



Genomadix Cube CYP2C19 System

Instructions For Use

For *In Vitro* Diagnostic Use

R_x ONLY



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NOTE: Throughout this document Genomadix Incorporated, may be referred to as Genomadix, or company and the Genomadix Cube may be referred to as the Cube.

Intended Use

The Genomadix Cube CYP2C19 System is a qualitative *in vitro* diagnostic test for the identification of a patient's CYP2C19 *2, *3, and *17 genotypes determined from genomic DNA obtained from a buccal swab sample.

The Genomadix Cube CYP2C19 System can be used to aid clinicians in determining therapeutic strategy for therapeutics that are metabolized by the cytochrome P450 2C19 gene product, specifically *2, *3, and *17 alleles. This test is not intended to be used to predict drug response or non-response.

Indications For Use

The Genomadix Cube CYP2C19 System is not indicated for stand-alone diagnostic purposes. The information provided from this test may supplement decision making and should only be used in conjunction with routine monitoring by a physician. 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 that allele, or the drug metabolic pathway, has not been clearly established.

The Genomadix Cube CYP2C19 Test Kit is indicated for use with the Genomadix Cube CYP2C19 Platform.

Product Overview

The Genomadix Cube CYP2C19 System is a sample-to-result DNA testing system that uses proprietary technology to integrate Deoxyribonucleic acid (DNA) extraction and amplification. Genotypes are determined using Polymerase Chain Reaction (PCR) and fluorescent probe detection.

The test protocol is based on the following major processes:

- a. Collection of human genomic DNA via swab(s)
- b. DNA extraction
- c. PCR amplification
- d. Detection of amplified PCR products via fluorescent oligonucleotide probes
- e. Fluorescent signal detection and analysis (determination of CYP2C19 *2, *3, and *17 genotypes)

NOTE: Steps (b) through (e) are automated by the CYP2C19 System.

THE GENOMADIX CUBE CYP2C19 SYSTEM

Item	Content	Purpose
Platform	Genomadix Cube	Instrument that automates the Genomadix Cube CYP2C19 Test.
	Computer	The user interface (software) for logging in to the CYP2C19 Platform, inputting patient information and other information associated with the test, automatically inputting Cartridge lot numbers, and the instructions for starting a test.
	Barcode scanner	Scans barcode information.
Test Kit	Swabs	Proprietary swabs used to collect a buccal sample from the inside of a patient's cheek and transfer it to the cartridge.
	Cartridge	Contains substances for DNA extraction, PCR amplification, and detection of the CYP2C19 alleles, in three separate tubes. Each tube is specific for the detection of one of the three CYP2C19 loci.

Materials Provided

Item	Part Number	Content	Quantity
Genomadix Cube CYP2C19 Test Kit	ASM-00095	Cartridges (ASM-00101; one per pouch)	20 Kits
		Swabs (ASM-00103; three per pouch)	

Additional Materials

Components	Part Number	Quantity
CYP2C19 External Control Cartridges	ASM-00099	10
CYP2C19 Training Kits	ASM-00116	5
Swab Kits	ASM-00103	20

Materials Required - Not Provided

- The Genomadix Cube CYP2C19 Platform (ASM-00096) is not provided with shipments of test kits.
- Personal protective equipment.
- Cleaning solutions.


Precautions - General


- For *in vitro* diagnostic use.
- For prescription use only.
- To be used by qualified personnel only.
- The Genomadix Cube CYP2C19 Test Kit was validated using the Genomadix Cube CYP2C19 Platform.
- The Genomadix Cube CYP2C19 System is to only be used with buccal samples collected using the swabs provided by Genomadix.
- Buccal samples must be collected and tested within the time constraints described in the Test Procedure.
- Proper sample collection and sample handling are essential for correct result, as well as to avoid errors related to sample misidentification and/or mishandling.
- Do not touch swab tip when handling swab sample.
- Do not use any kit components with visible damage.
- Do not use kit components after their expiration date.
- All test kit components are single use items. Do not use with multiple specimens.
- Dispose of kit components and patient samples according to all local regulations.
- Performance characteristics of this test have been established with specimen types listed in the Intended Use section only. The performance with other specimen types or samples has not been validated.
- Read the Genomadix Cube CYP2C19 System Operator's Manual before operating the Genomadix Cube CYP2C19 Platform.
- Refer to the Troubleshooting section of the Operator's Manual (CUS-00030) when problems are encountered.
- Do not connect any external accessory (USB stick, cell phone, etc.) or storage devices to the laptop, or Cube as it could impact product performance.
- The installation of any additional software or applications onto the Genomadix supplied laptop is prohibited.
- Do not plug/insert any personal or non-Genomadix provided accessories into the Laptop or Cube.
- To reduce the risk of electrical shock, do not disassemble any part of the Genomadix platform. Please contact technical support if you are having any issues.
- In environments with high density electromagnetic fields or areas that are susceptible to elevated levels of ESD discharge the operator may experience a temporary loss of essential performance which can be recovered by the operator. This is normal and expected for this type of equipment.
- The Genomadix Cube CYP2C19 Platform is not authorized for use in moist or wet environments.
- The Genomadix Cube CYP2C19 Platform is not authorized to be used with any type of flammable gasses (anesthetics) or oxygen rich environments.
- The Genomadix Cube CYP2C19 Platform is not intended to interoperate with any other medical device.
- If any component becomes damaged or inoperable, please stop use immediately, and contact your distributor for a replacement.

Storage and Handling Cartridges


- Store cartridges (test kit and external control cartridges) at -15°C to -80°C in the original box.
 - It is recommended to store the cartridges at the back of the freezer. This avoids exposure to room temperature when the freezer is opened.
- After removing the cartridge from the freezer, the test must be started within 15 minutes.
- Follow normal precautions for handling laboratory reagents (cartridges).
- Follow Good Laboratory Practices, as set out by your institution. These may include but are not limited to: wear protective clothing (e.g., disposable gloves); do not eat, drink, or smoke in the laboratory or test setup areas; wash hands thoroughly after handling buccal samples and reagent tubes.

Receiving Cartridges

 The cartridges are shipped frozen and may be shipped on dry ice. Dry ice can cause severe frostbite within seconds of contact. Avoid contact with skin and eyes. Always wear gloves. Do not ingest. Do not use in confined areas. Dry ice releases carbon dioxide vapors that can cause suffocation. For disposal, allow dry ice to sublimate in a well-ventilated area.





-80°C  -15°C Cartridges must be stored in a manual defrost freezer (one that does not say “frost-free”) at a temperature between -15°C and -80°C . It is recommended to store the cartridges at the back of the freezer in the original box. This avoids exposure to room temperature when the freezer door opens.

- When stored under these conditions, cartridges are stable until the expiry date marked on the product label.
- Do not take cartridges out of the freezer until you are ready to perform a test.

 Upon receipt of cartridge inspect the WarmMark temperature indicator. The purpose of the indicator is to ensure that the kits have remained frozen during shipping. The indicator window shows how much time the temperature indicator has been above -18°C .

The temperature indicator should be used as follows:

- Ensure the WarmMark indicator has a black region adjacent to “WarmMark”
- Ensure that the WarmMark indicator has no red colouring.

			
	<p>No colour to subtle colour in the “Brief” window.</p>		<p>If there is red dye present in the indicator window, cartridges may be thermally compromised; contact your distributor.</p>

NOTE: The temperature indicator is only to be used for shipping, it is not intended for the life of the product.

Specimen Collection, Storage and Handling

- The buccal swab collection must follow the instructions provided in the test procedure “Step 3: Collect Buccal Sample”
- The collected buccal samples can be stored up to 20 hours at 18°C to 25°C. If the collected samples cannot be tested within 20 hours, dispose of buccal samples collected. Obtain a new swab kit and collect new buccal samples from the patient.
- All patient specimens are potentially hazardous. Care should be taken when handling materials of human origin.
- If an INCONCLUSIVE or PSC result is displayed, restart at Step 1 of the workflow, collect a new set (second set) of swabs from the same patient to test using a new cartridge.
 - **NOTE:** If the patient being tested will not be available an hour after swab collection, it is recommended to collect two sets of swabs (six swabs total). Run a test using one set of swabs and store the second set according to the storage requirements listed in Specimen Collection, Storage and Handling. The second set of swabs shall be used if a repeat test is required. If the second set of swabs is not required, discard.

Contamination Prevention

- **Always** wear disposable gloves when handling swabs or cartridges. This prevents contamination of the test with your DNA.
- **Always** use a new pair of gloves for each new patient.
- **Never** open a cartridge after a test is started on the Cube.
 - Opening a cartridge after the test is finished could contaminate the environment with amplified DNA. This could lead to inaccurate results for future tests.
- **Never** attempt to open an external control cartridge at any time.
 - The external control cartridge contains a synthetic DNA template.
 - Opening an external control cartridge could contaminate the environment with this synthetic template. This could lead to inaccurate results for future tests.

To avoid PCR cross-contamination, the following precautions should be observed:

- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves
 - Use a new pair of gloves for each patient.
 - Use a new pair of gloves before handling the cartridge.
 - Change gloves after having contact with potential contaminants (such as specimens, other instruments, and/or amplified product).
- The CYP2C19 Test Kit is single use only. Use new materials for each patient test. At the end of each run, discard all remaining materials.

It is recommended to decontaminate the work surface prior to running a new test, in the event of a spill or if contamination is suspected. Follow these minimum recommended steps to decontaminate the working surface:

- a) Put on clean gloves.
- b) Absorb any spilled material with a paper towel.
- c) Spray 10% bleach solution on the area that may have been contaminated. Let it stand for ten minutes.
- d) Wipe the area with a clean paper towel. Change gloves.
 - In the event of a spill repeat steps (c) and (d).
- e) Spray the area with distilled water.
- f) Wipe the area dry with a new paper towel. Change gloves

Test Procedure

There are **8 steps** in performing the CYP2C19 test:

1. Obtain swabs.
2. Remove cover from swab.
3. Collect buccal sample.
4. Replace swab cover.
 - Repeat steps 2 to 4 for each swab.
5. Place cartridge in the Cube.
6. Insert the three swab tips into cartridge.
7. Tap cartridge to mix samples.
8. Start test.

If an **INCONCLUSIVE** or **PSC** result is displayed, restart at Step 1 of the workflow, collect a new set (second set) of swabs from the same patient to test using a new cartridge.

- If the patient being tested will not be available an hour after swab collection, it is recommended to collect two sets of swabs (six swabs total). Run a test using one set of swabs and store the second set according to the storage requirements listed in Specimen Collection, Storage and Handling. The second set of swabs shall be used if a repeat test is required. If the second set of swabs is not required, discard.



IMPORTANT:

- It is important to conform to the following time constraints:
 - After removing the Cartridge from the freezer, start the test within **15 minutes**.
 - After collecting the buccal sample, start the test within **20 hours**.
- Use a new pair of disposable gloves for each patient that you swab.
- Use a new pair of gloves before handling the cartridge.
- If you have accidentally touched the swab tip to an unintended object or believe for any other reason that the reaction has been contaminated with unintended material, discard the swabs and/or cartridge and collect new buccal samples.
- Proper sample collection and sample handling are essential for correct result, as well as to avoid errors related to sample misidentification and/or mishandling.

STEP 1: Obtain Swabs

- i) Obtain Swabs. Open pouch and remove all three swabs.



IMPORTANT:

- Wear gloves when handling swabs.
- Do not discard the pouch. After sample collection, you will need to place the swabs back into the pouch.
- If the patient being tested will not be available an hour after swab collection, it is recommended to collect two sets of swabs, in the event of an inconclusive (INC) or PSC.
- Tear open the pouch slowly. This will prevent you from inadvertently dislodging a swab tip from a swab.



STEP 2: Remove Swab Cover

- i) Pick up a swab in one hand and hold the white shaft like a pencil. The ideal position is halfway to three-quarters of the way away from the swab cover.

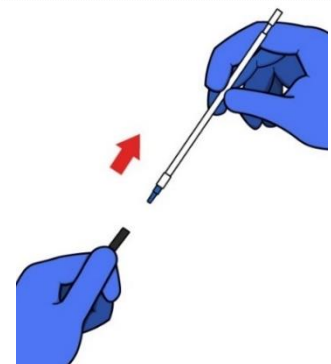
- ii) With your other hand, remove the swab cover.

- iii) Do not discard the swab cover. You will need to put it back onto the swab.



IMPORTANT:

- After removing the cover from the swab, ensure that the blue swab tip does not accidentally contact any surface.



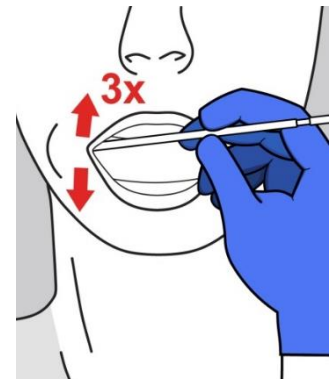
STEP 3: Collect Buccal Sample

- Scrape the inside of the patient's cheek with the blue swab tip. Use three (3) up-and-down strokes (6 strokes in total). Apply equal pressure for up and down strokes.



IMPORTANT:

- Apply the blue swab tip at a right angle to the inside of the cheek as though drawing vertical lines on the cheek with the swab tip.
- Use firm pressure to scrape the inside of the patient's cheek.
- The patient's cheek should be visibly pressed outwards to form a bulge.
- Proper sample collection and sample handling are essential for correct result, as well as to avoid errors related to sample misidentification and/or mishandling.



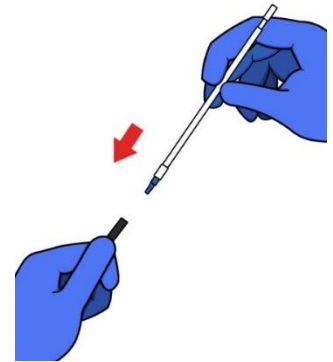
STEP 4: Replace swab cover

- i) Put the cover back over the swab tip.



IMPORTANT:

- Ensure that the swab tip does not accidentally contact any surface, including your gloved hand.



- ii) Repeat Steps 2 through 4(i) with the remaining two swabs.

- iii) Record or affix the patient information on the pouch and return the swabs to the pouch.

STEP 5: Place Cartridge into the Cube

- i) The cartridge is contained within a pouch that contains a barcode on the outside. Remove a cartridge from your freezer.

- ii) Log in to the CYP2C19 Platform software with your username and password.

- iii) Select **Run a test** and proceed through the screens of the software.

- iv) Enter patient information into the appropriate fields.

- v) Scan the barcode from the cartridge pouch using the barcode scanner.



- vi) Open the lid of the Cube.



- vii) Place the cartridge into the Cube.

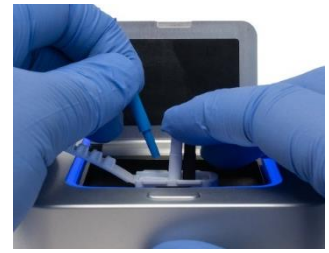


STEP 6: Insert Swab tip into Cartridge

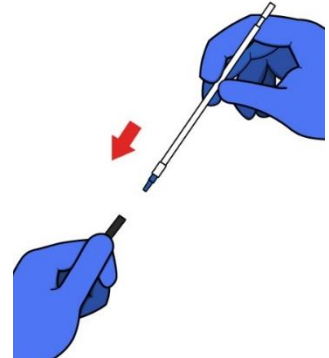
- i) Hold the cartridge down with one hand and remove a stopper from the cartridge with the other hand.



IMPORTANT: Ensure nothing falls into the uncapped cartridge tube.



- ii) Remove the swab cover from one of the buccal swabs used to swab the patient.

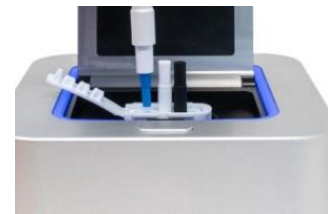


- iii) Insert the blue tip of the swab into the open cartridge tube.



IMPORTANT:

- To avoid loss of buccal material, avoid touching the swab tip to the sides of the cartridge.



- iv) Grip the swab shaft tightly with your fingers. Place your thumb on top of the swab. Push down on the top of the swab with your thumb. The blue swab tip will be ejected into the cartridge tube.

- v) Repeat Steps 6(i) through 6(iv) for the remaining samples.



- vi) Close the cartridge lid. It should be flush with the cartridge top.



STEP 7: Tap Cartridge to Mix Samples

- i) Grasp the closed cartridge between your thumb and forefinger and lift the cartridge from the Cube.
- ii) Tap the tubes of the cartridge **three** times using a sweeping motion with the index finger of your free hand.
- iii) Return the cartridge to the Cube and close the cube lid.



IMPORTANT:

- Tapping the cartridge facilitates contact between the liquid and the buccal material on the blue cap.



STEP 8: Start Test

- i) Select **Start** to begin the analysis.



IMPORTANT:

- Do not open the lid of the Cube during the test. Keep the lid closed at all times unless loading or unloading cartridges.



CYP2C19

Start

- ii) The test will finish in about an hour, at which time the results are displayed on the screen and can be exported.

If an **INCONCLUSIVE** or **PSC** result is displayed, restart at **Step 1**, collect a new set (second set) of swabs from the same patient and retest using a new cartridge.

- iii) After the test is finished, open the Cube lid and slowly remove the cartridge.

- iv) Discard the cartridge.



IMPORTANT:

- Never open a cartridge after it has been closed.
- Cartridges cannot be re-run.

External Control Procedure

Before beginning the External Control procedure, retrieve the External Control cartridge from your freezer and bring it to the Cube.





There are 3 steps in performing the External Control procedure:

1. Prepare External Control Cartridge
2. Insert cartridge into the Cube
3. Start run



IMPORTANT:

- External Control test must be started within **15 minutes** after removal from the freezer.
- Wear disposable gloves for performing Steps 1–3.
- Always wear a new pair of gloves when running an external control cartridge.
- Do not use the same pair of gloves for running patient tests.

STEP 1: Prepare External Control	
<p>i) The external control cartridge is inside a pouch with an orange barcode. Remove an External Control Kit from your freezer.</p> <p> IMPORTANT:</p> <ul style="list-style-type: none"> • <u>Never</u> open External Control Cartridges at any time. 	
<p>ii) Log in to the CYP2C19 Platform software using your username and password.</p>	
<p>iii) Select Run a Control and proceed through the screens of the software.</p>	
<p>iv) Scan the barcode on the cartridge pouch.</p>	
<p>v) Remove the External Control Cartridge from the pouch and allow it to thaw for about 2-3 minutes.</p>	
<p>vi) Tap the tubes of the cartridge three times using a sweeping motion with the index finger of your free hand.</p>	

STEP 2: Place Control Cartridge into the Cube

- i) Open the lid of the Cube.
- ii) Place the Control Cartridge into the Cube.
- iii) Close the lid of the Cube.



STEP 3: Start Run

- i) Select **Start**.
- ii) The test will finish in about an hour, at which time the results are displayed on the screen and can be exported.
- iii) After the test is finished, open the Cube lid and remove the Control Cartridge.
- iv) Discard the Control Cartridge.

Interpretation of Results

Genomadix Cube CYP2C19 Test

The Genomadix Cube CYP2C19 test is designed to detect the CYP2C19*2, *3, and *17 alleles. At the end of a run, the test report is presented on the screen. The test report indicates which genotypes were detected in the buccal sample.

There are four possible outcomes detected for each CYP2C19 allele:

CYP2C19*2	*1/*1	Patient is not a carrier of the *2 allele (wild-type)
	*1/*2	Patient carries one copy of the *2 allele (heterozygous)
	*2/*2	Patient carries two copies of the *2 allele (homozygous)
	INCONCLUSIVE	System is unable to detect the *2 genotype
CYP2C19*3	*1/*1	Patient is not a carrier of the *3 allele (wild-type)
	*1/*3	Patient carries one copy of the *3 allele (heterozygous)
	*3/*3	Patient carries two copies of the *3 allele (homozygous)
	INCONCLUSIVE	System is unable to detect the *3 genotype
CYP2C19*17	*1/*1	Patient is not a carrier of the *17 allele (wild-type)
	*1/*17	Patient carries one copy of the *17 allele (heterozygous)
	*17/*17	Patient carries two copies of the *17 allele (homozygous)
	INCONCLUSIVE	System is unable to detect the *17 genotype

The above test outcomes are combined to produce a combined patient genotype call, which is indicated in the test results as detailed in the table below:

Test outcomes and combined patient genotype call			
*2	*3	*17	Combined Genotype
*1/*1	*1/*1	*1/*1	*1/*1
*1/*2	*1/*1	*1/*1	*1/*2
*1/*1	*1/*3	*1/*1	*1/*3
*1/*1	*1/*1	*1/*17	*1/*17
*2/*2	*1/*1	*1/*1	*2/*2
*1/*2	*1/*3	*1/*1	*2/*3
*1/*2	*1/*1	*1/*17	*2/*17
*1/*1	*3/*3	*1/*1	*3/*3
*1/*1	*1/*3	*1/*17	*3/*17
*1/*1	*1/*1	*17/*17	*17/*17

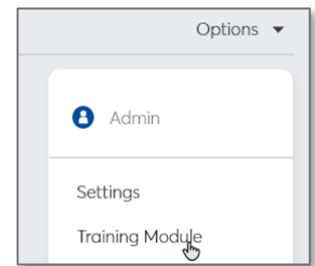
A *1/*1 genotype indicates only the absence of *2, *3, and *17 alleles. The Genomadix Cube CYP2C19 test only assesses CYP2C19 *2, *3, and *17, and does not provide information for other CYP2C19 alleles. Variants in these undetected alleles may or may not be present in the buccal sample.

If the test result is INCONCLUSIVE, the test should be repeated on the same patient with a new set of swabs and a new cartridge.

Training

The Company recommends all users complete the Training Module before performing any tests or controls on the Genomadix Cube CYP2C19 System. The training module comprises a video, a practical walkthrough of the procedure, and a quiz. Upon completion of the Training Module, the user is provided with a certificate of training that may be saved. The certificate of training is also saved on the computer and can be accessed by an administrator.

To access the Training Module, select **Options** on the upper right of the main screen and then select **Training Module**. The software will direct you through the various components of the training.



Quality Control

All quality control requirements and testing should be performed in conformance with local, state, and/or federal regulations or accreditation requirements. Genomadix recommends performing external controls based on test frequency. If testing daily, it is recommended to perform external controls weekly. If testing infrequently, it is recommended to perform external controls on the day of testing.

Internal Controls

The Genomadix Cube CYP2C19 System has two internal controls that run automatically during each test:

- Internal Positive Control (IPC)
- Positive System Control (PSC)

The IPC measures fluorescence signals at the end of the run. It ensures that sufficient buccal material was added to the Cartridge, and that the sample was correctly processed. If the IPC is triggered, the result will read INCONCLUSIVE. The test should be repeated on the same patient with a new set of swabs and a new cartridge (test kit).

The PSC assesses system performance at intermediate stages in the run and at the end of the run. It ensures that the Cube is working correctly and that the system and test are within acceptable performance limits. If the PSC is triggered at any time during the run, the test will abort and the screen will provide a warning message containing information about the status of the platform and how to proceed. If the PSC is triggered repeatedly, contact your distributor.

External Controls

The **External Control Cartridge** is designed to determine if the Genomadix Cube CYP2C19 System is performing correctly.

Each external control cartridge contains a synthetic DNA template and the necessary reagents for DNA extraction, PCR amplification, and detection of the CYP2C19 alleles. It does not require anything to be added to it.

It takes approximately one (1) hour to run an external control on the Genomadix Cube CYP2C19 System. After a successful external control test is finished, the results will indicate a "PASS". If the external control does not pass, the screen will indicate an external control "FAIL". Please run a new external control if this occurs.

Genomadix recommends running external controls under any of the following conditions:

- The IPC consistently generates inconclusive results.
- The PSC consistently generates warning messages.
- You are required to run External Controls in conformance with local, state, and/or federal regulations and/or accreditation requirements.

End User Performance Summary

Based on end user studies, a second pass test is required, when the first pass gives an Inconclusive result, to achieve a point error estimate (% agreement) of 99% or better, and a 95% One-sided Confidence Lower Limit of 95% or better.

Inconclusive (INC) and Positive System Control (PSC) results are system generated internal quality controls that run automatically during each test. Refer to the Quality Control section of this document for more details on the internal controls and the CYP2C19 Operator's Manual, for information on Troubleshooting.

First Pass Performance

End User Study	Samples Tested	# INC/PSC	Incorrect Calls	Correct Call	% Agreement	95% Two-sided Confidence Lower Limit	95% One-sided Confidence Lower Limit
Inter-laboratory Reproducibility	960	9	0	951	99%	98%	98%
Method Comparison	621	16	2	603	97%	96%	96%
Total of both studies	1581	25	2	1554	98%	98%	98%

Final (Second Pass) Performance

New swabs collected only for those subjects for whom an inconclusive result was obtained on first pass; for a second pass (repeat) testing. Twenty-two (22) second pass tests were conducted, of those twenty (20) were resolved and the correct call was obtained. Three subjects were not available to sample for a repeat test; therefore, the inconclusive result was applied to the final result.

End User Study	Samples Tested	# INC/PSC	Incorrect Calls	Correct Call	% Agreement	95% Two-sided Confidence Lower Limit	95% One-sided Confidence Lower Limit
Inter-laboratory Reproducibility	960	3 ^a	0	957	100%	99%	99%
Method Comparison	621	2	2	617	99%	98%	99%
Total of both studies	1581	5	2	1574	100%	99%	99%

^a The subjects were not available to be re-run after a first pass inconclusive result was obtained; therefore, the inconclusive was applied to the final result.

Inter-laboratory Reproducibility

The performance of the Genomadix Cube CYP2C19 System was evaluated at three clinical sites over five non-consecutive days per site, with a total of six operators (two per site). The following table outlines the operators involved in the study, who all completed the system training and did not have extensive experience with buccal swab collection.

SITE(S) - OPERATOR(O)	POSITION	TRAINING	BUCCAL SWAB COLLECTION	RUN SAMPLES ON CUBE
S1-O1	Quality Coordinator	Technical Training	YES	YES
S1-O2	Specialty Technologist	Technologist	YES	YES
S2-O1	Research Technologist	Technologist	YES	YES
S2-O2	Student Researcher	Student	YES	YES
S3-O1	Research Coordinator	Nurse	YES	YES
S3-O2	Research Coordinator	Nurse	YES	YES

The results were generated from two swabbing sessions, each subject was tested by Operator 1 and Operator 2, at each site. If the result of the first pass for a particular individual was an inconclusive result, the test was repeated (second pass). The second pass test is considered the final result and has been used when calculating the performance characteristics.

On the first pass, nine samples produced inconclusive results (Lot A (3), Lot B (5), Lot C (1)). New swabs were collected only for those subjects for whom an inconclusive result was obtained. A second pass test was performed on six of the nine samples, all six samples were resolved. Three samples were not available for a second pass test; first pass inconclusive result was applied to the final result.

The overall call rate after the second pass of the Genomadix Cube CYP2C19 System is 99.7%, with a 95% two-sided lower confidence limit of 99.1%.

Inter-Laboratory Reproducibility – Final (Second Pass) Results						
Sample Type ^a	# Samples Tested	# Inconclusive Calls	# Incorrect Calls	# Correct Calls ^b	Correct call rate (%)	95% two-sided Confidence Lower Limit
*1/*1	120	0	0	120	100	96.9
*1/*2	120	0	0	120	100	96.9
*1/*3	120	0	0	120	100	96.9
*1/*17	120	0	0	120	100	96.9
*17/*17	120	0	0	120	100	96.9
*2/*17	120	2 ^c	0	118	98.3	94.1
*2/*2	120	0	0	120	100	96.9
*2/*3	120	1 ^c	0	119	99.2	95.4
Total Samples (all sites, all lots)	960	3	0	957	99.7	99.1

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

^b In order for a sample to be deemed a correct, results of all three genotypes (*2,*3,*17) had to be correct.

^c The subjects were not available to be re-run after a first pass inconclusive result was obtained; therefore, the inconclusive was applied to the final result.

Test Kit Lot-to-Lot Reproducibility

Inter-Laboratory Reproducibility data above sorted by sample type, also was used to assess Lot-to-lot and site to site reproducibility using the three independent lots of test kit. as follows:

Test Kit Lot-to-Lot Reproducibility – Final (Second Pass) Results							
Test Kit Reagent Lots	SITE	# Samples Tested	# Inconclusive Calls	# Incorrect Calls	# Correct Calls	% Correct Call Rate	95% two-sided Confidence Lower Limit
Lot A	1 & 3	319	1	0	318	99.7	98.2
Lot B	1 & 2	321	2	0	319	99.4	97.8
Lot C	2 & 3	320	0	0	320	100	98.8

On the first pass, nine samples (Lot A (3), Lot B (5), Lot C (1)) produced inconclusive results. A second pass test was performed on six of the nine samples, all six results were resolved. The remaining three samples were not available for a second pass test. The first pass inconclusive result was applied to the final result. Overall, the results indicate reagent lots and each site perform equivalently.

Method Comparison

The CYP2C19 *2, *3 and *17 allele genotyping accuracy of the Genomadix Cube CYP2C19 system was evaluated by comparing genotype calls generated by the system and genotypes generated through bi-directional sequencing.

After excluding any poor-quality sequences (based on bi-directional sequencing), a total of 621 subjects samples were tested, utilizing 413 unique subjects.

After first pass the Genomadix Cube CYP2C19 platform resulted in a correct call rate of 96.9% (603/621) with 16 inconclusive results, and two incorrect calls. New swabs were collected from only those subjects for whom an inconclusive result was obtained. The second pass resolved 14 of the 16 inconclusive results. The final correct call to 99.4% with a 95% two-sided confidence lower limit of 98.4%.

Method Comparison - Final (Second Pass) Results						
Sample Type	# Samples tested	# Inconclusive calls	# Incorrect calls	# Correct calls	% Correct call rate	95% two-sided Confidence limit
*1/*1	208	0	0	208	100	98.2
*1/*2	177	0	1	176	99.4	96.9
*2/*2	38	0	1	37	97.2	86.5
*1/*17	111	1	0	110	99.1	95.1
*17/*17	21	0	0	21	100	84.5
*2/*17	37	0	0	37	100	90.6
*2/*3	9	1	0	8	88.9	56.5
*1/*3	17	0	0	17	100	81.6
*3/*17	2	0	0	2	100	34.2
*3/*3	1	0	0	1	100	20.7
Total	621	2	2	617	99.4%	95.5%

As part of the study, there were two unresolved inconclusive (no call) results, that were due to lack of DNA amplification.

The laboratory sequencing was not completed in advance, and the miscalls were not identified until after the completion of the testing at the site. Miscall investigation would have been required at the time the Genomadix testing was being completed on site. Root cause not identified. The probable root cause is that the samples were swapped by the operator, however this cannot be confirmed.

Proper sample collection and sample handling are essential for correct result, as well as to avoid errors related to sample misidentification and/or mishandling.

Analytical Sensitivity (Limit of Detection)

The Upper and Lower Limit of detection (LoD) for the Genomadix CYP2C19 System:

Result	Criteria	Buccal Collection	Technique Reference/Rationale	Detection (% agreement)
Lower LoD (LLoD)	The lowest quantity of buccal sample that can be collected on a CYP2C19 swab that can be consistently detected in ≥95% of samples tested.	Single inside cheek touch	Technique that will collect the minimum possible amount of buccal sample.	99% 117/118, one Inconclusive result
Upper LoD (ULoD)	The highest quantity of buccal sample that can be collected on a CYP2C19 swab that can be consistently detected in ≥95% of samples tested.	Fifteen (15) up-and-down strokes	5-fold increase over the IFU technique (5X Instructions for Use (IFU), workflow)	100%

The upper limit of detection was assessed by swabbing 5x (15 up-and-down strokes) the standard workflow (3 up-and-down strokes). The lower limit of detection was assessed by using a single stroke of the swab and a single cheek-touch, where the swab is pressed against the inside of the cheek without a stroke.

Seven different genotypes (*1/*1, *1/*2, *2/*17, *3 containing (*1/*3, *2/*3), *1/*17 and *17/*17) were used to assess the LoD.

If the result of the first test (first pass) generated an inconclusive result, a second set of swabs was collected from that individual using the same collection technique and the test was repeated (second pass). The second pass test is considered the final result.

First pass testing generated a total of seven inconclusive results: one 5x standard workflow sample, and six cheek touch samples. New swabs were collected, using the same swabbing method, only for the subjects for whom inconclusive results were obtained.

After second pass testing (one allowable retest), the one 5x standard workflow sample was resolved and five of the six, cheek touch samples, were resolved.

Upper and lower limit of detection – Final (Second Pass) Results							
Swabbing Method	Sample Type	# Samples Tested	# Inconclusive Calls	# Incorrect Calls	# Correct Calls ^b	Total Calls	Call Rate (%)
Cheek Touch ^a	*1/*1	32	0	0	32	117/118	99.2%
	*2/*17	14	0	0	14		
	*1/*2	36	0	0	36		
	*3 containing	13	1	0	12		
	*1/*17	9	0	0	9		
	*17/*17	14	0	0	14		
Single Stroke	*1/*1	5	0	0	5	20/20	100%
	*2/*17	5	0	0	5		
	*3 containing	5	0	0	5		
	*17/*17	5	0	0	5		
Standard Workflow (3 up-and-down strokes)	*1/*1	5	0	0	5	20/20	100%
	*2/*17	5	0	0	5		
	*3 containing	5	0	0	5		
	*17/*17	5	0	0	5		
5x Standard Workflow (15 up-and-down strokes)	*1/*1	5	0	0	5	20/20	100%
	*2/*17	5	0	0	5		
	*3 containing	5	0	0	5		
	*17/*17	5	0	0	5		

^a The cheek touch condition was run at a higher sample size to provide confidence on the lower limit of detection

^b Genotype determined by concordance with the normal collection condition

Interference

Interference from potential endogenous and exogenous interfering substances was evaluated using buccal swab samples collected from *1/*1, *2/*17, *17/*17, *1/*3, and *2/*3 individuals. Genotypes of all individuals were confirmed by bi-directional sequencing prior to initiation of the study. Buccal swab samples were collected from individuals after exposure to the substance or the substance was added to the buccal swab (blood) or the substance was added to the reaction (bacteria).

A total of 16 replicates were tested for each substance. If the result of the first test (first pass) was inconclusive, a second set of swabs was collected from that individual and exposed to the same interfering substance followed by repeat testing (second pass). The second pass test is considered the final result and has been used when calculating the performance characteristics. The interfering substances included in the study are detailed below.

On the first pass, four samples (Toothpaste (2), Sugar solution (1) and Tobacco (1)) produced inconclusive results. New swabs collected only for those four samples that obtained an inconclusive result. A second pass test (one allowable retest) was performed on these samples, all four samples were resolved after the second pass.

No interference detected from any of the exogenous or endogenous substances tested.

Interfering Substances – Final (Second Pass) Results						
Substance		# Samples Tested	# Inconclusive Calls	# Incorrect Calls	# Correct Calls	% Correct Calls
Baking Soda	30 mL, rinse 10 seconds	16	0	0	16	100
Chewing gum	1 piece, chew 1 minute	16	0	0	16	100
Cough Syrup	30 mL, rinse 10 seconds	16	0	0	16	100
Cranberry juice	30 mL, rinse 10 seconds	16	0	0	16	100
Denture paste	3 strips roof of mouth, remove after 5 minutes	16	0	0	16	100
Hard candy	1 piece fully dissolve	16	0	0	16	100
Horse meat	15g, chew 10 seconds	16	0	0	16	100
Mouthwash	20 mL, rinse 10 seconds	16	0	0	16	100
Salt solution	30 mL, rinse 10 seconds	16	0	0	16	100
Sugar solution	30 mL, rinse 10 seconds	16	0	0	16	100
Tobacco	5 inhale and exhale	16	0	0	16	100
Toothpaste	1.9cm brush 2 minutes	16	0	0	16	100
Bacteria (oral)	~ 9 x 10 ⁴ cells added to reaction	16	0	0	16	100
Whole blood	Swab dipped in a 1.7% blood/saliva mixture	16	0	0	16	100
Total		224	0	0	224	100%

Background Information

There is individual variability in the activity of drug-metabolizing enzymes. This variability is one of the main reasons that individuals sometimes respond differently to the same drug. Cytochrome P450 2C19 (CYP2C19) is a drug-metabolizing enzyme in the liver that is involved in the metabolism of many commonly prescribed drugs, which may include, antidepressants, antiepileptics, barbiturates, proton pump inhibitors, and antiplatelet medications.

Genetic polymorphisms in CYP2C19 are common and can result in variable levels of enzymatic activity ranging from poor metabolism to ultra-rapid metabolism. Individuals who possess poor or intermediate metabolizer genotypes may experience different drug effectiveness than normal individuals. Therefore, these individuals may require alternative therapeutic strategies for medications that are metabolized by CYP2C19.

The CYP2C19*1 allele encodes a protein with full functional enzyme activity. The CYP2C19*2 and *3 loss-of-function alleles encode non-functional proteins with no enzyme activity. An individual with two loss-of-function alleles is classified as a poor metabolizer. An individual with one loss-of-function allele is classified as an intermediate metabolizer. CYP2C19*2 and *3 alleles are responsible for the vast majority of poor metabolizer phenotypes. *2 and *3 genotype frequencies vary significantly between different ethnic groups, ranging from 2% - 5% in Caucasian and African populations to 13% - 23% in Asian populations.^[1]

The CYP2C19*17 allele is associated with ultra-rapid CYP2C19 enzyme activity. It is located within the upstream regulatory region of the CYP2C19 gene and enhances transcription of the gene product.^[2, 3] The allele frequency is reported to be 18% in Swedish and Ethiopian populations, 27% in a Polish population, and 4% in Chinese subjects.^[2]

The Genomadix Cube CYP2C19 System identifies the **CYP2C19*2**, **CYP2C19*3**, and **CYP2C19*17** alleles. The following table lists the alleles recognized by the system, the polymorphism for each allele, enzyme activity, and references^[4].

Alleles recognized by the Genomadix Cube CYP2C19 System			
CYP2C19 Allele	Polymorphism Recognized	Enzyme Activity	References
*1 ^a	None ^a	Normal	[5,6,7]
*2	19154G>A	None	[8,9,10,11]
*3	17948G>A	None	[8,10]
*17	-806C>T	Increased	[2,3]

^a*1 genotype for the Genomadix Cube CYP2C19 System indicates only the absence of *2, *3, and *17 alleles.









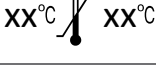




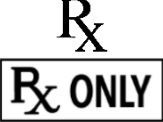
Other alleles of the CYP2C19 gene may be associated with absent or reduced enzyme activity but are significantly less frequent than the *2 and *3 alleles. For individuals with a CYP2C19*10 genotype (rs6413438) and single nucleotide polymorphism (SNP) rs576566073, please see Residual Risks and Limitations for more information.

Residual Risks and Limitations

- The Genomadix Cube CYP2C19 System identifies only the CYP2C19 *2, *3, and *17 alleles and the corresponding polymorphisms. The test reports only the genotypes for these alleles. Other CYP2C19 alleles, which are rare, or unknown at the time of development of this product, will not be identified by this product. These other alleles may result in either a *1 call (wild-type), a no-call, or a call of a genetically related allele included in this system. A *1 call or the call of a genetically related allele may result in a phenotype prediction that is different from the phenotype prediction that would be made if the presence of the rare allele were known. When the report lists the combined genotype as wild-type (*1/*1), only the tested alleles (*2, *3, *17) are wild-type.
- The CYP2C19*10 allele is a loss-of-function allele that has a predicted frequency of 0.037% based on the NCBI ALFA database¹². The presence of the CYP2C19*10 allele can interfere with the detection of the CYP2C19*2 allele. A homozygous *10/*10 will result in an inconclusive, while a *2/*10 will result in an incorrect *2 call.
- In samples with inadequate amount of DNA, the presence of the single nucleotide polymorphism (SNP) rs576566073 can interfere with the detection of the CYP2C19*17 allele. The frequency of the rs576566073 SNP is predicted to be 0.048% based on the NCBI ALFA database¹². A homozygous rs576566073 SNP sample may result in an inconclusive, while a *17 sample containing a heterozygous rs576566073 SNP may result in an incorrect *17 call at low input DNA concentrations.
- Based upon published information, the genotype information provided by the Genomadix Cube CYP2C19 System may be used by the clinician to predict an individual's CYP2C19 enzymatic activity. Clinicians should use caution and professional judgment in predicting phenotype and adjusting treatment strategy for patients who express *2, *3, and/or *17 alleles. 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 judgement in the interpretation of results from this test. Results from this type of test should not be used in predicting a patient's response to drugs for which the drug-metabolizing enzyme activity of that allele, or drug metabolic pathway, has not been clearly established.
- Results from the Genomadix Cube CYP2C19 System should be used and interpreted only in the context of the overall clinical diagnosis. Genomadix Inc. is not responsible for any clinical decisions that are taken.
- There is a risk that the swab tip may fall off in the patient's mouth. If this occurs, immediately instruct the patient to remove the tip from their mouth and obtain a new swab kit to collect the sample.
- If the operator/user is collecting samples from multiple patients at a time, care must be taken to properly record patient ID and segregate the samples as there is a risk that sample mix could lead to incorrect results.
- Care must be taken to input the correct patient identifier into the graphical user interface (laptop) as there is a risk that incorrectly entered patient ID could lead to incorrect results.
- Only operate the Genomadix Cube CYP2C19 Platform under the following conditions: 18 to 30 Degrees Celsius and 10 to 80% non-condensing, relative humidity.
- Only use the Genomadix supplied power adapters, power cords, and cables. If any become damaged or inoperable, please contact your distributor for a replacement. (Note: the use of 3rd party unapproved USB hubs or any other accessory is strictly prohibited.)
- Only use the Genomadix Cube CYP2C19 platform (laptop, Cube, cables, and barcode scanner) for Genomadix System purposes.
- In the event of a power failure while using the equipment the test will be automatically stop and the operator notified of an incomplete test.
- The Genomadix platform is not intended to be used with any other medical devices, or software.

Table of Symbols

Symbols are used throughout this document or on the product. The table below lists the symbols and a brief description of their meanings.

	Warning, Risk of Danger – This symbol is used to indicate that non-compliance with the instructions or procedures may lead to physical injury, or even death, or could damage the instrument.
	Important Note – This symbol is used to bring the user’s attention to an important annotation.
	Biohazard – This symbol is used to indicate that certain precautions must be taken when working with potentially infectious material.
	This symbol indicates the manufacturer of the device or system component.
	Date of manufacture.
	Do not reuse a product containing this symbol (use the product only once).
	Use until (indicates the expiry date of the product).
	Read the documentation before proceeding.
	Temperature limitations
	Part number or catalogue number.
	Lot number of product.
	Sufficient material for N tests.
	In Vitro Diagnostic (indicates the material is an In Vitro Diagnostic or part of an In Vitro Diagnostic Device).
	For prescription use only.

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