

BRIEF REPORT

Validation of the Spartan RX *CYP2C19* Genotyping Assay Utilizing Blood Samples

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The antiplatelet agent clopidogrel, a prodrug that requires bioactivation through the cytochrome P450 2C19 (*CYP2C19*) enzyme, is commonly prescribed post-percutaneous coronary intervention (PCI). Genetic variation in *CYP2C19* contributes to individual variability in clopidogrel response, and can lead to adverse cardiovascular events. Incorporating *CYP2C19* testing during routine clinical care helps identify high-risk patients, and provides the opportunity for pharmacotherapeutic interventions in the early post-PCI period. The Spartan RX *CYP2C19* System has emerged as an optimal genotyping assay for use in clinical care due to ease of use, utilization of buccal swabs, and rapid turnaround time. However, workflow constraints related to sample collection and processing, storage, time, and personnel were encountered when integrating testing into clinical care. To improve clinical workflow and successfully implement *CYP2C19* genotyping at our institution, we validated the Spartan RX System to return genotype utilizing blood samples. Our Molecular Diagnostic Laboratory tested 26 known reference materials and both blood and buccal swab samples from 23 patients and volunteers using the Spartan RX Assay. Genotype results were 100% concordant between DNA from blood and buccal swabs for all patients or volunteers, and consistent with expected results for the 26 reference materials. For reproducibility, three samples were tested in at least four separate runs, with all resulting genotypes in agreement between runs. Post-validation, the laboratory began offering *CYP2C19* testing during clinical care. DNA extracted from blood can serve as a genomic DNA source for the Spartan RX Assay. Alteration of the methodology allowed for clinical implementation to support genotype-guided therapy.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?

☑ Currently, the Spartan RX *CYP2C19* System can only utilize genomic DNA from buccal swab samples to return cytochrome P450 2C19 (*CYP2C19*) genotype. The stability of the reagents imposes special transportation requirements and a 60-minute time frame from sample collection for genotyping to be completed.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Can the Spartan RX System be adapted to return genotype using DNA extracted from blood samples in order to ease constraints related to integration of genotyping during clinical care?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study describes a successful validation of the Spartan RX *CYP2C19* System to utilize blood samples, opposed to buccal swab samples, as a DNA source.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ As genotype-guided therapy becomes more widespread and routine during clinical care, institutions are faced with how to integrate this testing. Complex clinical workflows can cause barriers and hinder effective implementation. Validating genotyping assays using alternative methodologies can eliminate these constraints, and allow for the successful implementation of genotyping strategies into clinical care.

Genetic variation can contribute to impaired drug response, leading to adverse outcomes. This is particularly true for clopidogrel (Plavix), a P2Y₁₂ antagonist, commonly used in combination with aspirin for dual-antiplatelet therapy after percutaneous coronary intervention (PCI).¹ Clopidogrel is a prodrug and requires metabolic bioactivation by the cytochrome P450 2C19 (*CYP2C19*) enzyme encoded by the *CYP2C19* gene. Patients harboring at least one nonfunctional allele in *CYP2C19* are at significantly

increased risk of major adverse cardiovascular events and stent thrombosis.^{2,3} Several alleles in *CYP2C19* are considered nonfunctional, leading to decreased or absent activation of clopidogrel, and high on treatment-platelet reactivity.^{5,6} The most common nonfunctional allele is *CYP2C19*2* (c.681G>A; rs4244285), a splice site variant that leads to the production of a truncated, nonfunctional protein.^{7,8} Thirty percent of patients undergoing PCI harbor a *CYP2C19*2* allele.^{9,10}

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The utility of pharmacogenomic test results to guide treatment decisions in clinical practice is becoming more apparent.¹¹ As institutions integrate genotype-guided therapy into clinical practice, most have initiated their efforts with implementation of genotype-guided antiplatelet therapy in patients undergoing PCI.^{12,13} This was a natural starting point supported by the large number of patients undergoing PCI each year,¹⁴ feasibility of testing along a timeline conducive for clinical care, US Food and Drug Administration (FDA) and Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines related to decreased clopidogrel efficacy in poor metabolizers,^{7,15} and the availability of the alternative P2Y₁₂ inhibitors prasugrel (Effient) and ticagrelor (Brilinta).

The Spartan RX CYP2C19 System (Spartan Bioscience, Ottawa, Ontario, Canada) is a CYP2C19 genotyping assay that utilizes buccal swab samples to interrogate *2, *3, and *17. The ease of use, utilization of buccal swabs, and quick turnaround time afforded by the system¹⁶ has supported its broad use for CYP2C19 genotyping.^{12,13} Although the system has significant advantages, substantial limitations were encountered when attempting to implement testing into clinical workflow. When a CYP2C19 test was ordered, the molecular laboratory had to prepare sample collection kits for transport. The kit was too large to be sent through the hospital tube system, requiring a coordinator to transport it for collection and return it for testing. Furthermore, because of storage and refrigeration requirements, samples had to be collected within 45 minutes of preparation. Post-collection, samples had to be returned to the laboratory and processed within 1 hour. These time constraints made coordination between the laboratory and research nurse crucial, and hampered clinical workflow. Laboratory personnel had to be available to process samples and begin the test within this limited time frame, regardless of other clinical laboratory tests that needed to be performed. Moreover, the analyzer requires 1 hour for processing and can only process one sample at a time. Because each kit requires that three samples be processed (*2, *3, and *17), complications arose if multiple patients required testing, or samples had to be re-collected due to missing genotype calls.

In addition to workflow limitations, patient-related issues also arose. Prior to buccal swab collection, the patient had to rinse his or her mouth. As initial implementation of testing was limited to patients who had undergone PCI, patients requiring sedation postsurgery were unable to perform this step. For others, improper rinsing techniques caused assay interference resulting in a no call for genotype. In these instances, another sample collection kit would have to be prepared and the process repeated.

These time constraints and required level of coordination can be a significant barrier for institutions trying to offer this test in their clinical laboratory or to outside providers. To eliminate these barriers and successfully implement CYP2C19 testing clinically, we wanted to determine if blood samples could be used with the Spartan RX Assay. Utilizing blood as an alternative genomic DNA source would significantly improve the accessibility and integration of testing into clinical workflow. Herein, we describe our validation of the Spartan RX CYP2C19 System for use with DNA extracted from blood samples.

METHODS

This study was approved by the University of Alabama at Birmingham Institutional Review Board. We evaluated and validated the performance of the Spartan RX Assay to return genotype using genomic DNA extracted from blood samples compared with buccal swab samples, the FDA-cleared method for returning CYP2C19 genotype.¹⁷ A validation method was performed to include accuracy, precision, reference interval, sensitivity, and specificity, as required by Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP).

Spartan RX CYP2C19 system

Individual sample collection kits are provided to collect buccal swab samples. Each kit contains individual swabs and reagent tubes unique for CYP2C19 *2, *3, and *17. Samples are analyzed on the Spartan RX Platform, a thermal cycling instrument that integrates DNA extraction, polymerase chain reaction (PCR), allele detection, and genotype calls. A PCR-based amplification of specific polymorphism sites of the CYP2C19 gene is performed using fluorescent-labeled oligonucleotide probes, which is read by optical detection channels to analyze emissions of the specific probes for *2, *3, and *17. The system has a reported sensitivity of 0.1 ng/μL.¹⁷

Blood and buccal swab sample preparation and processing

Both peripheral blood in EDTA and buccal swabs were collected from patients or volunteers. Buccal swab samples were collected and processed according to manufacturer's instructions.¹⁷ For blood samples, genomic DNA was extracted from 350 μL of blood, using the EZ1 DNA Blood 350 microliter kit and EZ1 BioRobot (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA concentration was quantitated using NanoDrop 2000 Spectrophotometry (ThermoScientific, Waltham, MA). For each blood sample, 0.5 μL of extracted DNA, instead of the buccal swab, was added to each reagent tube (*2, *3, and *17) in the Spartan RX collection kit.

Validation of the Spartan RX Assay

The assay was first validated for buccal swab samples as specified,¹⁷ followed by validation using DNA extracted from blood. Reagent tubes with genomic DNA or the buccal swab were loaded into the instrument. Samples went through the integrated DNA extraction process. During this process, DNA extracted from blood remained stable. After extraction, the PCR reaction started in the same tube. Validation criteria followed that of a laboratory developed test, because this was a modification to the FDA-cleared assay.

Performance of reference materials

Reference materials, provided as DNA, were used to compare the accuracy of the laboratory testing methodology to known results. Samples included 23 CAP survey specimens, and 3 DNA controls (NA06993 *1/*1, NA18564 *2/*3, and NA18943 *2/*3) purchased from the Coriell Institute (Camden, NJ). Concentrations of the reference

materials were 365 ng/μL (NA06993 *1/*1), 326 ng/μL (NA18564 *2/*3), and 355 ng/μL (NA18943 *2/*3) for the Coriell controls, and ~ 357 ng/μL for CAP survey specimens. Prior to testing, all reference materials were diluted 1:10 to produce a final concentration of ~ 40 ng/μL. This concentration was selected based on the average yield of DNA extracted from blood.

Reproducibility

For reproducibility, three patient or volunteer blood samples with different expected genotypes (*1/*1, *1/*2, and *2/*17) were selected for retesting over time.

RESULTS

Blood and buccal sample comparison

SpartanRXAssayperformancewascomparedbetweenblood and buccal swab samples for 23 patients or volunteers. DNA concentrations of the samples ranged from 37–82.1 ng/μL. Genotype concordance was 100% between blood and buccal swab across all samples tested. All of the most frequently observed genotypes (*1/*1, *1/*17, *1/*2, and *2/*17) in a US-based population were represented (Table 1). No samples with a *3 genotype were available for comparison.

Performance of reference materials

Across all tests, molecular laboratory results were consistent with expected genotypes for all CAP and Coriell samples (Table 2; 100% accuracy). With the exception of *3/*3, all CYP2C19 genotype combinations were

represented. No reference material was available for *3/*3 at the time of validation.

Reproducibility

Over a 6-month period, samples 21 (*1/*2), 22 (*1/*1), and 23 (*2/*17) were tested five, six, and four times, respectively. DNA concentrations of these samples ranged from 49.9 to 82.1 ng/μL. All retested samples resulted in 100% accurate calls across analytic runs.

Clinical testing

Blood samples require an additional DNA extraction step, adding ~ 25 minutes of processing time and 5 minutes of technician hands-on time. Testing is provided through the molecular diagnostic laboratory with a turnaround time of 24 hours, with the majority of results returned to the electronic health record the same day. On average, the overall turnaround time is 8 hours, with a range of 4–12 hours. Prior to clinical testing, the method validation documentation was reviewed and approved by the Quality Office and Medical Director. Validation records are maintained in the Quality Office and in the laboratory to be available to inspectors.

DISCUSSION

We validated the performance of the Spartan RX CYP2C19 Assay to return CYP2C19 genotype using genomic DNA

Table 1 Comparison of Spartan RX CYP2C19 performance between blood and buccal swab samples

Sample	CYP2C19 result (Buccal swab)	CYP2C19 result (Blood)	DNA concentration (ng/μL)
1	*1/*17	*1/*17	54.6
2	*1/*1	*1/*1	52.3
3	*1/*1	*1/*1	54.3
4	*1/*1	*1/*1	44.3
5	*1/*2	*1/*2	70.8
6	*1/*17	*1/*17	64.3
7	*1/*2	*1/*2	53.0
8	*1/*1	*1/*1	67.1
9	*1/*1	*1/*1	45.8
10	*2/*17	*2/*17	54.3
11	*1/*1	*1/*1	48.5
12	*1/*1	*1/*1	56.2
13	*1/*1	*1/*1	62.3
14	*2/*17	*2/*17	62.7
15	*1/*2	*1/*2	42.4
16	*1/*17	*1/*17	58.7
17	*1/*1	*1/*1	48.2
18	*1/*17	*1/*17	37.0
19	*1/*17	*1/*17	72.4
20	*1/*17	*1/*17	63.2
21	*1/*2	*1/*2	49.9
22	*1/*1	*1/*1	64.6
23	*2/*17	*2/*17	82.1

Table 2 CYP2C19 Test results of reference materials (DNA)

Specimen ID	CYP2C19 expected result	Molecular laboratory CYP2C19 result
Coriell NA 06993	*1/*1	*1/*1
Coriell NA 18943	*2/*3	*2/*3
Coriell NA 18564	*2/*3	*2/*3
CAP2014 PGX A01	*1/*2	*1/*2
CAP2014 PGX A02	*1/*2	*1/*2
CAP2014 PGX B03	*1/*17	*1/*17
CAP2014 PGX B04	*17/*17	*17/*17
CAP2015 PGX A01	*2/*3	*2/*3
CAP2015 PGX A02	*1/*1	*1/*1
CAP2015 PGX B03	*1/*1	*1/*1
CAP2015 PGX B04	*1/*2	*1/*2
CAP2016 PGX A01	*1/*1	*1/*1
CAP2016 PGX A02	*1/*17	*1/*17
CAP2016 PGX A03	*1/*2	*1/*2
CAP2016 PGX B04	*2/*17	*2/*17
CAP2016 PGX B05	*1/*1	*1/*1
CAP2016 PGX B06	*2/*2	*2/*2
CAP2017 PGX A01	*2/*17	*2/*17
CAP2017 PGX A02	*1/*17	*1/*17
CAP2017 PGX A03	*1/*2	*1/*2
CAP2017 PGX B04	*1/*1	*1/*1
CAP2017 PGX B05	*17/*17	*17/*17
CAP2017 PGX B06	*1/*1	*1/*1
CAP2018 PGX A01	*1/*1	*1/*1
CAP2018 PGX A02	*1/*1	*1/*1
CAP2018 PGX A03	*1/*2	*1/*2

extracted from blood, a modification to the FDA-cleared method utilizing buccal swabs. The need for an alternative testing method was brought to light after attempting to integrate *CYP2C19* testing into clinical workflow. Major barriers to effective implementation were encountered, including time constraints, personnel requirements and coordination, storage and sample stability, samples unable to be collected by bedside nurses, patients unable to provide samples, and sample recollection due to interference or improper techniques.

Using blood as a DNA source allows flexibility with regard to the preparation, collection, transport, and processing of samples. It is important to note, whereas the overall sample processing time increased by 30 minutes, technician hands-on time only increased 5 minutes. Because DNA does not have to be extracted within 1 hour of collection and can be stored postextraction until the technician has adequate time to perform the test, this increased time has ultimately increased the accessibility of testing. Furthermore, the nurse can collect samples with other clinical laboratories, making testing available to patients who require sedation or those unable to provide a buccal sample. Moreover, blood samples provide adequate DNA as not to necessitate recollection if retesting is required.

Although the Spartan RX *CYP2C19* System was developed as a “point-of-care” test, it is classified as high complexity according to CLIA regulations.^{18,19} Based on CLIA and CAP requirements, high complexity testing requires testing personnel to possess a state license, if required, and meet certain educational and/or training requirements to perform testing.^{20,21} Due to these requirements, the Spartan RX System needed to be placed in the molecular laboratory, instead of the emergency department or catheterization laboratory, creating more barriers related to time constraints and coordination requirements. Tests modified from FDA-cleared protocols automatically receive a high complexity classification, and are considered modified FDA-cleared assays. For these tests, the laboratory follows CAP validation guidelines that apply to laboratory developed tests. However, because the Spartan RX Assay is already considered high complexity, modification did not result in increased requirements. Furthermore, because reimbursement is based on the analyte tested (i.e., *CYP2C19*), no issues related to reimbursement have been noted.

The number of patients with nonfunctional alleles in *CYP2C19* and the relationship to adverse outcomes,^{2-4,9} emphasizes the importance of *CYP2C19* genotyping prior to prescribing antiplatelet therapy. Although genotyping capability in itself is important, turnaround time of the assay is also crucial to clinical implementation. On average, our laboratory returns results within 8 hours, a time frame conducive for informing genotype-guided antiplatelet therapy prior to patient discharge.

Our validation demonstrated that the Spartan RX *CYP2C19* Assay provides 100% accuracy for returning *CYP2C19* genotype using 37–82.1 ng/ μ L genomic DNA extracted from blood. No samples required special adjustments, and the 0.5 μ L of DNA used for testing was well above the assay’s limit of detection (0.1 ng/ μ L). Reproducibility was

demonstrated by retesting three different samples a minimum of four times each over a 6-month period.

For some institutions, buccal swabs may serve as the optimal DNA source due to the ease of use and rapid genotype results. In the outpatient setting, obtaining buccal swabs can prevent patients from having to receive a blood draw. However, in the inpatient setting, large institutions with complex clinical workflows may encounter difficulties implementing genotyping with buccal swabs into routine clinical care. Altering the methodology to utilize blood samples eased constraints and allowed for successful implementation of *CYP2C19* genotyping at our institution.

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