A Head-to-Head Comparison of Blood Versus Buccal Swab in Pharmacogenomics Tests

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Introduction

Pharmacogenomics (PGx) is the study of how an individual's genetic makeup influences their response to drugs, playing a pivotal role at the forefront of personalized medicine by offering a means to tailor medical treatments for effective and safe drug prescriptions. In the last decade PGx has been significantly integrated into clinical trials especially in the fields of oncology and mental health. The initial step in harnessing the power of PGx lies in the collection of genetic samples, a process pivotal to the accuracy and reliability of subsequent analyses. While blood and buccal swab samples have been commonly collected, there is a lack of comparison analysis.

In this study, the Agena MassARRAY® system is applied in testing buccal swab and blood samples collected from clinical subjects. Through comprehensively analyzing DNA yield and quality, sample stability and assay outcomes, and patient compliance, this research contributes to the strategic decision-making process in selecting optimal PGx sampling methods.

Materials & Methods

The Agena MassARRAY system was used to analyze single-nucleotide polymorphisms (SNPs) and copy number variations (CNVs) in buccal swab and blood samples. The primary workflow is illustrated in Figure 1. This assay operates on the principle of deoxyribonucleic acid (DNA) polymerase chain reactions (PCR) that utilized oligonucleotide primers designed to target specific loci in the genome. The DNA was procured with buccal swabs or blood samples from clinical subjects, and isolated by a spin column- or magnetic particle-based protocols (reference). The molecular weights of single-base extension amplicons were determined via a MassARRAY® Analyzer 4 MALDI-TOF with an automated 96 position chip preparation module. The specific terminators added were determined by matching the molecular weights of the subject sample amplicons to those of known sequences from their respective loci. CNV quantities were calculated by comparing the ratio of amplicons detected from copy number variable regions to the amount of amplicons detected from regions with established copy number quantities.

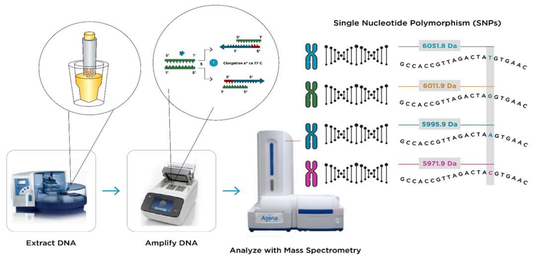

Figure 1 illustrates the primary genotyping workflow: DNA is extracted from buccal swab and blood samples using either spin column or magnetic bead-based protocols. This is followed by multiplex end-point PCR and a subsequent single-base extension reaction. Finally, the genotyping data is compiled by analyzing the molecular weights of the single-base extension amplicons using the MassARRAY system.

Sample Collection: PGx blood samples were collected using BD PAXgene Blood DNA Tubes and stored at -70°C within 8 hours. Buccal swabs were obtained with ORAcollect OC-100, containing stabilization and lysis buffer, and stored at room temperature.

Spin Column-based DNA Extraction: First, to lyse the sample it is mixed with a Proteinase K solution and incubated at 70°C. The binding conditions of the DNA are then adjusted through the addition of ethanol before the sample is loaded onto a NucleoSpin® Tissue Column. The sample is spun through the column and in the process binds the DNA to the silica membrane in the spin column. The silica membrane is washed twice to remove any contaminants that may inhibit amplification during PCR. Finally, the DNA is eluted into a collection tube and is ready to be used in subsequent steps. (Macherey-Nagel CAT 740952.50)

Magnetic beads-based DNA Extraction: To lyse the blood the sample is first mixed with a Proteinase K solution in the well of a 2ml 96-well assay block. The plate is incubated at 65°C before it is mixed with a volume of Multi-Sample DNA Lysis buffer. After lysis of the cells the sample is mixed with magnetic beads covered in a silica-like surface that binds the DNA floating in solution. Once this mixture is made the assay block is loaded onto a KingFisher Flex along with three assay blocks containing wash solutions and an elution plate. The KingFisher Flex transfers the beads out of the sample and washes them in the wash solutions before finally eluting them onto the elution plate. Finally, after the DNA is in the elution buffer the unbound magnetic beads are removed from the eluate. (MagMAX™ DNA Multi-Sample Ultra Kit CAT A25597)

PCR and SAP: After the genomic DNA is extracted, the loci associated with our genes of interested are amplified via PCR. In this step the samples are mixed with 69 unique primer pairs designed to amplify large portions of the target areas (>1 kb) areas adjacent to variable nucleotides. The samples are mixed with the primers and PCR reagents before being subjected to thermal cycling in a ProFlex PCR System. During the cycling individual regions in the DNA associated with our genetic variants of interest are doubled 45 times to create a high number of amplicons available for downstream applications. Following PCR samples are treated with shrimp alkaline phosphatase (SAP) to dephosphorylate the unincorporated nucleotides and stop them from being used in the following single-base extension reactions.

Genotyping: A single-base extension (SBE) reaction adds the mass-modified dideoxynucleotide terminators with thermosequenase to the PCR amplicons according to their template strands. The molecular weights of the amplicons are determined via matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. In this technique the amplicons are desorbed from MALDI matrix pads using a laser and accelerated towards a detector. Depending on the time necessary to reach the MALDI detector, its mass can be calculated. The specific terminators added in the SBE reaction are determined by matching the masses of ions reaching the detector with the masses of expected amplicons.

Table 1: List of tested genes implicated in drug metabolism pathways.

Gene	Portion Genotyped	Gene	Portion Genotyped
ABCB1	99.7%	Factor 5	99.7%
COMT	99.3%	MTHFR_1 rs1801131	99.0%
CYP1A2	97.8%	MTHFR_2 rs1801133	96.6%
CYP2B6	99.4%	OPRM1	99.3%
CYP2C19	95.2%	SLCO1B1	97.4%
CYP2C8	100.0%	VKORC1	99.2%
CYP2C9	97.6%	GLP1R_1* rs6923761	98.1%
CYP2D6	61.4%	GLP1R_2* rs1042044	93.4%
CYP3A4	98.5%	GLP1R_3* rs2300615	99.5%
CYP3A5	96.2%	PNPLA5*	99.7%
Factor 2	99.7%	SULT4A1*	99.1%

Results & Discussions

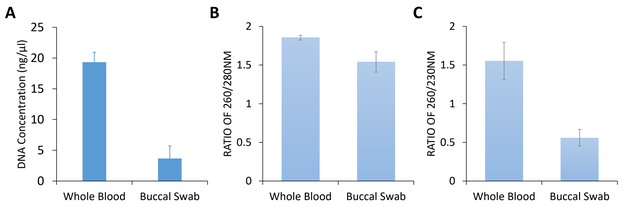

Figure 2: Comparative analysis of DNA extraction from whole blood and buccal swab samples using magnetic bead approach. (A) Compares DNA concentrations between the two sample types. The extracted DNA concentration was low, and Ideally should reach 10ng/µL; (B) Assesses DNA purity based on the 260/280 nm absorbance ratio, with a ratio near 1.8 indicative of purity and suggesting minimal protein contamination; (C) Evaluates DNA purity using the 260/230 nm absorbance ratio, where a ratio between 2.0 and 2.2 signifies purity, revealing the presence of organic contaminants in blood samples and higher contamination levels in buccal swabs.

DNA Extraction and Quality: Since 2016 we've tested buccal swab samples using several validated panels encompassing 69 to 101 single-nucleotide polymorphism (SNP) assays mostly associated with metabolism genes (Table 1). Over time, we added the capability to analyze whole blood. The quantity of DNA extracted from whole blood is always higher than that from buccal swab (Figure 2A). Whole blood typically has a higher cell density, including DNA-rich white blood cells, compared to the less consistent and sparser epithelial cells collected from buccal swabs.
The DNA purity analysis (ratio of 260/280nm) showed that the minimum protein contamination in both blood and buccal swab samples (Figure 2B). However, the low ratio of 260/230nm was observed at 0.56 in buccal swab samples (Figure 2C). Ideally the DNA concentration should reach 10 ng/µL in assessing the DNA purity. This may result in the low ratios in purity test. After DNA extraction, PCR was used to amplify DNA, resulting in the DNA increase. Thus, SNPs and CNVs can still be analyzed.

Assay Outcomes: The quality and quantity of DNA extracted from samples typically impact the call rate. Although variations in DNA extraction methods between buccal swabs and blood samples are evident, some assays deliver comparable results, indicating limitations in current DNA quantitation and purity tests that do not specifically target DNA sequences.
Assay performance differs depending on whether buccal swabs or blood samples are used. For example, a summary of results from several validated panels (Table 2) shows similar performance metrics, with call rates of 98.1% for buccal swabs and 93.2% for blood samples. However, the assay for CYP2D6 CNV demonstrated significant variability in blood samples, affecting its reliability. Enhancements in DNA extraction techniques, especially the spin column method, have improved DNA quality and now provide consistent validation of CYP2D6 CNV in assays (Figure 3).

Table 2: Comparative PGx Analysis of Buccal Swab and Blood Samples

	Whole Blood	Buccal
Sample Call Rate	93.2%	98.1
Intra-run Precision	99.9%	100.0%
Positive Control Concordance	99.0%	100.0%
Negative Control Call Rate	2.41%	0.00%
Dilution Range	1x-2x	1x-32x

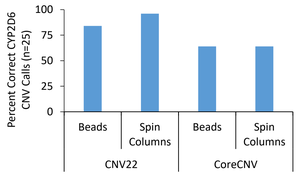

Figure 3: Percentage of accurate CYP2D6 CNV calls under varying experimental conditions at medium or high confidence levels. The comparison involves the average number of correct calls using either 5 or 22 primer pairs across the CYP2D6 gene (labeled as CoreCNV and CNV22, respectively), coupled with DNA extraction methods from whole blood using either spin column or magnetic bead-based techniques.

Stability: Analysis on sample stability reveals that blood samples maintain reasonable stability when stored at -20°C or below, exhibiting minimal degradation over a span of eight months. Conversely, when stored at room temperature or under fluctuating conditions, blood samples experience a rapid deterioration in DNA integrity. In comparison, buccal swab samples stand out for their exceptional stability across diverse storage conditions. Remarkably, even at room temperature, the integrity of DNA from buccal swabs remains significantly preserved for up to six months. This resilience against processing delays or deviations from optimal storage guidelines offers a substantial advantage, especially for samples requiring long-distance transport or prolonged storage prior to analysis (Supplementary data).

Patient compliance: The success of PGx testing is heavily reliant on accessibility and patient compliance. Studies indicate that patient compliance rates are significantly higher for PGx sample collection using buccal swabs (95%) than for blood sample collection (69%), as evidenced by data from over 30 clinical trials [1,2]. Additionally, the non-invasive nature of buccal swab collection improves patient willingness to participate in PGx testing, simplifies the collection process, reducing barriers to widespread adoption and facilitating more comprehensive genetic analysis.

Conclusion

- The two most common PGx samples: blood and buccal swab have been comparably analyzed through DNA extraction, sample stability, assay performance, and patient compliance.
- Blood samples are often preferred for high DNA quantity in extraction, whereas buccal swabs are favored due to better sample storage conditions and greater patient compliance during collection.

References

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2. Kumari, Meena et al. A randomized study of nurse collected venous blood and self-collected dried blood spots for the assessment of cardiovascular risk factors in the Understanding Society Innovation Panel. Scientific reports vol. 13,1 (2023). doi:10.1038/s41598-023-39674-6

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