

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k060070

B. Purpose for Submission:

New device

C. Measurand:

Controls for assays detecting Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations and variants

D. Type of Test:

Assayed quality control material

E. Applicant:

Maine Molecular Quality Controls, Inc. (MMQCI)

F. Proprietary and Established Names:

INTROL™ CF Panel I Control

G. Regulatory Information:

1. Regulation section:
866.5910 DNA quality control material for genetic testing
2. Classification:
De novo, Class II
3. Product code:
NZB, Quality control material, genetics, DNA
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
INTROL™ CF Panel I Control is intended for *in vitro* diagnostic use as a quality control to monitor analytical performance of the extraction, amplification and detection steps of diagnostic assays used in the detection of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations and variants. This product is intended to be extracted and analyzed routinely with each CFTR assay run.
2. Indication(s) for use:
INTROL™ CF Panel I Control is intended for *in vitro* diagnostic use as a quality control to monitor analytical performance of the extraction, amplification and detection steps of diagnostic assays used in the detection of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations and variants. This product is intended to be extracted and analyzed routinely with each CFTR assay run.

The INTROL™ CF Panel I Control is designed to monitor the detection of 38 CFTR mutations associated with cystic fibrosis, including the 23 mutations recommended for testing by American College of Medical Genetics (ACMG) and American College of Obstetricians and Gynecologists (ACOG). The INTROL™ CF Panel I Control also monitors 3 polymorphisms (I506V, I507V, F508C) and

the 5/7/9T variants.

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

The INTR^{OL}™ CF Panel I Control is designed to monitor the presence of 38 CFTR mutations associated with cystic fibrosis, including the 23 mutations recommended for testing by American College of Medical Genetics (ACMG) and American College of Obstetricians and Gynecologists (ACOG). The INTR^{OL}™ CF Panel I Control also monitors 3 polymorphisms (I506V, I507V, F508C) and the 5/7/9T variants.

4. Special instrument requirements:
Not applicable.

I. Device Description:

INTR^{OL}™ CF Panel I Control consists of synthetic CFTR DNA suspended in a matrix of synthetic DNA targets, carrier DNA of a non-human species, preservatives, dye, and stabilizers. The synthetic DNA contains all 27 CFTR gene exons plus intronic borders, and contains specific mutations and polymorphisms which are divided among 5 bottles (bottles a, b, c, d, and e). The 5 bottles exist in two versions: Version G106ac includes bottles (a), (b), and (c), while Version G106de includes bottles (a), (b), (d), and (e). The specific mutations present in each bottle are listed below in Table 1; all other CFTR sequence is wild type. CFTR mutations that are not listed cannot be detected in the INTR^{OL}™ CF Panel I Control.

Control sequences include the mutations, wild type alleles, and polymorphisms recommended for testing by the American College of Medical Genetics (ACMG) and American College of Obstetricians and Gynecologists (ACOG). CFTR mutations that are not listed cannot be detected in the INTR^{OL}™ CF Panel I Control.

CFTR DNA is stabilized in the matrix and released when processed through common extraction methods as if it were a whole blood specimen. Following extraction, the released DNA can be used in common amplification based molecular assays techniques. Because INTR^{OL}™ CF Panel I Control is designed to mimic the whole blood sample, the resulting copy number of the artificial CFTR segment, after extraction, will be similar to that found in a processed human whole blood sample (v/v).

Table 1. Composition of INTROL™ CF Panel I Control includes following combinations of CFTR mutations and polymorphisms (plus wild type sequence covering 27 CFTR exons).

Allele	Genotype	Allele	Genotype
Bottle a (both versions)		Bottle b (both versions)	
7T*	7T / 7T	E60X	Homozygous mutant
(I507V)*	I507V / WT	G85E*	Homozygous mutant
(F508C)*	F508C / WT	I148T	Homozygous mutant
S549N/ S549R	Heterozygous	621+1G>T*	Homozygous mutant
S1251N	Heterozygous	711+1G>T*	Homozygous mutant
Bottle c (Version ac only)		1078delT	Homozygous mutant
394delTT	Heterozygous	R334W*	Homozygous mutant
R117H*	Heterozygous	R347P*	Homozygous mutant
R347H	Heterozygous	9T*	9T / 9T
5T* / 7T*	Heterozygous	A455E*	Homozygous mutant
(I506V)*	I506V / WT	del F508*	Homozygous mutant
del I507*	Heterozygous	V520F	Homozygous mutant
R553X*	Heterozygous	1717-1G>A*	Homozygous mutant
2183AA>G	Heterozygous	G542X*	Homozygous mutant
Bottle d (Version de only)		G551D*	Homozygous mutant
394delTT	Homozygous mutant	R560T*	Homozygous mutant
R117H*	Homozygous mutant	1898+1G>A*	Homozygous mutant
R347H	Homozygous mutant	2143delT	Homozygous mutant
5T*	5T / 5T	2184delA*	Homozygous mutant
del I507*	Homozygous mutant	2789+5G>A*	Homozygous mutant
R553X*	Homozygous mutant	3120+1G>A*	Homozygous mutant
2183AA>G	Homozygous mutant	3199del6	Homozygous mutant
Bottle e (Version de only)		D1152H	Homozygous mutant
7T*	7T / 7T	R1162X*	Homozygous mutant
(I506V)*	I506V / I506V	3659delC*	Homozygous mutant
		3849+10kbC>T*	Homozygous mutant
		3876delA	Homozygous mutant
		3905insT	Homozygous mutant
		W1282X*	Homozygous mutant
		N1303K*	Homozygous mutant

*ACMG / ACOG Panel

J. Substantial Equivalence Information:

1. Predicate device name(s):
Not applicable.
2. Predicate 510(k) number(s):
Not applicable.
3. Comparison with predicate:
Not applicable

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

None referenced.

L. Test Principle:

Not applicable.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All INTROL™ CF Panel I Control products are tested by an FDA-cleared CFTR mutation detection method before being released for market distribution. Any mutations not tested by the FDA-cleared method are sequenced bidirectionally before product is released. All mutations must be detected.

a. *Precision/Reproducibility:*

The clinical study performed at the external sites evaluated reproducibility of INTROL™ CF Panel I quality control material with respect to within run, between run, between sites, between lots, and between methods.

External site evaluations:

The clinical laboratory study performed at the external sites evaluated reproducibility of INTROL™ CF Panel I Control quality control material with respect to within run, between run, between sites, between lots, and between methods.

Evaluation using different extraction methods:

# extraction methods	# laboratories	# successful laboratory extractions	percent successful
21	134	129	96% *

* Five laboratories didn't continue after DNA extraction because DNA quantitation method they used indicated that no DNA was extracted/isolated. Considering that the level of synthetic CFTR DNA present in the extracted control may not be detectable with certain quantitation methods, there is a possibility that extractions in these 5 laboratories may have been successful; however this could not be assessed because the assays were not performed.

INTROL™ CF Panel I quality control material has been tested using CFTR assays at 10 external sites, 8 of which were clinical laboratories representing intended user. Samples from 11 different manufacturing lots were tested at minimally 3 external sites in at least 3 separate runs. Results are summarized in the Table 2.

Table 2. External site evaluations.

Method	Site	# of Lots ¹	# of Runs	Total Calls	Percent Correct Calls
Tag-It™	1	10	9	223	100%
	2	3	9	138	100%
	3	1	1	6	100%
	4	1	1	7	100%
	5	1	1	4	100%
eSensor	6	5	1	30	100%
Other Amplification methods	7	5	1	38	100%
	8	3	1	31	100%
	9	1	2	7	100%
	10	5	40	649	100%
6 methods		11 Lots	66 Runs	1133 Calls	100% Correct

¹ Each bottle is processed independently and has its own lot number.

- b. *Linearity/assay reportable range:*
Not applicable.
- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Bidirectional sequencing of INTROL™ CF Panel I DNA is used to validate the presence of mutant or wild type sequence.

Product acceptance criteria and testing: All INTROL™ CF Panel I Control products are tested by an FDA-cleared CFTR mutation detection method before being released for market distribution. Any mutations not tested by the FDA-cleared method are sequenced bidirectionally before product is released. All mutations must be detected.

Upon receipt and after opening, the material should be stored at 2° – 8°C. INTROL™ CF Panel I Control material is shipped with a “Do not freeze” warning in the device labeling.

Unopened INTROL™ CF Panel I Control material is stable through the expiration date printed on each bottle when stored refrigerated (2° – 8°C). Opened material returned to the refrigerator (2° – 8°C) shortly after use is stable for thirty (30) days from the date of opening.

Real time stability study:

Results of the real time studies are in the tables below. Stability was tested using 4 different methods, including cleared Tag-It™ Cystic Fibrosis (CF) Kit from Tm Bioscience, and bidirectional sequencing. Testing was performed at 1, 2, 3, 4, and 12 months. In addition, real time testing was performed with 5 different lots of quality control material. At 12 months, all mutations and variants were tested using either the FDA cleared assay or bidirectional sequencing. Data from this ongoing study currently supports a shelf-life of 12 months.

Stability Testing	Number of Lots	Percent Correct Calls
Date of Manufacture	5	100%
1 month	5	100%
2 months	3	100%
3 months	1	100%
4 months	2	100%
12 months	3	100%

Stress testing:

Elevated Temperature Studies:

The study goal was to demonstrate product stability during prolonged shipping without refrigeration. A bottle of INTROL™ CF Panel I was removed from storage and mailed across the US and back without wet ice. Testing of the stressed product in the CFTR assay showed no loss of signal.

Two samples of INTROL™ CF Panel were compared after one was incubated at 60°C for 21-24 days, and the other incubated at 4°C, extracted and tested in duplicate in CFTR assay. There was no loss of signal after incubation at 60°C.

Freeze/Thaw Studies:

A bottle of INTROL™ CF Panel I was placed at -20°C for 48 hours, thawed and tested in CFTR assay. No loss of signal was detectable after one cycle of freeze/ thaw.

Open Vial Stability:

Two open vial studies were performed. In the first study, a bottle of INTROL™ CF Panel I was mixed, opened briefly, then stored at 2-8°C for 49 days. In the second study, a bottle of INTROL™ CF Panel I was mixed and opened with pipette simulation six times over 35 days. Both studies demonstrated no loss of signal when used in CFTR assay at the end of the test period.

Expected Results:

Expected results with the INTROL™ CF Panel I Control using two FDA-cleared CFTR assays are presented in Table 3.

Table 3. Results with the INTROL™ CF Panel I using two FDA cleared CFTR assays.

Method	Correctly Identified Alleles	No Call or other	Not Tested
Tag-It	All wt alleles 7T, I507V, F508C, S549R, G85E, I148T, 621+1G>T, 1078delT, R334W, R347P, 9T, A455E, delF508, V520F, 1717+1G>A, G542X, G551D, R560T, 1898+1G>A, 2184delA, 3120+1G>A, R1162X, 3659delC, 3849+10kbC>T, 3876delA, 3905insT, W1282X, N1303K, 394delTT, R117H, R347H, 5T/7T, I506, dell507, R553X, 2183AA>G	S549N MUT ¹ 711+1G>T No Call ² 2789+5G>A No Call ³	S1251N, E60X, 2143delT 3199del6, D1152H,
eSensor	All wt alleles 7T, I148T, 621+1G>T, 1078delT, R334W, R347P, 9T, A455E, delF508, 1717+1G>A, G542X, G551D, R560T, 1898+1G>A, 2184delA, 2789+5G>A, 3120+1G>A, R1162X, 3659delC, 3849+10kbC>T, W1282X, N1303K, R117H, 5T/7T, dell507, R553X,	G85E No Amp ³ 711+1G>T No Amp ⁴	I507V, F508C, S549N, S549R, S1251N, E60X, 1078delT V520F, 2143delT, 3199del6, D1152H, 3876delA, 3905insT, 394delTT R347H, I506V, 2183AA>G,

¹ Detected as homozygous mutant.

² Insufficient control sequence for one of the 711+1G>T amplicon primers.

³ Insufficient control sequence for one of the 2789+5G>A amplicon primers.

⁴ Mutation E60X interferes with a G85E amplicon primer.

- d. *Detection limit:*
Not applicable
- e. *Analytical specificity:*
Not applicable.
- f. *Assay cut-off:*
Not applicable.

2. Comparison studies:
 - a. *Method comparison with predicate device:*
Not applicable.
 - b. *Matrix comparison:*
Not applicable.
3. Clinical studies:
 - a. *Clinical Sensitivity:*
Not applicable.
 - b. *Clinical specificity:*
Not applicable.
 - 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:
Not applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. 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 866.5910 with special controls. The special control guidance document “Class II Special Controls Guidance Document: Quality Control Material for Cystic Fibrosis Nucleic Acid Assays” is available at <http://www.fda.gov/cdrh/oivd/guidance/1614.html>.