The VeriDose Core Panel: Strong Performance When Analyzing Challenging Pharmacogenetic Samples

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INTRODUCTION:

Pharmacogenetic (PGx) testing has experienced a rapid rise in popularity in recent years. It is estimated that the number of PGx tests ordered by physicians in the United States will grow at a compound annual growth rate of >23% from 2017 through 2020. PGx testing labs are often required to analyze challenging samples such as buccal swabs. This challenge is further intensified as buccal swabs are self-collected by consumers and mailed to the testing laboratory. The VeriDose® Core panel from Agena Bioscience® consists of 68 single nucleotide polymorphism (SNP) assays in 20 genes and 5 CYP2D6 CNV assays and is designed to be resilient to the lower quality DNA that is commonly extracted from challenging samples.

METHODS

When creating VeriDose Core, the following factors were considered to boost its resilience to low quality DNA: 1) PCR primers were designed to amplify shorter amplicons, 2) extend primers were optimized, and 3) content was moved between wells until optimal assay performance was achieved. Several different DNA extraction kits were tested (Figure 1), as differences in DNA quality were observed previously and shown to affect panel performance.

Software Design: The VeriDose Core Panel (Table 2) is accompanied by a reporting software that automatically analyzes each variation. A dedicated 2N control sample has to be present on each chip to anchor and normalize the data. By detecting peak height at each variation, the algorithm performs quality control and when all assays are present for a gene it will deduce the right haplotype and diplotype based on haplotype lookup tables generated based on PharmGKB and PharmVar descriptions.

Summary of key software features:

- Automatic analysis of peak heights to quality control each assay
- Automatic analysis for haplotype and diplotypes
- Easy to customize haplotype lookup tables with new data
- If run with CNV assays, calculation of overall copy number as well as copy number for non-hybrid alleles. See also poster G019
- All results displayed in easy to interpret reports (Table 3)

Figure 1. DNA extraction kits tested

1. Qiagen QIAmp DNA Mini Kit (#51304/51306)
2. Zymo Quick-DNA Microprep Plus (#D4074)
3. Invitrogen ChargeSwitch gDNA Buccal Cell Kit (#CS11021)
4. Stratec PSP SalivaGene DNA Kit (#1035200200) Using SalivaGene Swab Comfort (#1035231100)
5. Macherey-Nagel NucleoSpin Tissue (#740952)

RESULTS

Two experiments were conducted to assess the VeriDose Core panel's resilience to lower quality DNA. Five DNA extraction kits were tested. The panel performed well on DNA extracted by three of the kits, producing 99% or higher call rates (Table 1). Next, challenging DNA samples which did not produce results when analyzed using other PGx assays were analyzed using the VeriDose Core panel. Most samples were called, demonstrating that VeriDose Core is more resilient to lower quality DNA.

Experiment 1: Comparison of PGx74 and VeriDose Core Using HapMap DNA and DNA Derived from Buccal Swabs

DNA extracted from buccal swabs were analyzed using PGx74 and VeriDose Core. Two buccal swab samples were taken from each of up to 17 individuals. To create DNA of varying quality, the swabs from each individual were processed using five different commercially available extraction kits. Results were analyzed to determine results concordance and rate of assay failure.

Samples Tested

- ✓ **52 Coriell DNA samples run in duplicate**
- ✓ **155 buccal swab samples extracted using 5 different commercially available extraction kits (two reps per sample)**

Results

100% of the results which passed QC criteria for PGx74 and VeriDose Core were concordant, but overall call rate varied between the panels (Table 1).

When testing the HapMap samples, assay call rate for PGx74 was >99.6%. Out of a total of 8,275 possible calls, there were 246 manual calls. Call rate for VeriDose Core improved to >99.9% for the same sample set. Out of a total of 8,153 possible calls, there were 240 user calls. This shows that for high quality DNA, call rates are very similar between the panels.

For the buccal swab samples, a 95.6% call rate was observed across all extraction methods when analyzed using PGx74. This value improved to 98.8% when analyzed using VeriDose Core. Performance varied across extraction kits. The lowest performing kit produced an 81.8% call rate and the highest performing kit showed a 99.9% call rate when analyzed with PGx74 (Example output Figure 2). When analyzed using VeriDose Core, all kits showed >93% call rates, and three kits showed >99% call rate. This clearly showed that the VeriDose Core panel is more resilient to lower quality of DNA.

Table 1. Summary data for the PGx74 and VeriDose Core success rate for different extraction techniques

KIT	# SAMPLES TESTED	PGX74 OVERALL CALL RATE	VERIDOSE CORE OVERALL CALL RATE	AVG NG/µL	AVG TOTAL YIELD (µg)	AVG 260/280	ELUTION	SWAB
INVITROGEN	35	81.8%	95.5%	10.9	1.6	1.86	Kit Buffer	Polyester
MACHEREY-NAEGEL	28	100%	99.9%	20.5	2.1	1.85	Kit Buffer	Polyester
QIAGEN	35	99.9%	99.8%	7.2	1.1	2.26	Water	Polyester
STRATEC	26	99.9%	99.6%	17.7	1.8	1.85	Kit Buffer	Stratec Swab
ZYMO	35	93.5%	99.7%	22.2	2.2	1.92	Water	Polyester

Table 2. Gene Content of the VeriDose Core Panel

Gene	SNP	Variant	Gene	SNP	Variant	Gene	SNP	Variant
ABCB1	rs1045642	c.3435C>T	**CYP2C9**	rs1799853	*2	CYP2D6	rs5030865	*8
APOE	rs429358	C130R	**CYP2C9**	rs1057910	*3	CYP2D6	rs5030656	*9
APOE	rs7412	R176C	**CYP2C9**	rs56165452	*4	CYP2D6	CNV	CNV
COMT	rs4680	472G>A	**CYP2C9**	rs28371686	*5	CYP3A4	rs4987161	*17
CYP1A2	rs72547513	*11	**CYP2C9**	rs9332131	*6	CYP3A4	rs55785340	*2
CYP1A2	rs2069514	*1C	**CYP2C9**	rs7900194	*8	CYP3A4	rs35599367	*22
CYP1A2	rs762551	*1F	**CYP2D6**	rs1065852	*10	CYP3A5	rs28365083	*2
CYP1A2	rs12720461	*1K	**CYP2D6**	rs201377835	*11	CYP3A5	rs776746	*3
CYP1A2	rs56107638	*7	**CYP2D6**	rs5030862	*12	CYP3A5	rs10264272	*6
CYP2B6	rs28399499	*18	**CYP2D6**	rs5030865	*14	CYP3A5	rs41303343	*7
CYP2B6	rs3745274	*6	**CYP2D6**	rs72549357	*15	DRD2	rs1800497	Taq1A
CYP2C19	rs12248560	*17	**CYP2D6**	rs28371706	*17	F2	rs1799963	G20210A
CYP2C19	rs4244285	*2	**CYP2D6**	dup4125_4133	*18	F5	rs6025	R506Q
CYP2C19	rs4986893	*3	**CYP2D6**	rs72549353	*19	GLP1R	rs1042044	Leu260Phe
CYP2C19	rs28399504	*4	**CYP2D6**	rs16947	*2	GLP1R	rs6923761	Gly168Arg/Ser
CYP2C19	rs56337013	*5	**CYP2D6**	rs1135840	*2	GLP1R	rs2300615	c.510-1135T>G
CYP2C19	rs72552267	*6	**CYP2D6**	rs72549354	*20	MTHFR	rs1801131	Glu429Ala
CYP2C19	rs72558186	*7	**CYP2D6**	rs59421388	*29	MTHFR	rs1801133	Ala222Val
CYP2C19	rs41291556	*8	**CYP2D6**	rs35742686	*3	OPRM1	rs1799971	Asn40Asp
CYP2C9	rs28371685	*11	**CYP2D6**	rs3892097	*4	PNPLA5	rs5764010	C>T
CYP2C9	rs9332239	*12	**CYP2D6**	rs28371725	*41	SLCO1B1	rs4149056	*5
CYP2C9	rs72558187	*13	**CYP2D6**	rs5030655	*6	SULT4A1	rs763120	c.*1113A>G
CYP2C9	rs72558190	*15	**CYP2D6**	rs5030867	*7	VKORC1	rs9923231	1639 G>A

Table 3. Example output for the VeriDose Core panel

Sample	ABCB1	APOE	COMT	CYP1A2	CYP2B6	CYP2C19	CYP2C9	CYP2D6	CYP3A4	CYP3A5	DRD2	F2	F5	CNV	CNVQuality	HybridSta	CNVFunctionalOutcome	QCStatus
NA10831	A/A	E3/E3	A/G	*1A/*1F	*1/*1	*1/*17	*1/*2	1N *4/*5	*1/*1	*3/*3	WT/WT	WT/WT	WT/WT	1N	(1.05-0.01-HighConf)		1N(1.05)	PASS
NA10865	A/G	E3/E3	A/G	*1F/*1F	*1/*1	*17/*8	*1/*2	2N *1/*41	*1/*1	*3/*3	Taq1A/WT	WT/WT	WT/WT	2N	(2.26-0.13-MedConf)		2N(2.26)	PASS
NA11839	A/G	E2/E3	A/G	*1A/*1F	*1/*1	*1/*1	*2/*3	2N *1/*2	*1/*1	*1A/*3	WT/WT	WT/WT	WT/WT	2N	(2.13-0.02-HighConf)		2N(2.13)	PASS
NA12753	G/G	E3/E3	G/G	*1A/*1F	*1/*6	*1/*2	*1/*1	2N *2/*3	*1/*1	*3/*3	Taq1A/WT	WT/WT	WT/WT	2N	(2.13-0.04-HighConf)		2N(2.13)	PASS
NA17454	A/G	E3/E4	G/G	*1F/*1F	*1/*6	*1/*1	*1/*8	3N+ *1/*2	*1/*1	*1A/*3	Taq1A/Taq1A	WT/WT	WT/WT	3N+	(3.89-0.15-HighConf)		3N+(3.89)	PASS
NA18484	G/G	E3/E3	A/G	*1A/*1A	*1/*18	*1/*2	*1/*1	2N *1/*17	*1/*1	*1A/*7	Taq1A/Taq1A	WT/WT	WT/WT	2N	(2-0-HighConf)		2N(2)	PASS
NA18518	G/G	E3/E3	A/G	*1A/*1A	*1/*6	*17/*2	*1/*1	2N *17/*29	*1/*1	*1A/*6	WT/WT	WT/WT	WT/WT	2N	(1.87-0.05-HighConf)		2N(1.87)	PASS
NA18540	A/G	E3/E4	A/A	*1L/*1L	*1/*6	*1/*2	*1/*1	3N+ *10/*41	*1/*1	*1A/*3	Taq1A/Taq1A	WT/WT	WT/WT	3N+	(3.78-0.05-MedConf)		3N+(3.78)	PASS
NA18552	G/G	E3/E3	G/G	*1A/*1A	*1/*6	*1/*4A	*1/*1	2N *1/*14B	*1/*1	*3/*3	Taq1A/Taq1A	WT/WT	WT/WT	2N	(2.24-0.05-MedConf)		2N(2.24)	PASS
NA18565	A/A	E3/E3	A/G	*1F/*1F	*1/*1	*1/*1	*1/*1	3N+ *10/*10	*1/*1	*1A/*3	Taq1A/WT	WT/WT	WT/WT	3N+	(3.51-0.01-LowConf)		3N+(3.51)	PASS
NA18855	G/G	E3/E3	G/G	*1A/*1L or *1C/*1F	*6/*6	*1/*2	*1/*1	1N *1/*5	*1/*1	*3/*6	Taq1A/WT	WT/WT	WT/WT	1N	(1.2-0.07-HighConf)		1N(1.2)	PASS
NA19035	G/G	E3/E3	G/G	*1A/*1F	*1/*6	*17/*17	*1/*1	1N *2/*5	*1/*1	*1A/*7	Taq1A/WT	WT/WT	WT/WT	1N	(1.24-0.07-MedConf)		1N(1.24)	PASS
NA19143	G/G	E3/E3	A/G	*1A/*1F	*6/*6	*1/*1	*1/*6	2N *10/*2	*1/*1	*6/*7	Taq1A/WT	WT/WT	WT/WT	2N	(1.86-0.02-HighConf)		2N(1.86)	PASS
NA19178	G/G	E3/E3	G/G	*1L/*1L	*6/*6	*1/*6	*1/*5	2N *1/*1	*1/*1	*1A/*1A	Taq1A/WT	WT/WT	WT/WT	2N	(1.8-0.04-MedConf)		2N(1.8)	PASS
NA19239	G/G	E3/E3	A/G	*1A/*1L or *1C/*1F	*1/*6	*1/*17	*1/*1	2N *15/*17	*1/*1	*1A/*1A	WT/WT	WT/WT	WT/WT	2N	(2.22-0.02-MedConf)		2N(2.22)	PASS

Conclusion:

- ✓ Multiple DNA extraction kits show high call rates with VeriDose Core
- ✓ Main issue for extraction kits seems to be the size of the genomic DNA fragments recovered
- ✓ Shortening the PCR amplicon size made VeriDose Core much more resilient to low quality DNA
- ✓ SNP and CNV assays are performed & analyzed using one workflow
- ✓ Many samples otherwise failing are rescued
- ✓ Automated analysis allows quick reporting

Experiment 2: Analysis of Previously Unsuccessful Clinical Samples

DNA was extracted from clinical samples (buccal swabs) and was previously analyzed using PGx74. A subset (62 total) of samples failed to give a complete genotype for all assays. Repeat analysis did not resolve the issue. These samples were genotyped with VeriDose Core and results were analyzed to determine results concordance and rate of assay failure.

Samples Tested

- ✓ **62 Clinical samples derived from a total of 618 samples previously tested using PGx74**

Results

Of the 62 samples that failed using PGx74, 40 samples (65%) had a 100% call rate on VeriDose Core on the first run and had no need for any manual calling. Another eight samples could be rescued by manual calls. In total, 48 of the 62 samples (77%) could be genotyped completely with VeriDose Core. Only 14 samples out of the 62 that failed on PGx74, failed on VeriDose Core also and were shown to be poor quality DNA.

Figure 2. Example of genotyping success rate by PCR amplicon length
Top: example kits I & Z; Bottom example kits M, Q, or S