Preliminary Studies of the Agena Bioscience VeriDose *DPYD* Plus Assay

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BACKGROUND

The Agena Bioscience VeriDose *DPYD* Plus (Table 1) is a single-well assay that is designed to be compliant with the AMP-led joint consensus recommendations testing for Tier 1 and Tier 2 *DPYD* pan-ethnic variants. The VeriDose *DPYD* Plus Panel includes the AMP Tier 1 and Tier 2 recommendations as well as four additional variants. A preliminary assay was designed, tested for feasibility internally and sent to volunteer laboratories for evaluation.

Table 1. VeriDose *DPYD* Plus Panel Content

Nucleotide Change[1]	Star Allele	dbSNP rs#	AMP Tier	Allele Frequency
c.1905+1z>A	*2A	rs3918290	1	0-0.8
c.1679T>G	*13	rs55886062	1	0-0.06
c.1129-5923C>G	HapB3	rs75017182	1	0-2.4
c.1236G>A	HapB3	rs56038477		0-2.4
c.557A>G	N/A	rs115232898	1	0-2.6
c.868A>G	N/A	rs146356975	1	0-0.02
c.2279C>T	N/A	rs112766203	1	0-0.06
c.2846A>T	N/A	rs67376798	1	0-0.4
c.299_302del	*7	rs72549309	2	0-0.02
c.703C>T	*8	rs1801266	2	0-0.02
c.1314T>G	N/A	rs186169810	2	0-0.07
c.1475C>T	N/A	rs72549304	2	0-0.02
c.1774C>T	N/A	rs59086055	2	0-0.08
c.2639G>T	N/A	rs55674432	2	0-0.07
c.61C>T	N/A	rs72549310	NA	0-0.003
c.704G>A	N/A	rs755416212	NA	0-0.02
c.1024G>A	N/A	rs183385770	NA	0-0.007
c.2043_2058del	N/A	rs773499329	NA	0-0.1

Highlighted variants are also included in the VeriDose Core Panel v2.0.

[1]Reference sequence NM_000110.4

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MATERIALS & METHODS

Assays were designed using the online Agena Assay Design Suite (ADS, v2.0). Designed primer sequences were then aligned against GRCh38. PCR primers causing interactions were redesigned and re-analyzed until all interactions between primers in the same well were non-existent. Internal studies were performed on DNA extracted from buccal swabs, saliva, and whole blood samples using 2 different Agena MassARRAY Systems on different days using both the 96- and 384-well format. Additionally, multiple thermal cyclers were used. The following were guard banded to determine optimal parameters: annealing temperature (54- 64°C), PCR ramp rate (3-6°C/s), PCR reaction mix (0.75-1.25X), and unincorporated extension primers (0.75-1.25X). Performance of VeriDose Core 2.0 was compared to VeriDose *DPYD* Plus on Coriell DNA samples (Table 2) to ensure that all results were concordant. Reagents were sent to three external laboratories to determine accuracy, analytical sensitivity and specificity from orthogonally tested samples.

The alpha sites used DNA extracted from whole blood, saliva, buccal swabs and dried blood spots using a QIAmp DNA Blood mini kit, Qiagen DS DNA Mini kit, Qiagen EZ1 DNA Blood kit, and the Revvity DNA Blood 5K kit using the Chemagic MSM1 instrument. Thermal cyclers used included: Applied BioSystems Incorporated (ABI) ProFlex, ABI SimpliAmp, and ABI Verti 96.

The limit of input DNA concentration was tested. Results show that input DNA from 5ng-50ng/reaction yielded interpretable results. It is recommended that 10 ng DNA/reaction be used.

RESULTS

The assay performance was robust at all levels of guard banding. The external laboratory results (49 samples) were 100% concordant with previously tested results for 100% accuracy. The analytical sensitivity is 100% (95% CI; 88.3-100%) and the analytical specificity is 100% (95% CI; 94.7-100%).

1-well multiplex panel of 18 *DPYD* gene targets that include all AMP tier 1 & 2 variants, plus 4 biomarkers

Sample types: Saliva, Buccal swab and Whole blood

MassARRAY® Systems CPM 96-format and CPM 384-format, RUO only

The panel can work side-by-side with Corev2.0 workflow and use the PGx reporter

Table 2: Concordance of VeriDose Core 2.0 Panel and *DPYD* Plus performed on Coriell DNA samples.

	Corev2.0	DPYD+	Concordance	Corev2.0	DPYD+	Concordance	Corev2.0	DPYD+	Concordance	Corev2.0	DPYD+	Concordance	Corev2.0	DPYD+	Concordance	Corev2.0	DPYD+	Concordance
Sample	rs55886062	rs55886062		rs3918290	rs3918290		rs67376798	rs67376798		rs115232898	rs115232898		rs75017182	rs75017182		rs56038477	rs56038477	
HG00111	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
HG01680	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
HG01697	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
HG02373	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
HG02852	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
HG03225	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
HG03643	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA12753	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA12813	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA12878	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA16654	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA17012	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA17019	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA17039	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	C/T	C/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA17227	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	C/G	C/G	Concordant	C/T	C/T	Concordant
NA17229	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA17702	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18484	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18518	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18524	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18540	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18552	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18563	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18564	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18565	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA18855	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA19178	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA19207	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	C/T	C/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA19213	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA19226	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA19239	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA24009	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant
NA24027	A/A	A/A	Concordant	C/C	C/C	Concordant	T/T	T/T	Concordant	T/T	T/T	Concordant	G/G	G/G	Concordant	C/C	C/C	Concordant

CONCLUSIONS

VeriDose *DPYD* Plus Panel is designed to supplement the VeriDose Core 2.0 Panel. It should be noted that while six of the variants overlap in both assays, the designs for those variants are not the same. In a sense, *DPYD* Plus Panel and the Veridose Core 2.0 Panel are orthogonal assays. In very rare instances, it is possible that the results for a variant may not be the same in both assays due to an interfering variant under one of the primers or probes. One such example is c.1865G>A (rs201433243) that may interfere with VeriDose Core 2.0 *DPYD* genotyping for c.1905+1G>A and can cause allele drop out. It does not impact VeriDose *DPYD* Plus. The function of this variant is unknown. The frequency is 0.28% in individuals of European genetic ancestry. As often done in testing, the laboratory should investigate any discrepant results either by sequencing or another orthogonal method.

c.2657G>A (rs1801267; legacy name *9B), if present in cis (on the same chromosome), may interfere with testing causing allele drop out for c.2639G>T (rs55674432) as identified by the SeraCare *DPYD* control material. The variant frequency of c.2657G>A is 0.02% (https://gnomad.broadinstitute.org/variant/1-97098598-C-T?dataset=gnomad_r4) in individuals of East Asian genetic ancestry. Using the gnomAD prediction tool of co-occurrence (https://gnomad.broadinstitute.org/variant-cooccurrence?dataset=gnomad_r2_1&variant=1-97564172-C-A&variant=1-97564154-C-T), these two variants are not predicted to be in cis.

There was a second potential interfering variant identified under the PCR primer for c.2639G>T which is c.2656C>T (normal function; p.Arg886Cys; rs147545709; frequency 0.01% in South Asian genetic ancestry), while not tested, could also interfere if the variant was in cis. Again, using the gnomAD prediction tool of co-occurrence (https://gnomad.broadinstitute.org/variant-cooccurrence?dataset=gnomad_r2_1&variant=1-97564172-C-A&variant=1-97564155-G-A), these two variants are not predicted to be in cis.

It is possible that during studies, an individual may be unexpectedly identified as homozygous for a rare variant such as for c.2639G>T (rs55674432). This can occur by three possible methods: 1) a deletion is present in trans, 2) there is consanguinity present, or 3) there is an interfering variant in trans. If identified, one may need to test parents of the homozygous individual or other close family members to resolve which possible scenario has occurred.

Some of the variants in the *DPYD* Plus assay are known to be tri- and quad-allelic. *DPYD* Plus is intended only to detect the included variants (Table 1). It is possible that rare variant peaks may be present, but the software will not call the rare variants.

Overall, the Agena Bioscience *DPYD* Plus assay performed well in internal and external studies. The *DPYD* Plus assay is commercially available as Research Use Only.

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