Comparison of Extracted DNA vs. Non-extracted Sample (Direct) For the Detection of Factor V Leiden, Factor II, and MTHFR Single Nucleotide Polymorphisms From Whole Blood and Buccal Swabs

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Modified Abstract

Introduction/ Background: Molecular methods traditionally entail extraction of nucleic acids prior to performing amplification and detection steps. We have in development reagents that enable direct sample-to-answer detection of nucleic acids from various specimen types without performing any nucleic acid isolation or purification steps. We used reagents to detect factor V Leiden (FVL), factor II (FII), and methylenetetrahydrofolate reductase (MTHFR) single nucleotide polymorphisms (SNPs) from whole blood and buccal swab samples, and compared methods using nucleic acid extraction to the direct detection methodology.

Materials and Methods: Whole blood (previously tested by a reference method) and buccal swab samples were evaluated. Additional whole blood was also collected using EDTA, sodium citrate, or sodium heparin as an anticoagulant. Real-time PCR assays were performed to compare extracted human genomic DNA vs. extraction-free (direct) amplification of these samples. The master mix utilized for direct testing was optimized to provide robust amplification directly from specimens, even in the presence of potential inhibitory substances.

Results: Genotypes obtained for both alleles were in 100% agreement with those obtained by the reference method. In addition, complete concordance was obtained between extracted samples and those that were amplified directly without extraction. Direct detection was achieved with both whole blood (collected in different anticoagulants: EDTA, sodium citrate, and sodium heparin) and buccal swab samples, and none of the samples showed inhibition. Optimal testing time in this study (from sample to result) ranged from less than 40 minutes for extracted DNA to approximately 50 minutes for whole blood and buccal cells.

Conclusion: The direct method gave the same results as the traditional method that used extraction. Performance was the same for detecting and discriminating the various SNPs, including whole blood and buccal cells. The ability to directly amplify and detect nucleic acids from whole blood in the presence of different anticoagulants without previous extraction and purification demonstrates the robust nature of the amplification chemistry.

Methods

Nucleic acid preparation: 100 µL to 200 µL (depending on available volume) of each whole blood sample was extracted using the MagNA Pure LC automated system with a Total Nucleic Acid Isolation Kit (Roche Diagnostics, Indianapolis, IN), and eluted in 50 µL of elution buffer.

Whole blood preparation: Whole blood was either tested directly or diluted with 1X PBS Buffer.

Buccal (cheek) cell preparation: Buccal cells were scraped from one side of a person's inner cheek and stored in 500 µL 1X PBS Buffer.

Whole blood collection with different anticoagulants: Whole blood was collected in 3 different tubes; each tube contained a different anticoagulant (EDTA, sodium citrate, or sodium heparin).

Real-time PCR amplification and detection: Real-time PCR was performed with 1 µL to 2 µL of extracted DNA, buccal cells, or whole blood, in a total reaction volume of 10 µL using the 3M™ Integrated Cycler (3M, St Paul, MN). Each of the Focus Diagnostics primer sets targets one type of allele (wild type or mutant) specific for one of the 4 SNPs. Fluorescent signal for target-specific PCR products was detected at 60°C.

Methods (Cont.)

Method Comparison: Human genomic DNA was extracted from whole blood samples that were previously tested with a reference method for FII (n=270), FVL (n=268), or MTHFR (n=181) and tested with the appropriate Focus Diagnostics primer sets.

DNA vs. Whole Blood: DNA and whole blood from 543 patients were tested for both FII and FVL using the respective Focus Diagnostics wild type and mutant primer sets. DNA and whole blood from 184 patients were tested for both MTHFR SNPs, 677 and 1298, using the appropriate Focus Diagnostics reagents.

DNA, Whole Blood, and Buccal Cell Comparison: Focus Diagnostics MTHFR reagents were used to compare the detection of extracted DNA, whole blood, and buccal cells.

Anticoagulant testing: Focus Diagnostics MTHFR reagents were used to perform whole blood direct detection on blood collected in different anticoagulants: EDTA, sodium citrate or sodium heparin.

Results

Figure 1. Examples of amplification plots for the 3 MTHFR 1298 genotypes.

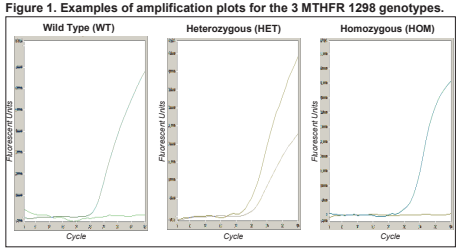


Table 1. Method comparison concordance for all 4 SNPs. The Focus Diagnostics reagents gave the same results as the reference method, detecting and identifying the genotype of each patient DNA in comparison to the reference results (Tables 1a through 1d).

Table 1a. Factor II Concordance

REF	Focus Diagnostics	WT	HET	HOM	Total
	WT	261	0	0	261
	HET	0	9	0	9
	HOM	0	0	0	0
	Total	261	9	0	270

Table 1b. Factor V Concordance

REF	Focus Diagnostics	WT	HET	HOM	Total
	WT	241	0	0	241
	HET	0	26	0	26
	HOM	0	0	1	1
	Total	241	26	1	268

Table 1c. MTHFR 677 Concordance

REF	Focus Diagnostics	WT	HET	HOM	Total
	WT	86	0	0	86
	HET	0	67	0	67
	HOM	0	0	28	28
	Total	86	67	28	181

Table 1d. MTHFR 1298 Concordance

REF	Focus Diagnostics	WT	HET	HOM	Total
	WT	85	0	0	85
	HET	0	83	0	83
	HOM	0	0	13	13
	Total	85	83	13	181

Results (Cont.)

Table 2. Concordance of extracted DNA vs. whole blood tested directly for all 4 SNPs. Extracted DNA and whole blood direct gave the same results. (Tables 2a through 2d).

Table 2a. Factor II Concordance

DNA	Whole Blood	WT	HET	HOM	Total
	WT	519	0	0	519
	HET	0	24	0	24
	HOM	0	0	0	0
	Total	519	24	0	543

Table 2b. Factor V Concordance

DNA	Whole Blood	WT	HET	HOM	Total
	WT	489	0	0	489
	HET	0	50	0	50
	HOM	0	0	4	4
	Total	489	50	4	543

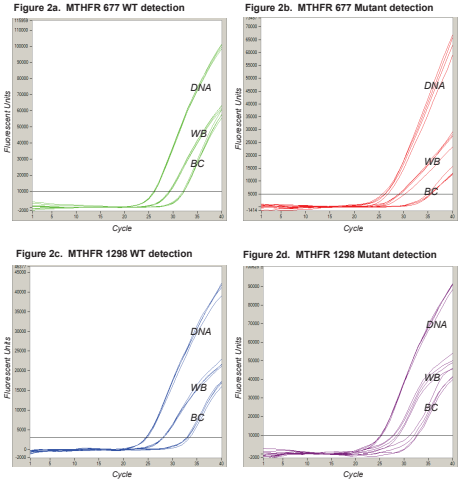
Table 2c. MTHFR 677 Concordance

DNA	Whole Blood	WT	HET	HOM	Total
	WT	88	0	0	88
	HET	0	68	0	68
	HOM	0	0	28	28
	Total	88	68	28	184

Table 2d. MTHFR 1298 Concordance

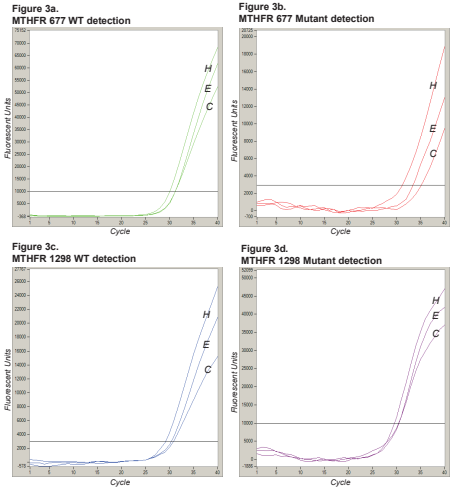
DNA	Whole Blood	WT	HET	HOM	Total
	WT	86	0	0	86
	HET	0	84	0	84
	HOM	0	0	14	14
	Total	86	84	14	184

Figure 2. Comparison of extracted DNA vs. direct testing of whole blood and buccal cells for detecting WT and mutant MTHFR SNPs. Amplification plots (Figures 2a through 2d) demonstrate the ability of the Focus Diagnostics MTHFR reagents to test different sample types: extracted DNA (DNA), whole blood (WB), and buccal cells (BC).



Results (Cont.)

Figure 3. Direct testing of whole blood using different anticoagulants. Amplification plots (Figures 3a through 3d) demonstrate the ability of the Focus Diagnostics MTHFR reagents to detect WT and mutant alleles of MTHFR directly from whole blood collected in different anticoagulants: EDTA (E), sodium citrate (C) and sodium heparin (H).



Conclusions

- Focus Diagnostics direct method gave the same results as the method using nucleic acid extraction.
- Whole blood and buccal cells can be genotyped directly without extraction/purification using the Focus Diagnostics reagents.
- Whole blood direct detection is possible with Focus Diagnostics reagents when collected in tubes containing anticoagulants such as EDTA, sodium citrate or sodium heparin.
- The Focus Diagnostics direct assay is currently in development, therefore, is not FDA cleared nor commercially available.

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