



Hospital of the University of Pennsylvania  
 Department of Pathology and Laboratory Medicine  
 Division of Laboratory Medicine

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**Section:** Point of Care Testing

Title: CYP2C19 genotyping utilizing the Spartan Rx System

Technical SOP

<b>Title</b>	CYP2C19 genotyping utilizing the Spartan Rx System	
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**TABLE OF CONTENTS**

1. TEST INFORMATION .....	2
2. ANALYTICAL PRINCIPLE .....	2
3. SPECIMEN REQUIREMENTS .....	2
4. REAGENTS .....	3
5. CALIBRATORS/STANDARDS .....	4
6. QUALITY CONTROL .....	4
7. EQUIPMENT and SUPPLIES .....	5
8. PROCEDURE .....	6
9. CALCULATIONS .....	8
10. REPORTING RESULTS AND REPEAT CRITERIA .....	9
11. EXPECTED VALUES .....	10
12. CLINICAL SIGNIFICANCE .....	10
13. PROCEDURE NOTES .....	12
14. LIMITATIONS OF METHOD .....	12
15. SAFETY .....	12
16. RELATED DOCUMENTS .....	13
17. REFERENCES .....	13
18. ADDENDA .....	13



Title: CYP2C19 genotyping utilizing the Spartan Rx System

**1. TEST INFORMATION**

Assay	Method/Instrument	Order Code
Spartan Rx CYP2C19	Genotyping / Spartan Rx	POC CYP2C19

**Synonyms/Abbreviations**

CYP2C19 genotyping

**Department**

Cardiac Catherization

**2. ANALYTICAL PRINCIPLE**

DNA extraction; PCR amplification of extracted DNA; Detection of amplified PCR products via fluorescent oligonucleotide probes; Fluorescent signal detection and analysis (determination of CYP2C19 \*2, \*3, and \*17 genotypes).

**3. SPECIMEN REQUIREMENTS**

**3.1 Patient Preparation**

Component	Special Notations
<b>Fasting/Special Diets</b>	Rinse mouth with water to remove food particles
<b>Specimen Collection and/or Timing</b>	n/a
<b>Special Collection Procedures</b>	n/a
<b>Other</b>	n/a

**3.2 Specimen Type & Handling**

Criteria	
<b>Type</b> -Preferred -Other Acceptable	Buccal swab
<b>Collection Container</b>	n/a
<b>Volume</b> - Optimum - Minimum	n/a



Title: CYP2C19 genotyping utilizing the Spartan Rx System

Criteria	
<b>Transport Container &amp; Temperature</b>	Store buccal swabs in -20C to -80C frost-free freezer Collect buccal sample within 8 minutes of removing from freezer and perform the test on the analyzer within 4 minutes of sample collection. This time can be extended to 45 minutes and 60 minutes, respectively when utilizing the cold block/ sample transport system.
<b>Stability &amp; Storage Requirements</b>	Room Temperature: 12 min
	Refrigerated: n/a
	Frozen: 45 min with cold block
<b>Timing Considerations</b>	Collect at any time.
<b>Unacceptable Specimens &amp; Actions to Take</b>	n/a
<b>Compromising Physical Characteristics</b>	n/a
<b>Other Considerations</b>	n/a

#### 4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering “SAFETY” for additional information.

##### 4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
CYP2C19 assay	Spartan Bioscience 51F02127
CYP2C19 external control kit	Spartan Bioscience 51F02039

##### 4.2 Reagent Preparation and Storage

**NOTES:**

**Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.**

Assay Kit	
<b>Reagent a</b>	CYP2C19 assay



Title: CYP2C19 genotyping utilizing the Spartan Rx System

<b>Reagent b</b>	CYP2C19 external control kit
<b>Container</b>	Enclosed in foil pouch
<b>Storage</b>	-20 to -80C
<b>Stability</b>	Manufacturer Expiration Date

## 5. CALIBRATORS/STANDARDS

### 5.1 Calibrators/Standards Used

The Spartan Rx does not require calibration

## 6. QUALITY CONTROL

### 6.1 Controls Used

Spartan external control kits contain reagent tube for \*2, \*3, \*17 alleles

### 6.2 Control Preparation and Storage

External control kits must be stored in -20 to -80C frost-free freezer

### 6.3 Frequency

- Run external control for each allele (\*2,\*3, \*17) once per week, each new shipment or lot before patient use on each Spartan Rx system

### 6.4 Tolerance Limits

Step	Tolerance Limits
1.	If the Spartan Rx user interface displays positive system control failure, please rerun the test. If the positive system control fails twice in a row, contact Spartan Bioscience and provide error codes ( <a href="mailto:support@spartanbio.com">support@spartanbio.com</a> ; Tel: +1-877-228-7756)

### 6.5 Review Patient Data



Title: CYP2C19 genotyping utilizing the Spartan Rx System

If the Spartan Rx user interface displays positive system control failure, please rerun the test. If the positive system control fails twice in a row, contact Spartan Bioscience and provide error codes ([support@spartanbio.com](mailto:support@spartanbio.com); Tel: +1-877-228-7756)

## 6.6 Documentation

Refer to department policies and procedures for QC documentation and to records management program for record retention requirements.

## 6.7 Quality Assurance Program

Step	Quality Assurance Program
1.	Proficiency testing of device users will be performed 3 times per year.
2.	Refer to Administrative and department policies and procedures for other quality assurance activities applicable to this procedure.

## 7. EQUIPMENT and SUPPLIES

### 7.1 Assay Platform

Spartan RX Analyzer

The Spartan RX Analyzer device is a multi-well thermal cycler with optical detection capability. The unit has two optical detection channels (labeled as green and red).

### 7.1 Equipment

Spartan Rx analyzer, netbook, printer, barcode scanner, and sample transport system.

### 7.2 Supplies

Sample collection kits for CYP2C19 \*2 (blue), \*3 (white), \*17 (black) alleles; each is color coded. Each kit contains a buccal swab and reagent tube. The reagent tube contains the materials needed for DNA extraction, PCR amplification, and fluorescent detection of the specific CYP2C19 allele.




Title: CYP2C19 genotyping utilizing the Spartan Rx System

## 8. PROCEDURE

**NOTE:** For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used.

8.1	Instrument Set-up Protocol
1.	Turn on analyzer first, wait for initialization before turning on netbook and printer
2.	Log onto computer – Username: RX Password: RX
3.	Use Operator ID Log On to perform patient testing. Use Spartan RX Admin Barcode to access the administrator settings menu to add lot numbers for the sample collection kits and external control kits by scanning the barcode on certificate provided with each box of tests
4.	Check the connection status. Green checks indicate you are able to connect the system. 

8.2	Specimen / Reagent Preparation
	POCT will be contacted by the Cath Lab staff when a patient is ready for testing.
1.	Cath Lab staff will supply a test requisition form with patient identifier on it.
2.	Put on gloves. Obtain sample collection kits (one of each color) and place in sample transport system with a cold block. Bring to patient.
3.	Cath Lab staff will accept the transport system and bring to the patient bedside. Ask patient to rinse his/her mouth with 15-30 ml of water.
4.	Open all sample collection kits and take out all buccal swabs and reagent tubes. Do not discard foil pouches, you will need to scan the barcodes when running the test.
5.	Pick up buccal swab in one hand and hold the white shaft like a pencil.



**Section:** Point of Care Testing

Title: CYP2C19 genotyping utilizing the Spartan Rx System

<b>8.2</b>	<b>Specimen / Reagent Preparation</b>
6.	With the other hand, pick up a reagent tube of the same color as the buccal swab and hold it between your thumb and forefinger.
7.	Remove colored cap from reagent tube using a screwing motion to remove cap.
8.	Pull off cover from buccal swab; ensure that the swab tip does not accidentally contact any surface.
9.	Scrape the inside of patient's cheek using colored tip of buccal swab. Use three up and down strokes (6 strokes total). Apply equal pressure for up and down strokes.
10.	Push color tip of buccal swab firmly down into reagent tube.
11.	Place sample into sample transport system.
12.	Repeat steps 5-11 for remaining color coded buccal swabs and reagent tubes
13.	Discard buccal swab and reagent tube covers.
14.	Bring samples and foil pouches to Spartan Rx analyzer.

<b>8.3</b>	<b>Test Run</b>
1.	Logon to Spartan Rx user interface using your username and password
2.	Enter patient information into appropriate fields (3 identifiers allowed)
3.	Scan barcodes from foil pouches
4.	Thaw liquid in reagent tubes by rolling tubes between your fingers
5.	Ensure that the reagent tube is securely fastened to swab tip.
6.	Hold top of buccal swab with one hand and use index finger of the other hand to firmly tap bottom of reagent tube to mix contents using a horizontal motion.
7.	Visually check for bubbles in bottom of tube after tapping. Re-tap tube to dislodge any bubbles.
8.	Check for bubbles. It is acceptable for there to be no bubbles or one big bubble at the top. If you see bubbles on the bottom or middle or foamy bubbles, then remove these bubbles by holding the middle of the swab and use your index finger to flick the bottom of the tube. Repeat flicking as necessary to remove bubbles.
9.	Open lid of Spartan Rx analyzer and insert reagent tubes of each color into its color coded well. Tubes should slide in when aligned correctly. Place thumb on top of swab and slide fingers upward releasing the swab shaft and leaving the tube and colored cap in the analyzer.
10.	Close lid of analyzer and press "play" button to begin test.
11.	Printer will automatically print results when test is finished.
12.	Put on gloves and open analyzer and remove reagent tubes and place in one hand. Wrap in gloves and discard



**Section:** Point of Care Testing

Title: CYP2C19 genotyping utilizing the Spartan Rx System

8.3	Test Run
13.	<p>Result test in Cerner</p> <ul style="list-style-type: none"><li>• Log into Cerner</li><li>• Select the Point of Care Entry Button</li><li>• Enter the Patient MRN and press SEARCH</li><li>• Choose the correct patient</li><li>• Place the Order<ul style="list-style-type: none"><li>○ Test Site – POC CYP2C19 Bench</li><li>○ Orderable – POC CYP2C19 Genotype</li><li>○ Specimen Type – Tissue</li><li>○ Performed Date – Type “T” for Today PRESS TAB Type” N” for Time as Now</li><li>○ Performed ID – Type in your user ID</li><li>○ Ordering Physician – Type in Provider who ordered the testing</li></ul></li><li>• Entering Results<ul style="list-style-type: none"><li>○ Using the Dropdown Box Select the Correct results for all alleles</li><li>○ Review results</li><li>○ Select Verify</li><li>○ Close out Cerner</li></ul></li><li>• All results must be reviewed by a second technologist.</li><li>• Results filed in POCT lab</li></ul>

8.4	Special Handling
1.	Never open reagent tubes after buccal sample is collected.
2.	Collect buccal sample within 8 minutes of removing from freezer and perform the test on the analyzer within 4 minutes of sample collection. This time can be extended to 45 minutes and 60 minutes, respectively when utilizing the cold block/ sample transport system.

8.5	External Control Procedure
1.	Gloves must be worn in compliance with standard precaution during testing.
2.	Obtain external control kits from freezer (one of each color) and place them in sample transport system with a cold block and bring to the Spartan Rx analyzer.
3.	Log in to Spartan rx user interface using your username and password
4.	Double click on green External Control icon (+ symbol)





**Section:** Point of Care Testing

Title: CYP2C19 genotyping utilizing the Spartan Rx System

8.5	External Control Procedure
5.	Scan the barcode on foil pouch containing external control kits
6.	Open foil pouches and remove the external control kit. NEVER open reagent tubes at any time.
7.	Thaw reagent tube by rolling tube between your fingertips
8.	Check for bubbles. It is acceptable for there to be no bubbles or one big bubble at the top. If you see bubbles on the bottom or middle or foamy bubbles, then remove these bubbles by holding the middle of the swab and use your index finger to flick the bottom of the tube. Repeat flicking as necessary to remove bubbles.
9.	Open lid of Spartan Rx analyzer and insert reagent tubes of each color into its color coded well. Tubes should slide in when aligned correctly.
10.	Close lid of analyzer and press “play” button to begin test.
11.	Printer will automatically print results in about 10 minutes when test is finished.
12.	Open analyzer and remove reagent tubes and place in a glove. Discard gloves

**9. CALCULATIONS**

n/a

**10. REPORTING RESULTS AND REPEAT CRITERIA**

**10.1 Interpretation of Data**

**See Addendum A for interpretation table**

There are four possible test outcomes for each CYP2C19 allele:

**CYP2C19\*2**

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

**CYP2C19\*3**

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



**Section:** Point of Care Testing

Title: CYP2C19 genotyping utilizing the Spartan Rx System

**CYP2C19\*17**

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

The above test outcomes are combined to produce an overall patient genotype call, as detailed in the table below:

*2	*3	*17	Overall Call
*1/*1	*1/*1	*1/*1	<b>*1/*1</b>
*1/*2	*1/*1	*1/*1	<b>*1/*2</b>
*1/*1	*1/*3	*1/*1	<b>*1/*3</b>
*1/*1	*1/*1	*1/*17	<b>*1/*17</b>
*2/*2	*1/*1	*1/*1	<b>*2/*2</b>
*1/*1	*3/*3	*1/*1	<b>*3/*3</b>
*1/*1	*1/*1	*17/*17	<b>*17/*17</b>
*1/*2	*1/*3	*1/*1	<b>*2/*3</b>
*1/*2	*1/*1	*1/*17	<b>*2/*17</b>
*1/*1	*1/*3	*1/*17	<b>*3/*17</b>

Note that a \*1/\*1 genotype indicates only the absence of \*2, \*3, and \*17 alleles. The Spartan RX CYP2C19 Assay only genotypes CYP2C19 \*2, \*3, and \*17, and no information is provided for other CYP2C19 alleles. Mutations in these undetected alleles may or may not be present in the buccal sample.

**10.2 Rounding - N/A**

**10.3 Units of Measure – N/A**

**10.4 Clinically Reportable Range (CRR) – N/A**

**10.5 Repeat Criteria and Resulting**

IF the result is ...	THEN...
Inconclusive	Repeat test for all alleles with new sample collection kits.
The sample's positive system control has failed. Please run a new sample	Repeat test for all alleles with new sample collection kits.



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Version: 1.0  
 Effective date: 10/06/2017

**Section:** Point of Care Testing

Title: CYP2C19 genotyping utilizing the Spartan Rx System

If the positive system controls fails two times in a row	Contact Spartan Bioscience at <a href="mailto:support@spartanbio.com">support@spartanbio.com</a> ; Tel: +1.613.228.7756
If the test is inconclusive two times in a row.	Contact Spartan Bioscience at <a href="mailto:support@spartanbio.com">support@spartanbio.com</a> ; Tel: +1.613.228.7756

## 11. EXPECTED VALUES

### 11.1 Reference Range(s)

n/a

### 11.2 Critical Values

n/a

## 12. CLINICAL SIGNIFICANCE

There is individual variability in the activity of drug-metabolizing enzymes. This variability is a major source of differences in drug exposure. CYP450 2C19 (CYP2C19) is a drug-metabolizing enzyme in the liver that is involved in the metabolism and elimination of many commonly-prescribed drugs, including antidepressants, antiepileptics, barbiturates, proton pump inhibitors, and antiplatelet medications.

Genetic polymorphisms in CYP2C19 are common. CYP2C19 enzyme activity ranges from poor metabolizers to ultra-rapid metabolizers. Individuals who possess poor or intermediate metabolizer phenotypes may exhibit different drug levels than normal individuals. Therefore, these individuals may require alternative therapeutic strategies for medications that are metabolized by CYP2C19.

The CYP2C19\*1 allele has fully functional enzyme activity. The CYP2C19\*2 and \*3 loss-of-function alleles encode non-functional proteins with no functional 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. The allele frequency of these mutations varies significantly between different ethnic groups, ranging from 2% to 5% in White and Black populations to 13% to 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.[3]



Title: CYP2C19 genotyping utilizing the Spartan Rx System

### 13. PROCEDURE NOTES

- **FDA Status:** 510 K cleared
- **Validated Test Modifications:** n/a

### 14. LIMITATIONS OF METHOD

#### 14.1 Analytical Measurement Range (AMR)

n/a

#### 14.2 Precision

n/a

#### 14.3 Interfering Substances

The presence of toothpaste in the mouth might interfere with the performance of the Spartan RX CYP2C19 Assay. Performing a water rinse prior to buccal swab collection is likely to remove such interfering substances.

### 15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety Standard Operating Procedures to learn requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor.



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**Section:** Point of Care Testing

Title: CYP2C19 genotyping utilizing the Spartan Rx System

## 16. RELATED DOCUMENTS

1. Spartan Rx Instructions for use- CYP2C19 assay package insert
2. Spartan Rx CYP2C19 Platform Operator's Manual

## 17. REFERENCES

1. Desta Z et al. (2002). Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet.* 41(12): 913–958.
2. Sim SC et al. (2006). A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther.* 79(1): 103–113.
3. Rudberg I et al. (2008). Impact of the ultrarapid CYP2C19\*17 allele on serum concentration of escitalopram in psychiatric patients. *Clin Pharmacol Ther.* 83(2): 322–327.

## 18. ADDENDA

Addendum	Title
A	Interpretation Table



Title: CYP2C19 genotyping utilizing the Spartan Rx System

**A. Table 1. Precision Medicine POC CYP2C19 recommendations post percutaneous coronary intervention (PCI)**

Genotype	Phenotype <sup>1</sup>	Description <sup>1</sup>	Interpretation provided with test result	CDS Alert for clopidogrel	Treatment <sup>2</sup>
CYP2C19 *1/*1	Normal metabolizer	Two normal function alleles	This result signifies that the patient has two copies of a normal function allele (*1/*1). The patient is predicted to be a normal metabolizer of CYP2C19 substrates.	N	Clopidogrel (Plavix) 75 mg per day
CYP2C19 *1/*17	Rapid metabolizer	One normal function and one increased function allele	This result signifies that the patient has one copy of a normal function allele (*1) and one copy of an increased function allele (*17). The patient is predicted to be a rapid metabolizer of CYP2C19 substrates.	N	
CYP2C19 *17/*17	Ultra-rapid metabolizer	Two increased function alleles	This result signifies that the patient has two copies of an increased function allele (*17/*17). The patient is predicted to be an ultra-rapid metabolizer of CYP2C19 substrates.	N	
CYP2C19 *1/*2	Intermediate metabolizer	One normal function allele and one no function allele	This result signifies that the patient has one copy of a normal function allele (*1) and one copy of a no function allele (*2). The patient is predicted to be an intermediate metabolizer of CYP2C19 substrates.	Y (see table 2)	Prasugrel (Effient) 10 per day
CYP2C19 *1/*3	Intermediate metabolizer	One normal function allele and one no function allele	This result signifies that the patient has one copy of a normal function allele (*1) and one copy of a no function allele (*3). The patient is predicted to be an intermediate metabolizer of CYP2C19 substrates.	Y	Or Ticagrelor (Brilinta) 90 mg twice daily
CYP2C19 *2/*17	Intermediate metabolizer	One increased function allele and one no function allele	This result signifies that the patient has one copy of an increased function allele (*17) and one copy of a no function allele (*2). The patient is predicted to be an intermediate metabolizer of CYP2C19 substrates.	Y	See contraindications table 3
CYP2C19 *3/*17	Intermediate metabolizer	One increased function allele and one no function allele	This result signifies that the patient has one copy of an increased function allele (*17) and one copy of a no function allele (*3). The patient is predicted to be an intermediate metabolizer of CYP2C19 substrates.	Y	
CYP2C19 *2/*2	Poor metabolizer	Two no function alleles	This result signifies that the patient has two copies of a no function allele (*2/*2). The patient is predicted to be a poor metabolizer of CYP2C19 substrates.	Y	
CYP2C19 *3/*3	Poor metabolizer	Two no function alleles	This result signifies that the patient has two copies of a no function allele (*3/*3). The patient is predicted to be a poor metabolizer of CYP2C19 substrates.	Y	
CYP2C19 *2/*3	Poor metabolizer	Two no function alleles	This result signifies that the patient has two copies of a no function allele (*2/*3). The patient is predicted to be a poor metabolizer of CYP2C19 substrates.	Y	