



INFINITI[®] CYP2C19 Assay
Package Insert

For *In Vitro* Diagnostic Use



FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 1600 Faraday Avenue , Carlsbad, CA USA 92008

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
Schiffgraben 41, 30175 Hannover, Germany



INTENDED USE

The INFINITI CYP2C19 Assay is an *in vitro* diagnostic test for the identification of a patient's CYP450 2C19 genotype in genomic deoxyribonucleic acid (DNA) obtained from EDTA-anticoagulated whole blood samples. The INFINITI CYP2C19 Assay is a qualitative assay for use in clinical laboratories upon prescription by the attending physician.

The INFINITI CYP2C19 Assay is indicated for use as an aid to clinicians in determining therapeutic strategy for therapeutics that are metabolized by the CYP450 2C19 gene product, specifically, *2, *3, and *17.

The INFINITI CYP2C19 Assay is not indicated to be used to predict drug response or non-response.

The information provided from this test may supplement decision making and should only be used in conjunction with routine monitoring by a physician. Because of the variability in the knowledge of clinical utility with specific drugs that are metabolized by CYP2C19, clinicians should use professional judgment in the interpretation of results from this test. Results from this type of assay should not be used in predicting a patient's response to drugs for which the drug metabolizing enzyme activity of the allele, or the drug metabolic pathway, has not been clearly established.

BACKGROUND INFORMATION

Individual variability in the activity of drug-metabolizing enzymes is a major source of differences in drug exposure and, hence, a major contributor to variation in drug response. CYP450 2C19 (CYP2C19) is an important drug-metabolizing enzyme with variability determined by genetic polymorphism. CYP2C19 is a member of the hepatic microsomal enzymes involved in the xenobiotics metabolism. This highly polymorphic liver enzyme is involved in the metabolism and elimination of many commonly prescribed drugs including antidepressants, antiepileptics, barbiturates, and proton pump inhibitors.

Genetic polymorphisms in CYP2C19 are common and can affect therapeutic response to drugs. The enzyme activity is expressed at highly variable levels: ranging from poor metabolizers to ultra-rapid metabolizers. CYP2C19 acts on 5-10% of drugs in current clinical use.

Detecting genetic variations in drug-metabolizing enzymes is useful for identifying individuals who may experience adverse drug reactions with conventional doses of certain medications. Individuals who possess CYP2C19 poor metabolizer variants may exhibit different pharmacokinetics (drug levels) than normal individuals. As a result, such individuals may require non-conventional therapeutic strategies for medications that are metabolized by CYP450 2C19.

Adjustment of therapeutic strategy could be beneficial based upon knowledge of these differences in metabolism, particularly for individuals possessing the poor metabolizer phenotype. Data is available in literature that supports phenotypic determinations for drugs that are metabolized by CYP2C19. The following table lists some clinically relevant drugs that are known substrates of CYP2C19 enzymes.

Antifungals	Cardiovascular	Gastroenterology	Neurology & Psychiatry
Voriconazole	Clopidogrel	Dexlansoprazole	Citalopram
	Prasugrel	Esomeprazole	Clobazam
	Ticagrelor	Lansoprazole	Diazepam
		Omeprazole	
		Pantoprazole	
		Rabeprazole	

The most common poor metabolizer phenotypes have been identified as **CYP2C19*2** and **CYP2C19*3**. CYP2C19*2 and CYP2C19*3, which encode for non-functional proteins, are responsible for the vast majority of poor metabolizer (PM) phenotypes. The allele frequency of PM phenotype varies significantly between populations, ranging from 2 to 5% in White and Black populations to 13-23% in Asian populations.^[1]



The effect of the CYP2C19*2 and CYP2C19*3 are well established and are well known.

A novel CYP2C19 gene variant, **CYP2C19*17** is the one phenotype that has an opposite effect. CYP2C19*17 has been reported to be associated with ultra-rapid CYP2C19 activity.^[2, 3] Two single-nucleotide polymorphisms are specific for the CYP2C19*17 allele (-806C>T and -3402C>T), and the ultra-rapid activity is linked to the recruitment of the transcription factor(s) to the mutated -806 sites.^[2, 3]

The frequency of the CYP2C19*17 allele is equally high (18%) in Ethiopians and Swedes. A lower frequency has been reported in a Japanese population (1.3%) and in Chinese subjects (4%). A study reported a CYP2C19*17 allele frequency of 27% in a Polish population.^[3]

The **INFINITI CYP2C19 Assay** detects mutations in the CYP450 2C19 *2, *3 and *17 alleles. Clinicians should use caution in predicting phenotype and adjusting treatment strategy for patients who express alleles that have not been investigated for activity in metabolizing a specific drug.

The following table lists the alleles recognized by the INFINITI CYP2C19 Assay, the single nucleotide polymorphisms recognized by the device for each allele, enzyme activity and references.^[4]

CYP2C19 Allele	Polymorphism Recognized by INFINITI CYP2C19 Assay	Polymorphisms Associated with the Allele ^b	Enzyme Activity	References
*1 ^a	None ^a	None ^a	Normal	Romkes <i>et al</i> , 1991 ^[5] Richardson <i>et al</i> , 1995 ^[6] Blaisdell <i>et al</i> , 2002 ^[7]
*2	19154G>A	-98T>C; 99C>T; 12122G>A; 12460G>C 12662A>G; 12834G>C; 19154G>A ; 19520A>G; 57740C>G; 79936T>A; 80160C>T; 80161A>G; 87275G>A	None	de Morais <i>et al</i> , 1994 ^[8] Ibeanu <i>et al</i> , 1998 ^[9] Fukushima-Uesaka <i>et al</i> , 2005 ^[10] Lee <i>et al</i> , 2009 ^[11]
*3	17948G>A	-889T>G; 12013T>G; 12122G>A; 12306G>A; 13166T>C; 17948G>A ; 18911A>G; 80161A>G; 80248G>A; 87313A>C	None	de Morais <i>et al</i> , 1994 ^[8] Fukushima-Uesaka <i>et al</i> , 2005 ^[10]
*17	-806C>T	-3402C>T; -806C>T ; 99C>T; 80161A>G	Increased	Sim <i>et al</i> , 2006 ^[2] Rudberg <i>et al</i> , 2008 ^[3]

a *1 genotype for the INFINITI CYP2C19 Assay indicates only the absence of *2, *3 and *17 alleles

b SNPs in **bold** are the major SNPs/alterations responsible for the phenotype of the corresponding allele. and are unique to the mutation.

No information is provided by the INFINITI CYP2C19 Assay for the following CYP450 2C19 alleles: *4, *5, *6, *7, *8, *9, *10, *11, *12, *13, *14, *15, *16. Mutations in these alleles may or may not be present in the sample.

Samples with 2C19*10 genotype may be miscalled by the INFINITI CYP2C19 Assay as 2C19*2. Both 2C19*2 and 2C19*10 are poor metabolizers.



TEST PRINCIPLE/ASSAY OVERVIEW

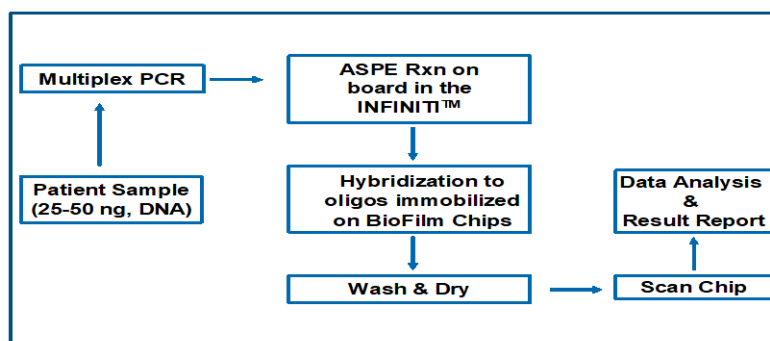
The INFINITI CYP2C19 Assay utilizes hybridization capture array with instrument performed automated detection of multiplex PCR products.

The INFINITI CYP2C19 Assay is designed to identify genotypes of 2C19 in deoxyribonucleic acid (DNA) obtained from EDTA-anticoagulated whole blood samples. The CYP2C19 Assay is based on five major processes:

- PCR amplification of purified DNA
- Labeling of the amplified product (allele specific detection primer extension)
- Hybridization of the labeled amplified product to a microarray
- Scanning of the microarray
- Signal detection

PCR amplification is performed off-line. The rest of the processes are automated by the AutoGenomics INFINITI Analyzer and INFINITI PLUS Analyzer.

A schematic overview of the assay is shown below.



DEVICE DESCRIPTION

The INFINITI CYP2C19 Assay utilizes AutoGenomics' proprietary film-based microarray technology for multiplex detection of the genotypes of CYP450 2C19 in DNA obtained from human blood samples.

The INFINITI CYP2C19 Assay is comprised of the following:

- R-Chip BioFilmChip Microarray
- Intellipac Reagent Module
- Amplification Mix
- Assay Protocol

The **BioFilmChip® Microarray** consists of a polyester film coated with proprietary multi-layer components designed for DNA analysis. The layers have been designed to provide a versatile surface to enhance test performance. The microarrays are designed to be assay specific. The INFINITI CYP2C19 Assay uses a microarray chip (R-Chip) which contains unused capture probes which could potentially be used for certain specific assays. Therefore, multiple assays can be developed using the same microarray.

The **Intellipac® Reagent Module** which acts as a communication link contains up to four reservoirs that house the test reagents and has an integrated memory chip. Information on the reagent such as lot number, expiration date and volume usage is stored in the memory chip.

The **Amplification Mix** provides the reagent for the PCR amplification of the DNA sample.



The assay is performed using the INFINITI PLUS Analyzer. The **Assay Protocol** (GAP) which specifies the assay steps, parameters and conditions, and the assay Header which specifies the algorithm, assay multipliers and ratios/cut-offs, reside in the CYP2C19 GAP/Header. The GAP/Header is loaded into the INFINITI PLUS Analyzer.

The **INFINITI PLUS Analyzer** is an instrument used for clinical multiplex systems intended to measure and sort multiple signals from a clinical sample. The Analyzer is designed to measure fluorescence signals of labeled DNA target hybridized to BioFilmChip microarrays. The Analyzer automates the CYP2C19 Assay and integrates all the discrete processes of sample (PCR amplicon) handling, reagent management, hybridization, detection, and results analysis. The assays are processed automatically and the spots are read by the built-in confocal microscope. Results are analyzed and presented as genotype calls.

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

The INFINITI PLUS Analyzer is CE-marked.

The INFINITI 2C19 Assay is available in two formats:

- INFINITI CYP2C19 - tests one sample per microarray chip
- INFINITI CYP2C19 (Quad Format) - tests four samples per microarray chip

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. No test method can offer complete assurance that HCV, HIV or other infectious agents are absent.
Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).
- Upon receipt of samples, visually inspect sample condition. Specifically, look for abnormal signs that indicate that sample integrity has been compromised (e.g., evaporation, decrease in volume, precipitation, spills, discoloration, sedimentation, separation, turbidity, etc.). If you observe or suspect any sample abnormality, do not perform any test.
- Samples should be handled with extreme caution to prevent contamination, spillage, sample mix-up. Sample containers should be labeled clearly to prevent mix-up.
- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures

- Follow normal precautions for handling laboratory reagents.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling

- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations
- Safety data Sheets (SDS) are available upon request from AutoGenomics Customer Service



Sample Preparation

- Refer to the safety instructions in the package insert provided with the DNA extraction kit used.
- It is critical to perform the PCR properly, ensuring proper pipetting of reagents. In addition, proper sealing of the PCR tubes should be ensured by pressing down on the lid.
- The thermocycler used for PCR should be properly calibrated.
- Visually inspect each PCR product for indication of evaporation, e.g., low volume or discoloration.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately.

INFINITI PLUS Analyzer

- **Read the Operator's Manuals before operating the instruments.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

STORAGE / STABILITY

BioFilmChip Microarray: 24 months Refrigerated (2 to 8°C)

Intellipac Reagent: 18 months Refrigerated (2 to 8°C)

Note: Do not use after Intellipac has been opened for 4 weeks

Amplification Mix: 18 months Frozen (-30 to -15°C)

Note: Specific product expiration date is printed on the product label

INFINITI CYP2C19 MATERIALS PROVIDED (SUFFICIENT FOR 48 TESTS)

- Product Number 03-1220-02: INFINITI CYP2C19 BioFilmChip Microarray Magazine: 4 magazines per package; 12 microarray chips per magazine
- Product Number 03-2220-02: INFINITI CYP2C19 Intellipac Reagent Module: 2 modules per package; 24 tests per module. Each Intellipac module contains
 - 1.1 ml ASPE Master Mix:
 - dNTPs
 - Labeled-dCTP
 - Allele Specific Primers
 - Extension Reaction Buffer
 - 2.6 ml Hybridization Buffer
 - SSC
 - EDTA
- Product Number 03-3220-02: INFINITI CYP2C19 Amplification Mix: each package contains 2 x 500 µl vials of amplification mix. Amplification Mix contains
 - dNTPs
 - PCR Primer Mix
 - MgCl₂
 - PCR Reaction Buffer
- Product Number 12-0330-00: Wash buffer

INFINITI CYP2C19 (QUAD FORMAT) MATERIALS PROVIDED (SUFFICIENT FOR 192 TESTS)

- Product Number 03-1230-02: INFINITI CYP2C19 (Quad Format) BioFilmChip Microarray Magazine: 4 magazines per package; 48 tests per magazine
- Product Number 03-2230-02: INFINITI CYP2C19 (Quad Format) Intellipac Reagent Module: 2 modules per package; 96 tests per module. Each Intellipac module contains:
 - 4 x 1.1 ml ASPE Master Mix
 - dNTPs
 - Labeled-dCTP
 - Allele Specific Primers



- Extension Reaction Buffer
- Product Number 03-3230-02: INFINITI CYP2C19 (Quad Format) Amplification Mix: each package contains 4 x 1ml vials of amplification mix. Amplification Mix contains:
 - dNTPs
 - PCR Primer Mix
 - MgCl₂
 - PCR Reaction Buffer
 - Product Number 12-0030-00: Hybridization Buffer 2: 6 x 30ml bottles. The hybridization buffer consists of:
 - SSC
 - EDTA
 - PVP and PEG
 - Sorbitol and Tween 20
 - **FOR INFINITI PLUS Analyzer:** Product Number 12-0330-00: Wash Buffer 4 x 125mL bottles

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- DNA Extraction Kits - The INFINITI CYP2C19 Assay can detect the CYP450 2C19 allelic mutations using genomic DNA isolated from blood with sufficient purity, i.e., with the ratio of absorbance at 260nm to absorbance at 280nm of ≥ 1.60 . Any DNA extraction method that meets this specification may be used. The INFINITI CYP2C19 Assay has been tested with several commercially available kits. The user can contact AutoGenomics for further information.
- Distilled Water (DNase and RNase free)
- Platinum Taq DNA Polymerase (Invitrogen, Catalog # 10966-018)
OR
INFINITI Taq DNA Polymerase 2 (30 μ l Catalog # 12-0480-00)

EQUIPMENT

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml micro-centrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Pipette Tips
- **FOR INFINITI PLUS Analyzer:**
 - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
 - AutoGenomics Product Number 11-0100-00: 48-Well Plates
 - AutoGenomics Product Number 11-0110-00: 48 Well Plate Lid (reusable)
 - AutoGenomics Product Number 11-0070-00: Tip Barrel Plugs

SPECIMEN COLLECTION AND STABILITY

- This test is to be used only with whole blood collected in EDTA. Specimens should be kept refrigerated (2°C to 8°C) and extracted within nine (9) days from the day the specimen was collected. Do not freeze/thaw blood samples.



ASSAY PROCEDURE

DNA Extraction

- Follow the instructions provided with the DNA extraction kit used.
- Extracted DNA samples should be kept refrigerated (2°C to 8°C) and assayed within two (2) days from the day the specimen was extracted. Extracted DNA samples can be stored frozen (-15°C to -30°C) for up to 5 years. Do not freeze/ thaw samples more than three times.

DNA Controls

It is required to run known positive controls and a negative control should also be included in each test run.

PCR Reaction

Observe the following precautions during PCR.

- It is critical to perform the PCR properly, ensuring proper pipetting of the reagents.
- Proper sealing of the PCR tubes is critical to the PCR. Proper sealing is achieved by making sure that the lid is properly seated and by pressing on the silicon lid. If evaporation occurs, sample temperature may vary and could affect the quality of the PCR product. This could result in a "no call".
- Visually inspect each PCR product for indication of evaporation, e.g., low volume or discoloration.
- The PCR product cannot be stored prior to loading it onto the microarray. Use immediately.
- Keep Taq DNA polymerase on ice.
- Completely thaw reagents at room temperature then immediately place on ice.
- Vortex the amplification mix tube for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.
- Filter tips and gloves must be used when handling specimens and controls.

Note:

- For the INFINITI PLUS Analyzer use the 48WP.

Note: When using the Quad format, always load the samples in multiples of four and in consecutive order. For example, if loading 8 samples, load wells A1 to A8. Do not load the B wells in this example.

1. Vortex the amplification mix tube for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.
- 2.
2. Prepare the PCR master mix.

Amplification mix	17.9 μ l
Platinum Taq polymerase or DNA Polymerase 2	0.1 μ l
<hr/>	
Total volume of PCR Master mix	18.0 μ l

Note: Calculate the amount of each reagent needed based on the number of reactions.

3. Gently vortex the PCR master mix then dispense 18 μ l of master mix into wells of the well plate.
4. Add 2 μ l of sample DNA (approximately 25ng/ μ l) to each well.

PCR master mix	18.0 μ l
Sample DNA	2.0 μ l
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Total volume of amplification reaction	20.0 μ l

Note: Sample DNA should not be less than 10 ng/ μ l and not more than 200 ng/ μ l.



- Place the well plate, sealed with 8-well flat strip caps, in a thermocycler and immediately commence the amplification reaction using the following program.

PCR Program for the thermal cycler:

Step No.	Temperature °C	Time	No. of Cycles
1	94	2 min.	N/A
2a	94	30 sec.	12x
b	62-56 (-0.5°C/cycle)	30 sec.	
c	72	30 sec.	
3	94	30 sec.	30x
	55	30 sec.	
	72	30 sec.	
4	72	2 min.	N/A
	4	Hold	

Note: After each cycle in step 2b the temperature is decreased by 0.5°C. When using an Eppendorf Mastercycler EP with the ramp rate set at 75%, the total cycling time is 1 hour and 45 minutes (\pm 5 min). If using other thermocycler models we recommend adjusting the ramp rate in order to obtain an equivalent total cycling time.

Sample Loading

- Carefully remove the 8-well flat strip caps to avoid splashing.
- Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
 - INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (see instructions in the INFINITI PLUS Analyzer Operator's Manual) (Product No. 11-0110-00, reusable).
- Load the following: assay specific magazines, Intellipac, INFINITI Static Free Pipette tips, and buffer.
 - FOR INFINITI PLUS Analyzer:**
Follow the INFINITI PLUS Analyzer Operator's Manual for checking and replacing Wash buffer.

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI PLUS Analyzer Operator's Manual (Product No. EM-34041)

QUALITY CONTROL

- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

It is recommended that positive samples for each mutation (heterozygous and/or homozygous), a negative control (a sample that does not contain the mutations, i.e., a wild type sample) and a non-template-control be included with each run.

All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.



LIMITATIONS

The INFINITI CYP2C19 Assay identifies **only** the 2C19 *2, *3 and *17 alleles and corresponding genotype polymorphisms. The INFINITI CYP2C19 Assay reports only the genotype for these alleles. When the report lists all the genotypes as wild-type, only these alleles are wild-type.

No information is provided by the INFINITI CYP2C19 Assay for the following CYP450 2C19 alleles: *4, *5, *6, *7, *8, *9, *10, *11, *12, *13, *14, *15, *16. Mutations in these alleles may or may not be present in the sample.

Samples with 2C19*10 genotype may be miscalled by the INFINITI 2C19 Assay as 2C19*2. Both 2C19*2 and 2C19*10 are poor metabolizers.

Based upon published information, the genotype information provided by the INFINITI CYP2C19 Assay may be used by the clinician to predict an individual's CYP2C19 enzymatic activity. Clinicians should use caution in predicting phenotype and adjusting treatment strategy for patients who express alleles that have not been investigated for activity in metabolizing a specific drug.

The results obtained from the INFINITI CYP2C19 Assay should be used and interpreted only in the context of the overall clinical diagnosis. AutoGenomics is not responsible for any clinical decisions that are taken.

INTERPRETATION OF RESULTS

The INFINITI CYP2C19 Assay is designed to detect and genotype CYP450 2C19 *2, *3, and *17. The assay report lists the alleles and provides which genotype was detected in the sample: Wild-Type, Mutant, and Heterozygous. If a wild-type is detected, the INFINITI CYP2C19 Assay report will show "W" for the allele. If the Mutant is detected, the report will show CYP2C19 [*2-Mut], CYP2C19 [*3-Mut], CYP2C19 [*17-Mut] for the corresponding allele. If Heterozygous, the report will show CYP2C19 [*2-Het], CYP2C19 [*3-Het], CYP2C19 [*17-Het] for the corresponding allele.

For example,

- If the sample is *1/*3, the report will show CYP2C19 [*3-Het] and "W" for *2, and *17
- If the sample is *2/*2, the report will show CYP2C19 [*2-Mut] and "W" for *3, and *17
- If the sample is *2/*17, the report will show CYP2C19 [*2-Het], CYP2C19 [*17-Het] and "W" for *3
- If the sample is wild-type for the alleles detected, the report will show "W" for all alleles listed

When the assay is not completed, and no genotype call is made, the assay will need to be repeated. The report displays a message which indicates the reason why no genotype call was made. When an error occurs (e.g., "low DNA"), an Error Log is generated which identifies the problem. Please refer to the Trouble Shooting section of the INFINITI Analyzer Operator's Manual.

Because the INFINITI CYP2C19 Assay only genotypes CYP450 2C19 *2, *3, and *17, no information is provided for other CYP450 2C19 alleles. Mutations in these undetected alleles may or may not be present in the sample

Samples with 2C19*10 genotype may be miscalled by the INFINITI CYP2C19 Assay as 2C19*2. Both 2C19*2 and 2C19*10 are poor metabolizers.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASPE primer specificity was determined by the correct calls made by the assay using known genomic samples. Capture probe specificity was determined by hybridizing different oligos and demonstrating that correct oligo hybridizes to the known spot.



Limit of Detection (analytical sensitivity)

The analytical sensitivity (Limit of Detection) of the INFINITI CYP2C19 Assay was assessed by analysis of whole blood samples at serial dilutions representing 500ng, 400ng, 200ng, 100ng, 50ng, 20ng, 10ng and 5ng DNA input (per test) to determine the lowest level of genomic DNA (ng input per test) that would give a $\geq 90\%$ correct call rate of the allele with no incorrect calls.

The following whole blood samples were used in the LOD studies: *1/*1, *1/*2, *1/*3, *1/*17, *2/*2 and *2/*17. Sample genotype was determined by bi-directional sequencing.

Two extraction methods were used in the studies: Qiagen QIAamp DNA Blood Kit and the PSS Magtration System. The concentration and A_{260}/A_{280} were determined by spectrophotometry for the extracted DNA.

A total of 1,560 tests were completed for the LOD studies. A $\geq 90\%$ correct call rate with no incorrect calls for the allele was obtained at DNA input levels from 400ng/test to 20ng/test. There was one incorrect call at the 5ng DNA input level. The incorrect call was probably due to the low DNA concentration, therefore, we are not recommending DNA input level at this low concentration.

The lowest detectable level for the INFINITI CYP2C19 Assay is **20ng DNA per test**. This is less than one-half (1/2) the recommended DNA input level of 50ng/test, and four times the level at which an incorrect call was made by the assay. We believe that establishing the lowest detectable level at 20ng DNA/test is conservative and should preclude an incorrect call. Table 1 provides a summary of the LOD studies for the INFINITI CYP2C19 Assay.

Table 1 Limit of Detection

Genotype ^a	Sample ID	ng DNA input per test	Replicates tested	Correct calls	Incorrect calls	No calls	% correct calls 1 st time run	95% One-sided Confidence Lower Limit
*1/*1	AG44 AG105 AG208 AG209 AG211 AG219	500	40	34	0	6	85.0%	72.7%
		400	40	38	0	2	95.0%	87.0%
		200	40	38	0	2	95.0%	87.0%
		100	40	40	0	0	100%	98.8%
		50	40	39	0	1	97.5%	91.4%
		20	40	39	0	1	97.5%	91.4%
		10	40	38	0	2	95.0%	87.0%
		5	40	39	0	1	97.5%	91.4%
	Total		320	305	0	15	95.3%	92.8%
*1/*2	AG81 AG197 AG210 AG223	500	20	19	0	1	95.0%	82.9%
		400	20	20	0	0	100%	97.5%
		200	20	20	0	0	100%	97.5%
		100	20	20	0	0	100%	97.5%
		50	40	37	0	3	92.5%	83.1%
		20	40	36	0	4	90.0%	79.5%
		10	40	38	0	2	95.0%	87.0%
		5	40	39	0	1	97.5%	91.4%
	Total		240	229	0	11	95.4%	92.6%



Genotype ^a	Sample ID	ng DNA input per test	Replicates tested	Correct calls	Incorrect calls	No calls	% correct calls 1 st time run	95% One-sided Confidence Lower Limit
*2/*2	AG82 AG199 AG214 AG233	500	40	40	0	0	100%	98.8%
		400	40	39	0	1	97.5%	91.4%
		200	40	37	0	3	92.5%	83.1%
		100	40	39	0	1	97.5%	91.4%
		50	40	37	0	3	92.5%	83.1%
		20	40	38	0	2	95.0%	87.0%
		10	40	37	0	3	92.5%	83.1%
		5	40	37	0	3	92.5%	83.1%
Total			320	304	0	16	95.0%	92.5%
*1/*3	AG152 AG162	500	40	40	0	0	100%	98.8%
		400	40	40	0	0	100%	98.8%
		200	40	40	0	0	100%	98.8%
		100	40	39	0	1	97.5%	91.4%
		50	40	40	0	0	100%	98.8%
		20	40	40	0	0	100%	98.8%
		10	40	40	0	0	100%	98.8%
		5	40	38	1	1	95.0%	87.0%
Total			320	317	1	2	99.1%	97.9%
*1/*17	AG74 AG94 AG180 AG235	500	40	36	0	4	90.0%	79.5%
		400	40	38	0	2	95.0%	87.0%
		200	40	40	0	0	100%	98.8%
		100	40	40	0	0	100%	98.8%
		50	40	40	0	0	100%	98.8%
		20	40	38	0	2	95.0%	87.0%
		10	40	40	0	0	100%	98.8%
		5	40	39	0	1	97.5%	91.4%
Total			320	311	0	9	97.2%	95.2%
*2/*17	AG191	100	20	20	0	0	100%	97.5%
	AG195	5	20	20	0	0	100%	97.5%
	Total			40	40	0	0	100%

^a determined by bi-directional sequencing

Assay Accuracy - Percent Agreement vs. Bi-directional Sequencing

The INFINITI CYP2C19 Assay was compared to bi-directional sequencing as the comparator method. Three sites were used for the comparison studies. Each site tested its own patient samples with the INFINITI CYP2C19 Assay. Patient samples were de-identified to protect patient's identity.

- A total of 317 samples were tested.
- There were no incorrect calls
- Six samples (1.9%) had to be repeated because of "no call" due to NTCE Error. NTCE is reported when the quality or quantity of the DNA in the sample/PCR product is poor. All six no calls gave correct calls upon the repeat for a 100% agreement of the INFINITI CYP2C19 Assay with bi-directional sequencing. The repeat test was done on the same extracted DNA. Only one repeat was done for each sample.



The results of the comparison studies comparing the INFINITI CYP2C19 Assay to bi-directional sequencing are provided in Table 2.

Table 2 Agreement between INFINITI CYP2C19 Assay with Bi-directional Sequencing

Genotype ^a	Number Tested	Replicates per Sample	Number of Correct Genotype Calls	Number of Incorrect Calls	No Calls	Agreement	95% One-sided Confidence Lower Limit
*1/*1	105	1	103	0	2	98.1%	95.0%
*1/*2	80	1	77	0	3	96.2%	91.5%
*2/*2	12	1	12	0	0	100%	95.8%
*1/*3	8	1	8	0	0	100%	93.8%
*3/*3	1	1	1	0	0	100%	50.0%
*1/*17	74	1	73	0	1	98.6%	95.3%
*17/*17	16	1	16	0	0	100%	96.9%
*2/*3	4	1	4	0	0	100%	87.5%
*2/*17	16	1	16	0	0	100%	96.9%
*3/*17	1	1	1	0	0	100%	50.0%
Total	317	1	311	0	6	98.1%	96.4%

a Genotype determined by bi-directional sequencing; *1/*1 samples are wild-type for *2, *3 and *17

Assay Inter-Laboratory Reproducibility

A three-site study was conducted to demonstrate the reproducibility of the INFINITI CYP2C19 Assay. The study involved three identical lots of the INFINITI CYP2C19 Assay, four operators, and four instruments (one site ran two sets of reproducibility studies, each with a different operator and instrument).

The sites ran identical samples comprised of 12 whole blood samples. The sites were blinded to sample identity. At each site, each sample was run in duplicate per day/operator for five non-consecutive days. A total of 430 tests were run. Of the 430 samples assayed, 14 samples (3.3%) had to be repeated due to "No Calls". The samples were repeated using the same extracted DNA. These NTCE errors might have been caused by temperature gradient during PCR, improper sealing of the PCR tubes, or operator pipetting error. The genotype calls from the repeat assays were 100% correct. There was one incorrect call (*1/*2 instead of *2/*2). The root cause of the incorrect call was not definitively determined. Results of the inter-laboratory reproducibility study are summarized in Table 3a and Table 3b.



Table 3a Inter-Laboratory Reproducibility of the INFINITI CYP2C19 Assay by Genotype Call

Genotype ^a	Samples Tested	Site ^b	Replicates per Site	Replicates with Genotype Calls made by INFINITI ^c	Correct Calls	Incorrect Calls ^d	No Calls ^e	% Correct Calls ^f	95% One-sided Confidence Lower Limit
*1/*1	2	1	40	39	39	0	1	97.5	91.4
		2	20	20	20	0	0	100	97.5
		3 ^g	10	10	10	0	0	100	95.0
		total	70	69	69	0	1	98.6	95.1
*1/*2	3	1 ^h	40	40	40	0	0	100	98.8
		2	30	30	30	0	0	100	98.3
		3	30	30	30	0	0	100	98.3
		total	100	100	100	0	0	100	99.5
*2/*2	2	1	40	39	38	1	1	95.0	87.0
		2	20	20	20	0	0	100	97.5
		3	20	14	14	0	6	70.0	47.4
		total	80	73	72	1	7	90.0	82.8
*1/*3	1	1	20	20	20	0	0	100	97.5
		2	10	10	10	0	0	100	95.0
		3	10	10	10	0	0	100	95.0
		total	40	40	40	0	0	100	98.8
*1/*17	2	1	40	39	39	0	1	97.5	91.4
		2	20	17	17	0	3	85.0	66.9
		3 ⁱ	10	9	9	0	1	90.0	66.4
		total	70	65	65	0	5	92.9	86.1
*17/*17	2	1	40	40	40	0	0	100	98.8
		2	20	19	19	0	1	95.0	82.9
		3 ^j	10	10	10	0	0	100	95.0
		total	70	69	69	0	1	98.6	95.1
Total	12	All	430	416	415	1^d	14^e	96.5	94.7

^a determined by bi-directional sequencing; *1/*1 samples are wild-type for *2, *3 and *17

^b Internal site (Site 1) had two sets, one operator each

^c Excludes samples with No Calls

^d Initial result was incorrect (*1/*2 instead of *2/*2). The root cause of the incorrect call was not definitively determined.

^e One no call was due to Registration Spot Error - this error is reported when the microarray chip is not properly aligned. Repeat test gave the correct call

13 reported NTCE Error - NTCE is reported if the quality or quantity of the DNA in the sample/PCR product is poor. Repeat tests gave the correct calls

^f Samples with correct calls/samples tested

^g Site 3: one sample had A260/A280 of 1.16 (1.6 is required), therefore only one sample was tested

^h Site 1: one sample had A260/A280 of 1.46 (1.6 is required), therefore only two samples were tested

ⁱ Site 3: one sample had A260/A280 of 1.46 (1.6 is required), therefore only one sample was tested

^j Site 3: one sample had A260/A280 of 1.45 (1.6 is required), therefore only one sample was tested



Table 3b Inter-Laboratory Reproducibility of the INFINITI CYP2C19 Assay by Sample

Sample ID	Genotype ^a	Replicates per sample	Replicates with Genotype Calls made by INFINITI ^b	Correct Calls ^c	No Calls ^d	Incorrect Calls ^e	Correct Call Rate ^f (%)	95% One-sided Confidence Lower Limit
1	*2/*2	40	38	37	2	1	92.5	83.1
2	*2/*2	40	35	35	5	0	87.5	76.0
3	*17/*17	40	39	39	1	0	97.5	91.4
4	*1/*17	40	37	37	3	0	92.5	83.1
5	*1/*3	40	40	40	0	0	100	98.8
6	*1/*2	40	40	40	0	0	100	98.8
7	*1/*2	40	40	40	0	0	100	98.8
8	*1/*1	30	29	29	1	0	96.7	88.6
9	*17/*17	30	29	29	1	0	96.7	88.6
10	*1/*1	40	40	40	0	0	100	98.8
11	*1/*17	30	29	29	1	0	96.7	88.6
12	*1/*2	20	20	20	0	0	100	97.5
All		430	416	415	14	1	96.5	94.7

^a Determined by bi-directional sequencing. *1/*1 samples are wild-type for *2, *3 and *17

^b Excludes samples with No Calls

^c A sample with correct call indicates a correct call at all loci.

^d One no call was due to Registration Spot Error - this error is reported when the microarray chip is not properly aligned. Repeat test gave the correct call; 13 reported NTCE Error - NTCE is reported if the quality or quantity of the DNA in the sample/PCR product is poor. Repeat tests gave the correct calls

^e Initial result was incorrect (*1/*2 instead of *2/*2). The root cause of the incorrect call was not definitively determined.

^f Samples with correct calls/samples tested

Additional reproducibility studies were conducted at the same three sites to demonstrate the reproducibility of the INFINITI CYP2C19 Assay for additional samples including *2/*17 and *2/*3. The study involved three lots of the INFINITI CYP2C19 Assay. The sites ran identical samples comprised of six (6) genomic whole blood samples. The sites were blinded to sample identity. At each site, each sample was run in triplicate per day/operator for five non-consecutive days.

A total of 255 tests were completed. Overall correct call rate for the first time run was 97.6%. There were no incorrect calls. There were six no calls:

- One no call was due to Registration Spot Error - this error is reported when the microarray chip is not properly aligned.
- Five were due to NTCE error. NTCE is reported if the quality or quantity of the DNA in the sample/PCR product is poor. These NTCE errors might have been caused by temperature gradient during PCR, improper sealing of the PCR tubes, or operator pipetting error.

Results of the inter-laboratory reproducibility studies are summarized in Table 4a and Table 4b.



Table 4a Inter-Laboratory Reproducibility of the INFINITI CYP2C19 Assay by Genotype calls

Genotype ^a	Samples Tested	Site	Replicates per Site	Replicates with Genotype Calls made by INFINITI ^b	Correct Calls	Incorrect Calls	No Calls ^c	% Correct Calls ^d	95% One-sided Confidence Lower Limit
*1/*1	1	1	15	15	15	0	0	100	96.7
		2	15	15	15	0	0	100	96.7
		3	15	15	15	0	0	100	96.7
		Total	45	45	45	0	0	100	98.9
*1/*2	1	1	15	15	15	0	0	100	96.7
		2	15	15	15	0	0	100	96.7
		3	15	13	13	0	2	86.7	66.1
		Total	45	43	43	0	2	95.6	88.4
*1/*3	1	1 ^e	0	n/a	n/a	n/a	n/a	n/a	n/a
		2	15	15	15	0	0	100	96.7
		3	15	15	15	0	0	100	96.7
		Total	30	30	30	0	0	100	98.3
*1/*17	1	1	15	15	15	0	0	100	96.7
		2	15	15	15	0	0	100	96.7
		3	15	15	15	0	0	100	96.7
		Total	45	45	45	0	0	100	98.9
*2/*3	1	1	15	15	15	0	0	100	96.7
		2	15	15	15	0	0	100	96.7
		3	15	12	12	0	3	80.0	56.4
		Total	45	42	42	0	3	93.3	84.9
*2/*17	1	1	15	15	15	0	0	100	96.7
		2	15	15	15	0	0	100	96.7
		3	15	14	14	0	1	93.3	77.4
		Total	45	44	44	0	1	97.8	92.4
Total	6	All	255	249	249	0	6	97.6	95.6

^a Determined by bi-directional sequencing; *1/*1 samples are wild-type for *2, *3 and *17

^b Excludes samples with No Calls

^c One no call was due to Registration Spot Error - this error is reported when the microarray chip is not properly aligned; 5 reported NTCE Error - NTCE is reported if the quality or quantity of the DNA in the sample/PCR product is poor.

^d Samples with correct calls/samples tested

^e Site 1: DNA concentration was 8 ng/μl, below the LOD of 10 ng/μl, therefore no sample tested



Table 4b Inter-Laboratory Reproducibility of the INFINITI CYP2C19 Assay by Sample

Sample ID	Genotype ^a	Replicates per sample	Replicates with Genotype Calls made by INFINITI ^b	Correct Calls ^c	No Calls ^d	Incorrect Calls	Correct Call Rate ^e (%)	95% One-sided Confidence Lower Limit
1	*1/*3	30	30	30	0	0	100	98.3
2	*1/*17	45	45	45	0	0	100	98.9
3	*1/*1	45	45	45	0	0	100	98.9
4	*2/*17	45	44	44	1 ^b	0	97.8	92.4
5	*2/*3	45	42	42	3	0	93.3	84.9
6	*1/*2	45	43	43	2	0	95.6	88.4
All		255	249	249	6	0	97.6	95.6

^a Genotype determined by bi-directional sequencing; *1/*1 samples are wild-type for *2, *3 and *17

^b Excludes samples with No Calls

^c A sample with correct call indicates a correct call at all loci.

^d One no call was due to Registration Spot Error - this error is reported when the microarray chip is not properly aligned; 5 reported NTCE Error - NTCE is reported if the quality or quantity of the DNA in the sample/PCR product is poor.

^e Samples with correct calls/samples tested

Table 5 provides a summary of the combined reproducibility studies.

Table 5: Summary of the Inter-Laboratory Reproducibility of the INFINITI CYP2C19 Assay by Genotype calls

Genotype ^a	Samples Tested	Replicates per Sample	Replicates with Genotype Calls made by INFINITI ^b	Correct Calls ^c	No Calls ^d	Incorrect Calls ^e	Correct Call Rate ^f (%)	95% One-sided Confidence Lower Limit
*1/*1	3	115	114	114	1	0	99.1	97.0
*1/*2	4	145	143	143	2	0	98.6	96.4
*2/*2	2	80	73	72	7	1	90.0	82.8
*1/*3	2	70	70	70	0	0	100	99.3
*1/*17	3	115	110	110	5	0	95.7	91.5
*17/*17	2	70	69	69	1	0	98.6	95.1
*2/*3	1	45	42	42	3	0	93.3	84.9
*2/*17	1	45	44	44	1	0	97.8	92.4
Total	18	685	665	664	20	1	96.9	95.6

^a Genotype determined by bi-directional sequencing; *1/*1 samples are wild-type for *2, *3 and *17

^b Excludes samples with No Calls

^c A sample with correct call indicates a correct call at all loci.

^d Two no calls were due to Registration Spot Error - this error is reported when the microarray chip is not properly aligned; 18 reported NTCE Error - NTCE is reported if the quality or quantity of the DNA in the sample/PCR product is poor.

^e Initial result was incorrect (*1/*2 instead of *2/*2). The root cause of the incorrect call was not definitively determined.

^f Samples with correct calls/samples tested



Interference

Interference from potential interfering substances was evaluated using eight (8) whole blood samples. The potential interfering substances were added separately to the whole blood sample prior to DNA extraction and testing with the INFINITI CYP2C19 Assay. Genotype results were compared to those obtained from non-spiked samples. Sample genotype was verified by bi-directional DNA sequencing. The interference studies demonstrated that the INFINITI CYP2C19 Assay performance was not affected by the addition of the following substances.

Substance Added	Concentration
Bilirubin (conjugated)	60mg/dl
Bilirubin (unconjugated)	60mg/dl
Triglycerides (Intralipid)	3000mg/dl
Human albumin	6g/dl

No studies were conducted with oral anti-coagulants, and no claims are being made.

Sample Carry-Over

No sample carry-over was detected when 300ng of a positive sample was followed by 10ng of a second positive sample, and when 300ng of a positive sample was followed by a “No Template Control” or water. All genotype calls were 100% correct.

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