K051824

AUG 1 8 2005

Invader® UGT1A1 Molecular Assay
510(k) Summary

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1. SUBMITTER'S NAME, ADDRESS, TELEPHONE NUMBER, CONTACT PERSON, AND DATE SUMMARY WAS PREPARED

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2. DEVICE NAME

Trade or Proprietary Name: Invader® UGT1A1 Molecular Assay

Common or Usual Name: UGT1A1 Molecular Assay

Classification Name: 21 CRF 862.3360 - Drug Metabolizing Enzyme Genotyping System

3. IDENTIFICATION OF THE LEGALLY MARKETED DEVICE TO WHICH IS CLAIMED EQUIVALENCE

The Invader® UGT1A1 Molecular Assay is equivalent to the AmpliChip CYP450 Test for CYP2C19 (510(k) Premarket Notification K043576, submitted December 03, 2004.

4. DESCRIPTION OF THE DEVICE

The Invader® UGT1A1 Molecular Assay is an *in vitro* diagnostic test which utilizes sequence specific Invader® DNA probes, a structure-specific cleavage enzyme and a universal fluorescent resonance energy transfer (FRET) system combined with interpretative software and third party microtiter plate reader instrumentation. Invader® is the term used to generically refer to the patented chemistry on which the Invader® UGT1A1 Molecular Assay is based. The assay is designed to identify specific nucleic acid sequences and query for the presence of known sequence polymorphisms through the structure-specific cleavage of a series of probes that are specifically complementary to TA repeat sequences in the "TATA Box" of the UGT1A1 promoter region.

In the Invader® UGT1A1 Molecular Assay, two oligonucleotides (a discriminatory Primary Probe and an Invader® Oligo) hybridize in tandem to the target DNA to form an overlapping structure. The 5'-end of the Primary Probe includes a 5'-flap that does not hybridize to the target DNA. The 3'-nucleotide of the bound Invader® Oligo overlaps the Primary Probe, but need not hybridize to the target DNA. The Cleavase® enzyme recognizes this overlapping structure and cleaves off the unpaired 5'-flap of the Primary Probe, releasing it as a target-specific product. The Primary Probe is designed to have a melting temperature close to the reaction temperature. Therefore, under the isothermal assay conditions, Primary Probes, which are provided in excess, cycle on the target DNA. This allows for multiple rounds of Primary Probe cleavage for each target DNA, and amplification of the number of released 5'-flaps.

In the secondary reaction, each released 5'-flap can serve as an Invader® Oligo on a fluorescence resonance energy transfer (FRET) Cassette to create another overlapping structure that is recognized and cleaved by the Cleavase® enzyme. When the FRET Cassette is cleaved, the fluorophore and quencher are separated, generating detectable fluorescence signal. Similar to the initial reaction, the released 5'-flap and the FRET Cassette cycle, resulting in amplified fluorescence signal. The initial and secondary reactions run concurrently in the same well.

The biplex format of the Invader® UGT1A1 Molecular Assay enables simultaneous detection of two DNA sequences, a non-varying segment of the human alpha actin (ACTA1) gene and the TA repeat in the TATA box of the human UGT1A1 gene, in a single well. The biplex format uses two different discriminatory Primary Probes, each with a unique 5'-flap, and two different FRET Cassettes, each with a spectrally distinct fluorophore. By design, the released 5'-flaps will bind only to their respective FRET Cassettes to generate a target-specific signal.

The Invader® UGT1A1 Molecular Assay utilizes four independent wells per sample (one well for each of the TA Oligo mix reactions), to make a single genotype call. Each well contains a TATA box specific probe and an alpha actin probe. The alpha actin probe serves

as an internal control to confirm the validity of a given result when a particular TATA box polymorphism is absent.

5. INTENDED USE OF THE DEVICE

The Invader® UGT1A1 Molecular Assay is an *in vitro* diagnostic test for the detection and genotyping of the *1 (TA6) and *28 (TA7) alleles of the UDP glucuronosyltransferase 1A1 (UGT1A1) gene in genomic DNA from whole peripheral blood as an aid in the identification of patients with greater risk for decreased UDP-glucunorosyltransferase activity.

6(a). SUMMARY OF THE TECHNOLOGICAL CHARACTERISTICS OF THE DEVICE COMPARED TO THE PREDICATE DEVICE

The technological characteristics of the Invader® UGT1A1 Molecular Assay are summarized in Table 1 (below):

Table 1. Comparison of Technological Characteristics

	AmpliChip CYP450 Test for CYP2C19 (K043576)	**Invader® UGT1A1 Molecular Assay**
DNA sequence detection	1) Detects specific DNA sequences through recognition of DNA targets	1) Same as predicate
Reaction conditions	1) Utilizes thermal cycling 2) Utilizes target DNA amplification 3) Reactions occur on a single glass slide	1) No thermal cycling; isothermal reaction at 63° C 2) Utilizes signal amplification 3) Reactions occur in multiple plastic microtiter wells
Assay results	1) Assay signal results are interpreted by a software program and are assigned a genotype that is presented to the end user in a report format.	1) Same as predicate

6(b). PERFORMANCE DATA FROM THE NON-CLINICAL STUDIES
6(b)(1). DISCUSSION OF NON-CLINICAL TESTS

6(b)(1)(i) Limit of Detection

The lower limit of detection of the Invader® UGT1A1 Molecular Assay was determined by analysis of dilutions of 3 genomic DNA samples at 50, 100 and 150 ng DNA/µL. The concentration of the DNA samples was determined by use of a PicoGreen® double stranded DNA Quantitation kit. The UGT1A1 genotypes in the DNA samples were 6/6, 6/7, and 7/7 as determined through bi-directional DNA sequence analysis. The lowest level of genomic DNA at which there was 100% correct detection of the UGT1A1 TATA box polymorphisms, as confirmed through the bi-directional DNA sequencing, was 50 ng/reaction. The 95% one-sided lower confidence limit was 92.8% (120/120 genotype calls).

The upper limit of detection of the Invader® UGT1A1 Molecular Assay was determined by analysis of three genomic DNA samples at 80 ng DNA/µL. The concentration of the DNA samples was determined by use of a PicoGreen® double stranded DNA Quantitation kit. The UGT1A1 genotypes in the DNA samples were 6/6, 6/7, and 7/7 as determined through bi-directional DNA sequence analysis. The genotype call agreement between the Invader® UGT1A1 Molecular Assay and the bi-directional sequencing result was 100%, (120/120 genotype calls) with the 95% one-sided lower confidence limit of 97.5%.

6(b)(1)(ii) Genotype Detection

Method comparison studies were performed using bi-directional DNA sequencing as the comparator for the Invader UGT1A1 Molecular Assay. Genotype detection was evaluated using genomic DNA samples at approximately 300 ng/reaction. The percent agreement for genotype detection of the Invader® UGT1A1 Molecular Assay was calculated by determining the percentage of tested samples with the correct genotype assigned as compared to the total number of samples tested of that genotype. The results for each UGT1A1 allele analyzed in this manner are presented in Table 2.

Table 2. Agreement between Invader® UGT1A1 Molecular Assay and Bi-directional DNA Sequencing

UGT1A1 Genotype*	Number tested	Replicates per sample	Number of Correct genotype calls$^{\nabla}$	Number of Invalid calls$^{\nabla}$	Agreement	95% One-Sided Confidence Lower Limit
*1 ($TA_{6/6}$)	73	1	73	0	100%	96.0%
*28 heterozygous ($TA_{6/7}$)	109	1	109	0	100%	97.3%
*28 homozygous ($TA_{7/7}$)	30	1	30	0	100%	90.5%
Others	73	1	73	0	100%	96.0%
Total	285	1	285	0	100%	98.95%

* Genotype determined through bi-directional DNA sequencing
$^{\nabla}$ Calls produced on first run

6(b)(1)(iii). Repeat Rate

Two hundred eighty five (285) blood samples containing various UGT1A1 TATA box promoter region polymorphisms were tested (see Table 2). A correct genotype was obtained on the first attempt for all samples, yielding a repeat rate of 0%. Repeat rate performance results were also derived from the external study in which three sites extracted (in triplicate) the same 20 samples and analyzed each replicate on each of 5 separate days. Of the 900 possible genotype calls, valid calls were obtained on 851 sample points on the first attempt. This yields a repeat rate of 5.4% (see Table 3).

Table 3. Invader® UGT1A1 Molecular Assay Repeat Rate for the Reproducibility External Study

Site	Number of Repeats	Repeat Rate out of 300 sample points
1	28	9.3%
2	0	0%
3	21	7.0%
Total	49	5.4% (n=900)

6(b)(1)(iv). Stability (Freeze-Thaw)

The stability of the Invader UGT1A1 Molecular Assay was evaluated using identical sets of assay components that were subjected to multiple freeze-thaw cycles. All testing was performed using the same lots of reagents in the manufactured kit format, which includes all oligonucleotide mixtures, reaction buffer, and enzyme. Kits were subjected to differing numbers of freeze thaw cycles (1, 3, 5, 10) and evaluated using 20 genomic DNA samples

of known genotype, as determined though bi-directional DNA sequence analysis. There was 100% agreement between each freeze-thaw cycle for all samples tested.

6(b)(1)(v). Stability (storage)

The stability of the Invader® UGT1A1 Molecular Assay was evaluated using three (3) different genomic DNA samples with three different lots of assay components. The Invader® UGT1A1 Molecular Assay accurately identified the UGT1A1 genotypes as compared to bi-directional DNA sequencing when stored under the storage conditions specified on the label (-20°C). The assay also demonstrated 100% agreement with bi-directional DNA sequencing genotypes when stored outside of the recommended storage conditions, including a simulated shipping stress condition (37° C for 48 hours / -20° C for 19 days). The one month time point for intended storage conditions (-20°C) was successfully completed with 100% agreement between the Invader® UGT1A1 Molecular Assay and bi-directional DNA sequencing. Testing is ongoing to establish stability dating out to 18 months.

6(b)(1)(vi). Lot-to-lot reproducibility

Whole blood samples (n = 40) underwent DNA extraction and subsequent bi-directional DNA sequence analysis. The same DNA samples were then analyzed using the Invader® UGT1A1 Molecular Assay using three different lots of the reagents. The observed agreement between all three lots of the Invader® UGT1A1 Molecular Assay and bi-directional DNA sequencing was 100% (120/120). The 95% one-sided lower confidence limit was 92.8%.

6(b)(1)(vii). Sample preparation equivalency

The performance of the Invader® UGT1A1 Molecular Assay using genomic DNA samples extracted from two differing Qiagen DNA purification kits was evaluated. Sixty human genomic DNA samples of known genotype (confirmed through bi-directional DNA sequencing) were isolated from whole blood using both the Qiagen® QIAamp® 96 DNA Blood Kit and the Qiagen QIAamp DNA Blood Mini Kit and were tested with the Invader® UGT1A1 Molecular Assay. There was 100% agreement in genotype calls between the two data sets.

6(b)(1)(viii) Reproducibility

The reproducibility of the Invader® UGT1A1 Molecular Assay was assessed using twenty (20) blood samples comprised of the UGT1A1 genotypes listed in Table 4.

Table 4. Inter-laboratory Reproducibility of the Invader® UGT1A1 Molecular Assay

UGT1A1 Genotype[1]	# tested	1st run tests per site	Site	1st Run Results					# Invalid after 2nd run[2]	# Incorrect for all runs	Correct Call Rate for all runs
				Genotype Calls	Correct Calls	Incorrect Calls	# Invalid[2]	Correct Call Rate			
*1 ($TA_{6/6}$)	6	90	1	82	79[3]	3[3]	8	87.8%[3]	2	3[3]	94.4%[3]
			2	90	90	0	0	100%	0	0	100%
			3	84	84	0	6	93.3%	1	0	98.9%
*28 hetero-zygous ($TA_{6/7}$)	5	75	1	69	69	0	6	92%	0	0	100%
			2	75	75	0	0	100%	0	0	100%
			3	70	70	0	5	93.3%	1	0	98.7%
*28 homo-zygous ($TA_{7/7}$)	4	60	1	53	50[3]	3[3]	7	83.3%[3]	1	3[3]	93.3%[3]
			2	60	60	0	0	100%	0	0	100.0%
			3	55	55	0	5	91.7%	0	0	100.0%
Other	5	75	1	68	63[3]	5[3]	7	84.0%[3]	1	5[3]	92.0%[3]
			2	75	75	0	0	100%	0	0	100.0%
			3	70	70	0	5	93.3%	0	0	100.0%
Total	20	900		851	840[3]	11[3]	49	93.3%[3]	6	11[3]	98.1%[4]

[1] Genotype determined using bi-directional sequencing
[2] Invalid = reported to user as "Low Signal". Insufficient signal generated to make a genotype call
[3] Nine samples were misidentified at site 1 on day 5. The data are reported using genotype calls based on sample identification error.
[4] Correct call rate for all runs is based on 883/900 genotype calls.

A total of 900 sample points were generated, 300 per site, with genotype results reported for 894 of the 900 sample points (99.3%). Correct genotype calls for UGT1A1 were obtained for 840/900 (93.3%) samples after the 1st run. The one-sided 95% confidence lower limit is 91.8%. After retesting 49 samples, 43/49 were correctly identified after the 2nd run. The total correct genotype calls for the combined first and second runs was 883/900 (98.1%, with a 1-sided 95% confidence lower limit of 97.2%). Nine samples were misidentified at site 1 on day 5. As a result, the 3 replicates for each sample produced genotype results that were inconsistent with the bi-directional sequencing result for those samples. Discrepancy resolution by resequencing of the template in the original Invader®

assay confirmed that the genotypes present in the assay wells were consistent with the reported genotypes of the assay.

From the 49 repeated genotype tests, 20 were due to an invalid Positive Control result, 20 were due to an invalid Negative Control result, and the remaining nine were due to "Low Signal" as a result in the 1st run. After retesting, 6 genotype reactions were still reported as "Low Signal".

6(b)(2). Interference Studies

Sixteen (16) whole blood samples were spiked with bilirubin (8mg/dL), lipids (mono-, di-, and triglycerides) (150mg/dL), and dipotassium EDTA (0.36mg/dL), underwent DNA extraction, and were tested with the Invader® UGT1A1 Molecular Assay. Genotype results were compared to those obtained from non-spiked samples, with 100% genotype call agreement between spiked vs. non-spiked samples. All genotype results were verified using bi-directional DNA sequencing.

Additionally, DNA extracts from 16 whole blood samples were spiked with hemoglobin (0.0125%), 1% Qiagen® AW2 buffer, and 5% Qiagen® AW2 buffer and tested with the Invader® UGT1A1 Molecular Assay. Genotype results were compared to those obtained from non-spiked samples. There was 100% genotype call agreement between spiked vs. non-spiked samples with the hemoglobin and 1% AW2 buffer interferants; 5% AW2 buffer resulted in 1 incorrect genotype call and 1 "low signal" result. All genotype results were verified using bi-directional DNA sequencing.

6(b)(3) Conclusions

The Invader® UGT1A1 Molecular Assay accurately identifies the UGT1A1 genotype of clinical specimens using DNA purified from human whole blood samples.

7. CLINICAL VALIDITY

Individual differences in drug response are common and variability in drug response among patients affects the disposition (absorption, distribution, metabolism and excretion) of a given drug. This is particularly true of drugs with a narrow therapeutic index. Sequence variations in genes involved in drug response have been shown to produce differences in

drug disposition that alters the expected relationship between the dose of a drug and its concentration in the blood or the length of time it stays in the blood. A polymorphism of seven TA repeats TA(7) in the TATA box of the UGT1A1 promoter region has been found to produce reduced gene expression and reduced glucuronidation in human liver microsomes resulting in decreased drug metabolism and increased toxicity (see Table 6). Adjustment of drug dosage may be beneficial based upon knowledge of these differences in metabolism, particularly for individuals possessing the TA(7) genotype.

An FDA advisory committee assessed the scientific and clinical evidence correlating the UGT1A1*28 genotype with greater risk for irinotecan induced toxicity. Table 5 shows the prevalence of the UGT1A1*1 and UGT1A1*28 alleles in Caucasians and the associated risk of toxicity, based on a prospective study of 66 patients receiving irinotecan treatment (adapted from FDA's presentation to the advisory committee). Patients who are homozygous for the UGT1A1*28 allele are at 50% risk of experiencing toxicity.

Table 5. Summary of Allele Prevalence and Risk of Toxicity

Group	Prevalence	Risk of Toxicity
All Patients	----	10%
Patients that are 7/7	10%	50%
Patients that are 6/7	40%	12.5%
Patients that are 6/6	50%	0%

The committee concluded that there is enough clinical and scientific evidence to correlate UGT1A1*28 genotype with increased risk for irinotecan toxicity. The updated CAMPTOSAR® (irinotecan) package insert recommends that individuals who are homozygous for the UGT1A1*28 allele are at an increased risk for neutropenia following initiation of CAMPTOSAR® treatment. A reduced initial dose should be considered for patients known to be homozygous for the UGT1A1*28 allele.

Table 6. UGT1A1 Allele Frequency

Allele	Population		
	Caucasian	**Asian**	**African**
$(TA)_6$	61.3%	84%	47%
$(TA)_7$	38.7%	16%	42.6%

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Mr. Sam Rua
Third Wave Technologies, Inc.
502 South Rosa Road
Madison, WI 53719-1256

AUG 18 2005

Re: k051824
Trade/Device Name: Invader® UGT1A1 Molecular Assay
Regulation Number: 21 CFR 862.3360
Regulation Name: Drug metabolizing enzyme genotyping system
Regulatory Class: Class II
Product Code: NTI
Dated: August 11, 2005
Received: August 15, 2005

Dear Mr. Rua:

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 Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. 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 Parts 801 and 809); and 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 (301) 594-3084. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). 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 (301) 443-6597 or at its Internet address http://www.fda.gov/cdrh/dsma/dsmamain.html.

Sincerely yours,

Carol C. Benson

Carol C. Benson, M.A.
Acting Director
Division of Chemistry and Toxicology
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): K051824

Device Name: Invader® UGT1A1 Molecular Assay

Indications For Use:

The Invader® UGT1A1 Molecular Assay is an *in vitro* diagnostic test for the detection and genotyping of the *1 (TA6) and *28 (TA7) alleles of the UDP glucuronosyltransferase 1A1 (UGT1A1) gene in genomic DNA from whole peripheral blood as an aid in the identification of patients with greater risk for decreased UDP-glucunorosyltransferase activity.

Prescription Use ✓ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

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

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K051824