	**16**	0	0	16	100%
S549R (T>G)	**990**	138	828	24	**12**	0	0	12	100%
G551D	**955**	127	792	36	**95**	11	72	12	100%
R553X	**956**	134	792	30	**58**	4	36	18	100%
A559T	**997**	139	828	30	**18**	0	0	18	100%
R560T	**972**	132	792	48	**59**	5	36	18	100%[a]
1898+1G>A	**969**	135	828	6	**32**	4	0	28	100%
1898+5G>T	**1003**	139	828	36	**30**	0	0	30	100%
2183AA>G	**973**	139	828	6	**34**	0	0	34	100%
2184delA	**973**	139	828	6	**36**	0	0	36	100%
2307insA	**1003**	139	828	36	**28**	0	0	28	100%
2789+5G>A	**946**	136	792	18	**85**	3	36	46	100%
3120+1G>A	**939**	135	792	12	**89**	1	36	52	100%
Y1092X	**961**	127	828	6	**58**	0	0	58	100%
M1101K	**947**	119	792	36	**66**	0	36	30	100%
R1162X	**937**	121	792	24	**80**	4	36	40	100%
3659delC	**942**	126	792	24	**83**	5	36	42	100%
S1255X	**973**	139	828	6	**28**	0	0	28	100%
3849+10kbC>T	**898**	94	792	12	**96**	8	36	52	100%[b]
3876delA	**1003**	139	828	36	**30**	0	0	30	100%
3905insT	**968**	134	828	6	**31**	1	0	30	100%
W1282X	**939**	111	792	36	**74**	12	36	26	100%[b]
N1303K	**901**	97	792	12	**95**	5	36	54	100%
5T/7T/9T	**N/A**	N/A	N/A	N/A	**1023**	137	828	58	100%
I506V	**139**	139	0	0	**12**	0	0	12	100%
I507V	**139**	139	0	0	**12**	0	0	12	100%
F508C	**136**	136	0	0	**14**	2	0	12	100%

a. One replicate of a genomic sample gave a "No Call" for this locus. Upon re-run this replicate gave a HET call, consistent with sequencing results.

b. 2 clinical samples were misidentified during transfer to sequencing plates (one sample identified as a WT by the Tag-It™ assay at this locus was initially identified as a HET by sequencing and a second sample identified as a HET by the Tag-It™ assay was initially identified as a WT by sequencing). Upon re-sequencing, sequencing calls at this locus were concordant with Tag-It™ results.

DNA sequencing provided unambiguous, comparable, genotyping calls at all 43 loci for 68 clinical samples. The mutations and variants detected in these 68 clinical samples are listed in Table 4 below. There were an additional 69 clinical samples genotyped both by the Tag-It™ Cystic Fibrosis Kit and DNA sequencing that were excluded from Table 4 below because DNA sequencing only provided unambiguous calls at a subset of the 43 loci probed. Some possible explanations for ambiguous sequencing results include: 1) poor quality DNA (e.g. impure samples or low concentrations) resulting in a decreased yield in PCR product; 2) PCR products with GC rich sequences; or 3) PCR products forming secondary structures. In those 69 excluded samples, there was 100% concordance with sequencing at the subset of loci which were comparable (Table 3). For the 68 samples included in Table 4, the Tag-It™ Cystic Fibrosis Kit accurately identified all 43 alleles probed, based on 100% concordance with DNA sequencing for all calls made.

Table 4: Accuracy of the Tag-It™ Cystic Fibrosis Kit (100% based on concordance with unambiguous bi-directional dideoxy DNA sequencing at all 43 loci probed, 95% CI: 99.9% - 100%)

Clinical Sample	Disease causing mutations detected and genotyping call	T-Tract variants detected	Other variants detected	Concordant Calls
1	None	7T	None	43/43
2	3659delC HET	7T/9T	None	43/43
3	R560T HET	7T	None	43/43
4	R560T HET	5T/7T	None	43/43
5	G542X HET	7T/9T	None	43/43
6	ΔF508 HET	7T/9T	None	43/43
7	3849+10kbC>T HET	7T	F508C	43/43
8	3849+10kbC>T HET	7T	None	43/43
9	W1282X HET	7T	None	43/43
10	W1282X HET	7T	None	43/43
11	3849+10kbC>T HET	7T/9T	F508C	43/43
12	R334W HET	7T	None	43/43
13	1891+1G>A HET	7T	None	43/43
14	R1162X HET	7T	None	43/43
15	R334W HET	7T	None	43/43
16	G542X HET	9T	None	43/43
17	G542X HET	7T/9T	None	43/43
18	2789+5G>A HET	7T	None	43/43
19	621+1G>T HET	7T/9T	None	43/43
20	621+1G>T HET	7T/9T	None	43/43
21	1717-1G>A HET	7T	None	43/43
22	ΔI507 HET	7T	None	43/43
23	ΔF508 HET	7T/9T	None	43/43
24	N1303K HET	7T/9T	None	43/43
25	1717-1G>A HET	7T	None	43/43
26	N1303K HET	7T/9T	None	43/43
27	N1303K HET	7T/9T	None	43/43
28	G551D HET	5T/7T	None	43/43
29	G551D HET	7T	None	43/43
30	G551D HET	7T	None	43/43
31	3659delC HET and ΔF508 HET	7T/9T	None	43/43
32	3649+10kbC>T and ΔF508 HET	9T	None	43/43

Clinical Sample	Disease causing mutations detected and genotyping call	T-Tract variants detected	Other variants detected	Concordant Calls
33	R117H HET and ΔF508 HET	7T/9T	None	43/43
34	3659delC HET and ΔF508 HET	7T/9T	None	43/43
35	R1162X HET and ΔF508 HET	7T/9T	None	43/43
36	R334W MUT	7T	None	43/43
37	711+1G>T HET and 3905insT HET	7T	None	43/43
38	G542X HET and ΔF508 HET	9T	None	43/43
39	1717-1G>A HET and ΔF508 HET	7T/9T	None	43/43
40	347P HET and ΔF508 HET	7T/9T	None	43/43
41	R117H HET and ΔF508 HET	7T/9T	None	43/43
42	R117H HET and ΔF508 HET	5T/9T	None	43/43
43	R117H HET and 1898+1G>A HET	5T/7T	None	43/43
44	R347P HET and ΔF508 HET	7T/9T	None	43/43
45	R117H HET and ΔF508 HET	5T/9T	None	43/43
46	R553X HET and ΔF508 HET	7T/9T	None	43/43
47	ΔF508 MUT	9T	None	43/43
48	ΔF508 MUT	9T	None	43/43
49	ΔF508 MUT	9T	None	43/43
50	621+1G>T HET and 3120+1G>A HET	7T/9T	None	43/43
51	ΔF508 HET and W1282X HET	7T/9T	None	43/43
52	ΔF508 MUT	9T	None	43/43
53	ΔF508 MUT	9T	None	43/43
54	R553X HET and ΔF508 HET	7T/9T	None	43/43
55	ΔF508 HET and R347P HET	7T/9T	None	43/43
56	N1303K HET and ΔF508 HET	9T	None	43/43
57	ΔF508 HET and R117H HET	5T/9T	None	43/43
58	1717-1G>A HET and ΔF508 HET	7T/9T	None	43/43
59	553X HET and ΔF508 HET	7T/9T	None	43/43
60	G551D HET and R117H HET	7T	None	43/43
61	R117H MUT	5T	None	43/43
62	G542X HET and ΔF508 HET	9T	None	43/43
63	G551D HET and ΔF508 HET	7T/9T	None	43/43
64	621+1G>T HET and ΔF508 HET	9T	None	43/43
65	G542X HET and R117H HET	5T/9T	None	43/43
66	G551D HET and ΔF508 HET	7T/9T	None	43/43
67	2789+5G>A HET and ΔF508 HET	7T/9T	None	43/43
68	1717-1G>A HET and ΔF508 HET	7T/9T	None	43/43
Total number of calls concordant with sequencing Accuracy: 100% (95% CI: 99.9% to 100%)				**2924/2924**

In summary, Table 5 below demonstrates that there was 100% agreement between the Tag-It™ assay and DNA sequencing on the overall genotype of clinical samples with either 2 identifiable mutations (compound heterozygous samples or homozygous mutant samples), 1 identifiable mutation (heterozygous samples) or no identifiable mutations (wild-type samples). This assessment is based on comparison of genotyping calls at each of the 39 disease causing mutations and 4 variants included on the Tag-It™ panel.

Table 5: Agreement on Overall Genotypes of Clinical Samples based on analysis of calls at 39 loci associated with disease causing mutations

Tag-It™ Cystic Fibrosis Kit	DNA Sequencing		
	Clinical Samples with 2 identifiable disease causing mutations	Clinical Samples with 0 or 1 identifiable disease causing mutations	**Total**
Clinical Samples with 2 identifiable mutations	38	0	38
Clinical Samples with 0 or 1 identifiable mutations	0	30	30
Total	38	30	68

b. Matrix comparison:
Not applicable

3. Clinical studies:
   a. Clinical Sensitivity:
   The clinical sensitivity of the Tag-It™ Cystic Fibrosis Kit can be estimated based on the published studies of mutation frequencies in various ethnicities listed in Table 7 below.

   b. Clinical specificity:
   The clinical specificity of the kit is considered to be high based on published literature and the results of analytical studies described in this submission.

   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.

4. Clinical cut-off:
   Not applicable.

5. Expected values/Reference range:
   Cystic Fibrosis (CF) is the most common autosomal recessive disorder in the Caucasian population, with an incidence of approximately 1 in 3,200 live births. The incidence of CF in other ethnic groups varies, as seen in Table 6 below.

Table 6. Incidence of Cystic Fibrosis in different Ethnic Groups

Ethnic Group	Incidence of Cystic Fibrosis
North American Caucasian	1 in 3200
Ashkenazi Jewish	1 in 3300
Hispanic	1 in 9500
African American	1 in 15 300
Asian American	1 in 32 100
Native American (Pueblo)	1 in 3970
Native American (Zuni)	1 in 1347

The Tag-It™ Cystic Fibrosis Kit tests for CFTR mutations which have been established as causing CF in peer-reviewed publications. Some of these mutations are also included in the ACMG/ACOG recommendations for CF testing. Table 7 summarizes the CF

mutation carrier detection rate for individuals from different ethnic groups.

Table 7: CF Mutation Carrier Detection Rate for individuals from different ethnic groups

Mutations Panel (*currently recommended by the ACMG/ACOG)	Mutation frequencies among individuals with clinically diagnosed cystic fibrosis (%)[a]				
	Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish
ΔF508*	72.42	54.38	44.07	38.95	31.41
ΔI507*	0.88	0.68	1.87	0.00	0.22
G542X*	2.28	5.10	1.45	0.00	7.55
G85E*	0.29	0.23	0.12	0.00	0.00
R117H*	0.70	0.11	0.06	0.00	0.00
621+1G>T*	1.57	0.26	1.11	0.00	0.00
711+1G>T*	0.43	0.23	0.00	0.00	0.10
1078delT	0.02	0.09	0.00	0.00	0.00
R334W*	0.14	1.78	0.49	0.00	0.00
R347P*	0.45	0.16	0.06	0.00	0.00
A455E*	0.34	0.05	0.00	0.00	0.00
1717-1G>A*	0.48	0.27	0.37	0.00	0.67
R560T*	0.38	0.00	0.17	0.00	0.00
R553X*	0.87	2.81	2.32	0.76	0.00
G551D*	2.25	0.56	1.21	3.15	0.22
1898+1G>A*	0.16	0.05	0.06	0.00	0.10
2184delA*	0.17	0.16	0.05	0.00	0.10
2789+5G>A*	0.48	0.16	0.00	0.00	0.10
3120+1G>A*	0.08	0.16	9.57	0.00	0.10
R1162X*	0.23	0.58	0.66	0.00	0.00
3569delC*	0.34	0.13	0.06	0.00	0.00
3849+10kbC>T*	0.58	1.57	0.17	5.31	4.77
W1282X*	1.50	0.63	0.24	0.00	45.92
N1303K*	1.27	1.66	0.35	0.76	2.78
394delTT	0.20	NF[b]	NF[b]	NF[b]	NF[b]
Y122X[c]	NF[b]	NF[b]	NF[b]	NF[b]	NF[b]
R347H	0.20	NF[b]	NF[b]	NF[b]	NF[b]
V520F	0.20	NF[b]	NF[b]	NF[b]	NF[b]
A559T	NF[b]	NF[b]	1.00	NF[b]	NF[b]
S549N	0.02	1.20	NF[b]	NF[b]	NF[b]
S549R(T>G)	0.10	NF[b]	NF[b]	NF[b]	NF[b]
1898+5G>T[d]	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
2183AA>G	0.40	0.40	NF[b]	NF[b]	NF[b]
2307insA	NF[b]	NF[b]	0.50	NF[b]	NF[b]
Y1092X	0.30	0.40	NF[b]	NF[b]	NF[b]
M1101K	0.50	NF[b]	NF[b]	NF[b]	NF[b]
S1255X	NF[b]	NF[b]	1.00	NF[b]	NF[b]
3876delA[e]	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
3905insT	0.30	NF[b]	0.50	NF[b]	NF[b]
Mutation Detection Rate	**90.5**	**73.8**	**67.5**	**48.9**	**94.0**

a) Mutation frequencies based on the ACMG 2004 Policy Statement (Watson M.S., G.R. Cutting et al., 2004) and a study of 5,840 CF chromosomes (Heim R.A., E.A. Sugerman et. al., 2001)

b) NF: Not Found in by Heim et al. (2001); c) NA: Not Analyzed by Heim et al. (2001)

c) Y122X accounts for about 48 percent of the CF mutations in the Reunion Islands, where Y122X and ΔF508 together account for 70 percent of the CF mutations (Bienvenu, Bousquet et al. 1993)

d) The 1898+5G>T mutation has been found in several Chinese and Taiwanese CF patients (Zielenski, Markiewicz et al. 1995; Wu, Shu et al. 2000; Alper, Shu et al. 2003)
e) 3876delA was first discovered in seven Hispanic patients associated with a severe CF phenotype (Wang, Bowman et al. 2000). In a small study of 29 Hispanic American CF patients, this mutation was found in 10 percent of the CF alleles (Wang, Bowman et al. 2000)

N. Instrument Name:

Luminex 100 IS (Integrated System); other names used: Luminex® 100 xMAP™ System; Tag-It™ Data Analysis Software TDAS CF-I for the Tag-It™ Cystic Fibrosis Kit (for use with the Luminex® 100 xMAP™ System)

O. System Descriptions:

The Tag-It™ Data Analysis Software CF-I (TDAS CF-I) is the software component of the Tag-It™ Cystic Fibrosis Kit which provides data analysis to aid in determining sample genotypes based on the mutations and variants probed by the kit.

The Tag-It™ Cystic Fibrosis Kit is designed to simultaneously probe for 43 mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The kit also incorporate multiplex Polymerase Chain Reaction (PCR) and multiplex Allele Specific Primer Extension (ASPE) with Tm Bioscience's proprietary Universal Tag sorting system on the Luminex®100 Platforms. These components of the kit are described in more detail in the Package Insert for Kit Reagents, which is included on the Tag-It™ Cystic Fibrosis Kit CD.

Prior to running a Tag-It™ Cystic Fibrosis Kit, user has to ensure that the appropriate template (Tag-It Cystic Fibrosis flex) required to process samples on the Luminex® 100 IS instrument is installed on the Luminex® computer. Instructions for installing the template can be found in the product insert of the Tag-It™ Cystic Fibrosis Kit or in Section A below.

Samples processed according to the product insert for kit reagents will generate fluorescent signals on the Luminex® 100 IS instrument for each variation screened. These fluorescence values are saved into an 'Output.csv' file, which may be opened and analyzed with TDAS CF-I to determine the genetic calls for each sample.

Genotyping calls are made by TDAS CF-I by using a combination of thresholds that require each signal (i.e. the Median Fluorescence Intensity, MFI, recorded in the Luminex® output.csv file) to be significantly higher than background and the allelic ratios calculated from these MFI values to fall within empirically derived ranges. Briefly, for each allele of a given sample, the NET MFI is set to be the larger of zero and the value obtained by subtracting the 'no target' (PCR negative control) MFI values from the respective MFI values of the sample. In order to exclude samples containing insufficient or degraded DNA or samples generating sub-optimal results, acceptance criteria are defined such that, for each variation within the assay, MFI units for at least one allele are required to be at least 10x the 'no target' MFI for that allele, at least 300 MFI units and at least 5% of the highest signal obtained for the sample. For samples in which all mutations meet these data requirements, the genotype is then determined based on allelic ratios, obtained by dividing the NET MFI of an allele (mutant or wild-type) by the sum of the NET MFIs for all alleles at a locus (wild-type plus mutant for bi-allelic variations). Allelic ratios represent the fraction of the total net MFI signal for a given variation attributed to the presence of a particular allele. Allelic ratios

are used as opposed to net signals because they normalize for variability in signals between loci and between samples. By setting threshold values, the allelic ratios are used to discriminate between genotype calls. In most cases, an allele is considered present (detected) if its allelic ratio is at least 0.30. In order to make a 'WT' (only wild-type allele detected) call, the wild-type allelic ratio must be at least 0.85 (actual value can be different between the variations). The exact values for the thresholds have been empirically determined for each individual variation by running multiple genomic samples.

1. Modes of Operation:
   Batch

2. Software:
   TDAS CF-I is designed to specifically address the interpretation of data generated using the Tag-It™ Cystic Fibrosis Kit. It is essential that the Luminex® 100 IS System be initiated using the **assay-specific** template (*i.e.* 'Tag-It Cystic Fibrosis flex'), which includes unique bead/variation names that are used to identify the assay. Failure to use the correct Luminex® 100 xMAP™ template file to read the assay data will make it impossible for TDAS CF-I to analyze the data. Users should not interrupt the reading of the plate before all wells for a session have been read and should not edit the output.csv file created by the Luminex® 100 xMAP™ system.

   TDAS CF-I does not interpret the data of the wells for which the Luminex® 100 xMAP™ IS system gives a 'Sample Empty' or 'User cancel' note in the 'Notes' column. TDAS CF-I uses the notes in the 'Notes' column to determine whether there was a problem encountered during the reading of the wells. 'Notes' column should not be edited before, during or after the data reading step, otherwise TDAS CF-I will not be able to correctly interpret the 'Notes' column.

   Files supplied on the Tag-It™ Cystic Fibrosis Kit CD

- Installation executable that installs Tag-It™ Data Analysis Software CF-I (TDAS CF-I)
- Luminex®100 IS System data acquisition template for Luminex IS 2.1/2.2
- Example Tag-It™ Cystic Fibrosis Kit 'Output.csv' files
- TDAS CF-I User Manual
- Version history of TDAS CF-I
- The Tag-It™ Cystic Fibrosis Kit Product Insert for TDAS CF-I (this document)
- The Tag-It™ Cystic Fibrosis Kit Product Insert for Kit Reagents

   Software Version

   User has to ensure that the appropriate version of the software is being used for the Tag-It™ Cystic Fibrosis Kit, as specified on the carton containing Tag-It™ Cystic Fibrosis Kit reagents.

   Required Items NOT supplied with the CD

   A Personal Computer with the following minimum requirements is required to run the software:

- Operating System: Microsoft® Windows 2000 or XP
- CPU: Pentium III 750MHz or equivalent
- Memory: 128 MB or more of RAM

- Disk Space: At least 200 MB of free space
- CD-ROM: 24X or faster CD ROM drive

The data acquisition template and the software can only be used in conjunction with the Tag-It™ Cystic Fibrosis Kit.

Detailed use of the software is described in the TDAS CF-I User Manual provided on the Tag-It™ Cystic Fibrosis Kit CD. The genetic calls are made using a combination of thresholds that requires each signal to be significantly higher than the background and that the allelic ratios for each wild-type and mutant pair corresponding to each call fall within empirically derived ranges.

TDAS CF-I will display, for each sample, the calls for each variation. For example, the possible calls for a given bi-allelic variation of a specific sample may be:

- WT: only the wild-type allele has been detected;
- HET: both the wild-type and the mutant alleles have been detected;
- Mu D: the mutant allele has been detected;
- No Call: a call could not be made.

A 'No Call' can occur for various reasons, and a short explanation is provided in the last column (the 'Notes and explanations' column). It is recommended that a sample with a 'No Call' be re-run once from the start.

File Opening

TDAS CF-I is designed to accept the Luminex® 100 xMAP™ IS system output in the form of a csv file under the condition that the Luminex® template file provided with TDAS CF-I on the Tag-It™ Cystic Fibrosis Kit CD has been used with the Luminex® 100 xMAP™ IS software to generate the csv file. TDAS CF-I is compatible with Luminex® Data Collector Version 2.1 or 2.2 output.

Assay Interpretation Selection

TDAS CF-I recognizes from the csv data file that the Tag-It™ Cystic Fibrosis Kit has been run and therefore interprets the data accordingly. This is accomplished through the use of unique variation names, hence the necessity of using the Tag-It™ Cystic Fibrosis Kit Template file for the Luminex100® IS System to generate the CSV data file.

Views

A number of views are available to visualize the data, calculated values and variation calls. The summary view contains the variation calls for all variations and every sample and therefore provides full genetic information for that run of the assay. For more detailed information, each sample has a full details view that provides the raw data, which includes MFI signals and background signals, calculated values, which include net signals, and allelic ratios for all variations. A variation full details view is also available that includes the same information as the sample views but for a specific variation rather than for a specific sample.

Printing

The summary and detailed views can be printed, either in the default size (which may need several pages) or scaled to fit horizontally on one page ('Scale printout to fit in page width' option). All the views can be printed in color or black and white. By unchecking the 'Send color data to the printer' option, the data will be printed in black and white with the lines having alternative white and light gray backgrounds (instead of using the grayscale versions of the colors with black-and-white printers).

Data and Results Exporting

To allow further analysis which may include combining multiple run data to produce a study report, the summary data and full data can be exported in csv format that can be opened in common spread sheet programs. All data can be selected to be exported to a PDF file as well, which can also include graphical representations of the data. For more details on how to use these features, please refer to the TDAS CF-I User Manual included on the Tag-It™ Cystic Fibrosis Kit CD.

The Tag-It™ Data Analysis Software CF-I (TDAS CF-I) is the data analysis and report generation tool provided by the sponsor. This software provides an algorithm for data analysis, and will be used exclusively for the Tag-It™ Cystic Fibrosis Kit. It is optimized to process the data that are generated from the Tag-It™ Cystic Fibrosis assay protocol. The software will work when installed on PC compatible workstations in conjunction with the Luminex® 100 xMAP™ IS software. This software has been validated by the sponsor.

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
Yes ___X____ or No _____

3. Specimen Identification:
   Users must fill in Batch Information by providing a unique batch Name, Description and Creator. Users have to enter appropriate patient information, i.e. number of samples, and sample IDs.

4. Specimen Sampling and Handling:
   Genomic DNA preparation, multiplex (16-plex) PCR, amplicon treatment, multiplex ASPE, bead hybridization and incubation with reporter are performed semi-manually. Subsequently, beads are analyzed by the Luminex® 100 xMAP™ system, and data generated analyzed by Tag-It™ Data Analysis Software CF-I (TDAS CF-I) to provide final genotype for the sample.

5. Calibration:
   Calibrators:
   Before using the Luminex® 100 xMAP™ IS System to read any samples prepared by the Tag-It™ assay, the Luminex® system must be prepared and calibrated following the procedures described in the Luminex® 100 xMAP™ User Manual.

   Luminex® Template File:

The Template 'Tag-It Cystic Fibrosis flex' required for running the samples on the Luminex® 100 xMAP™ Instrument has to be installed on the computer that controls the Luminex® 100 xMAP™ system.

6. Quality Control:

Negative Controls:

It is required that a ddH_2O control be included with each run of the Tag-It™ Cystic Fibrosis Kit. The TDAS CF-I software uses this ddH_2O sample to determine background signal levels and if any PCR carryovers contaminations may have affected the run. TDAS CF-I assumes that the last sample on a plate is the negative control.

Positive Controls:

It is recommended to routinely include in each assay run positive controls for cystic fibrosis mutations tested for by the Tag-It™ Cystic Fibrosis Kit. Since the most common mutation is the ΔF508 allele, which accounts for 30 to 88 percent of all CF mutations depending on the ethnic group (Gibson, Moskowitz et al. 2001), it is recommended that a control sample with this mutation be included with every run. Tm Bioscience recommends the use of genomic DNA controls similar to the specimen type whenever feasible although spiked controls (using synthetic DNA) may be used when specimen samples are not available.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10, Labeling for in vitro diagnostic products.

R. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.5900, Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation detection system, with special controls. The special control guidance document "CFTR Gene Mutation Detection System" will be available shortly.