Challenges of Quality Control in a Genetics Testing Lab

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Quality Control in Genetics Testing

Mark Manak – Warfarin and Thrombophilia

1. Genetic testing
2. Quality Control Considerations
3. Types of Controls
4. Controls for Warfarin and Thrombophilia
5. How Controls are used

Todd Christensen – Cystic Fibrosis

1. Special challenges of multiplex testing
2. Use of oligo based controls
3. Single tube control for many different alleles

Help ensure proper operation and accuracy of results each day.

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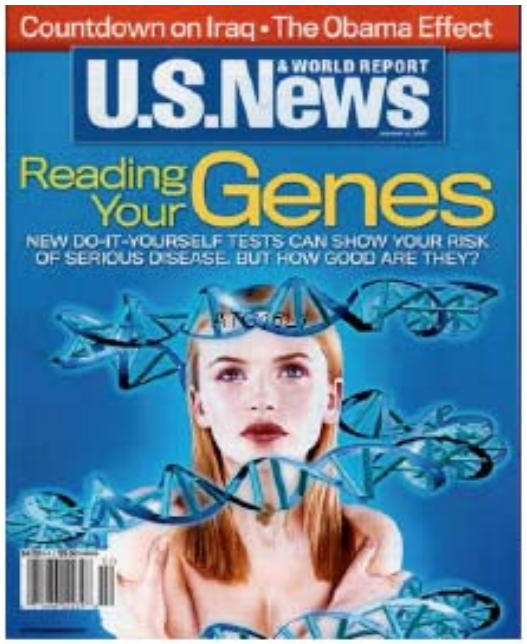
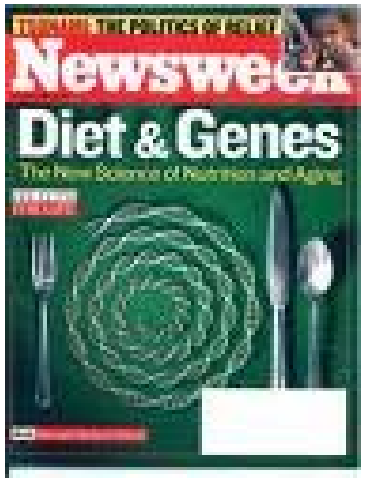
Genetic Testing in the News





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Genetic Tests

Chromosomal abnormalities

- Aneuploidy - Duplication
- Deletion, Inversion, Translocation, Mosaicism

Single gene disorders

- Dominant
- Recessive
- X-linked

Complex/multifactorial anomalies

- Most genetic diseases
- Many Cancers

Personalized medicine

- Bleeding disorders
- Targeted drug therapy

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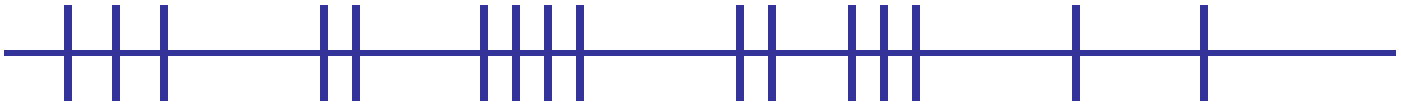
Genetic Mutations

SNP - Single Nucleotide Polymorphisms

……. G G T A A C T G ……
……. G G C A A C T G ……

~2 million SNPs identified in the human genome.



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Warfarin

Warfarin (Coumadin®)

Most widely prescribed oral anti-coagulant

Prescribed following MI, atrial fibrillation, stroke, venous thrombosis, prosthetic heart valve replacement, and following major surgery

> 2 million surgical and cardiac patients in US take Warfarin daily to prevent blood clotting.

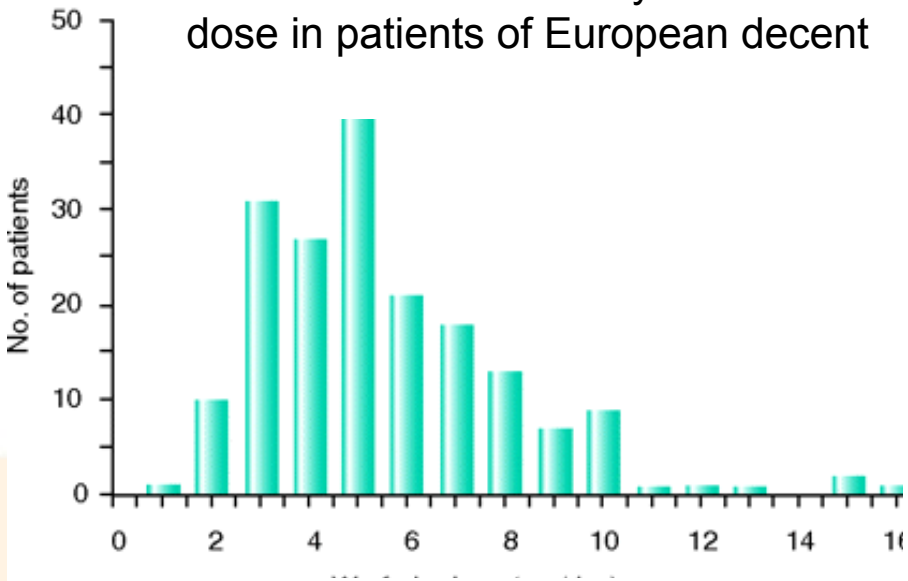
Finding the correct dose is very difficult

- Narrow therapeutic range
- Large inter-individual variation

Mistakes can be life-threatening

- Major bleeding episodes in 1-2% of patients
- Death in as many as 0.1-0.7%

Inter-individual variability in Warfarin dose in patients of European decent



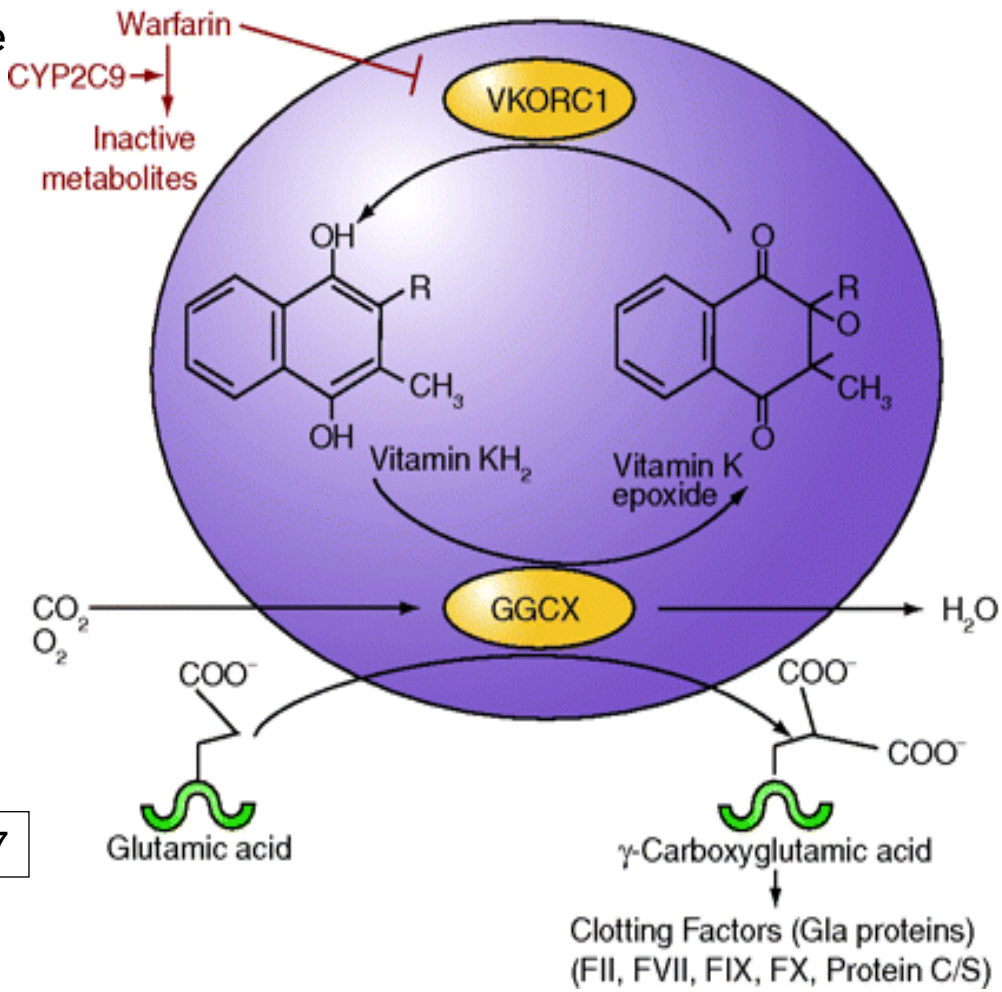
Mechanism of action of Warfarin

CYP2C9:

- Cytochrome P450 Enzyme
- Metabolizes S-Warfarin

VKORC1:

- Vitamin K Epoxide Reductase Complex 1
- Inhibits Vitamin K cofactor in clotting



From: Mol Interventions 6: 223-227

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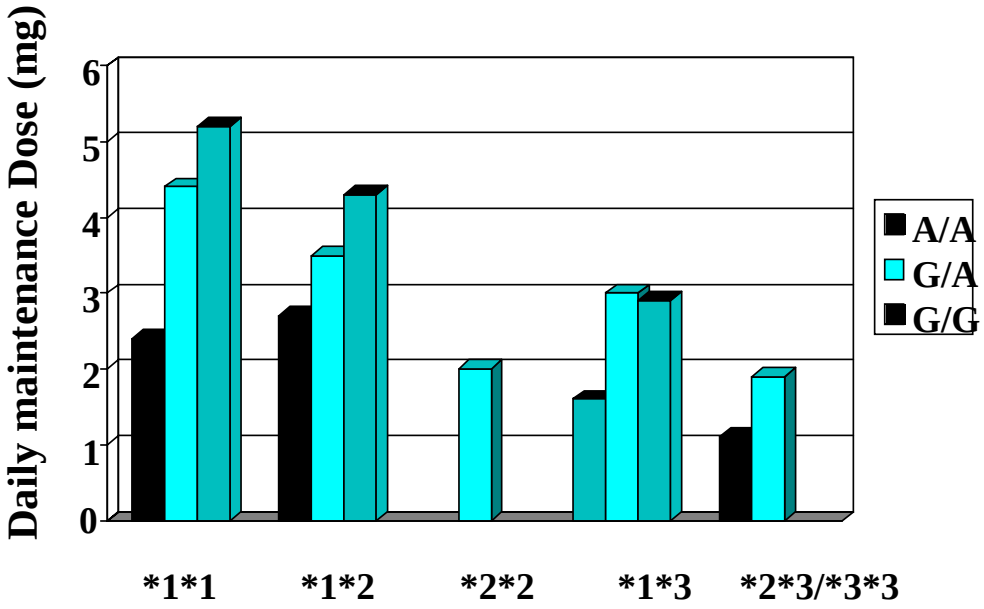
Effect of Mutations on Warfarin Metabolism

CYP2C9 allele frequencies

	2*	3*
European	11%	9%
Asian	0%	2%
African-American	3%	1%

CYP2C9*1 (WT)	Normal
CYP2C9*2 (Arg144Cys)	Intermediate
CYP2C9*3 (Ile359Leu)	Low



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Sconce et al, Blood, October 2005

Mutations in VKORC1 Leading to Warfarin Resistance

Amino acid change	Daily Dose	Resistance Phenotype
Val29Leu	14 mg	Moderate
Ala41Ser	16 mg	
Arg58Gly	32–36 mg	Major
Val66Met	27–35 mg	
Leu128Arg	> 45 mg	Severe
Val45Ala	Target INR never reached	

Differences in CYP2C9 and VKORC1 genes influence how much Warfarin is optimal for each person

Genetic Testing enables faster and more precise dosing.

From Mol Interventions 6: 223-227

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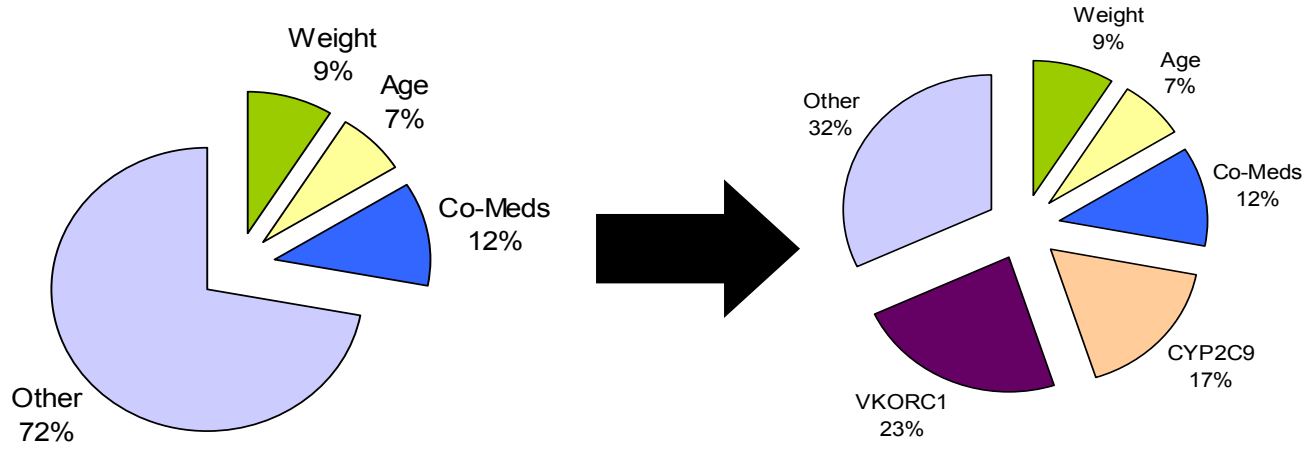
Factors affecting warfarin dosing

Dosing regimens based on age, gender, weight, etc.

Once an individual's dosing has been determined (trial and error), then genetic testing no longer an issue– only affects new patients.



Mutations in CYP2C9 and VKORC1 genes have profound effect on sensitivity to Warfarin

Allows faster, better targeted dosing in a cost effective manner

In August of 2007, FDA approved a relabeling of Warfarin that indicates new Warfarin patients should undergo genetic testing:

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Warfarin Testing Platforms

Company	Analytes Tested	Platform	FDA cleared?
Nanosphere	CYP2C9 *2, *3 VKORC1 (-1639G>A)	Verigene (IVD)	Yes
Osmetech	CYP2C9 *2, *3, *4, *5, *6 and *11 VKORC1 (-1639G>A)	eSensor	Yes
Luminex	CYP2C9 *2, *3, *4, *5, and *6	Tag-It/xTag	No. RUO
Autogenomics	In FDA Cleared test: CYP2C9 *2 and *3 and VKORC1 (-1639G>A) Other tests also have CYP2C9*4, *5, *6, and *11, multiple VKORC1	RealTime PCR	Yes
~~Third Wave~~	~~CYP2C9 *2, *3~~ ~~VKORC1 (-1639G>A)~~	~~Invader~~	~~ASR~~
Third Wave recently announced that they will discontinue their ASR Warfarin assay			
Paragon Dx	CYP2C9 and VKORC1 Alleles not specified	Cepheid Smart Cycler	Yes
"In-House"	Various	Various	No

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Regulatory Considerations

FDA Oversight of Genetic Tests

Data and regulatory issues must be addressed prior to approval

Submission depends upon claims requested
- At least a 510(k), possibly a PMA

Provide information on
- Device design, analytical studies, software and instrumentation,
- Comparison studies, clinical studies, and effectiveness

FDA guidance on Analyte Specific Reagents (ASRs) **
- Used to identify or quantify substances in biological specimens
- Viewed as an IVD when combined with specific performance claims
- Genetic tests utilizing ASRs are not exempt from premarket notification

“In-house" developed tests are subject to FDA regulation

*www.fda.gov/cdrh/oivd/guidance/1590.pdf
** http://www.fda.gov/cdrh/oivd/guidance/1549.pdf

CLIA Categorization of Tests

Tests graded for complexity from 1 to 3 for each of 7 criteria (1 being lowest complexity)

Waived Tests

- Simple, accurate with little likelihood of erroneous results
- No harm to patient if performed incorrectly
- All tests approved for home use

Tests of Moderate Complexity

- Score of ≤12 points; moderate complexity

Tests of High Complexity

- Score of >12 points;

Clinical Laboratory Improvement Amendments (CLIA)

Clinical predictive value
Pre- and post-test delivery and reporting issues
Laboratory equipment and reagent validation
Personnel training
Analytic sensitivity and specificity

Test validation by analysis of positive and negative samples in parallel with a predicate method

Continued monitoring of test sensitivity and specificity through use of well-characterized positive and negative controls,

Positive control run for each analyte tested

CLIA does not currently have a genetics specialty

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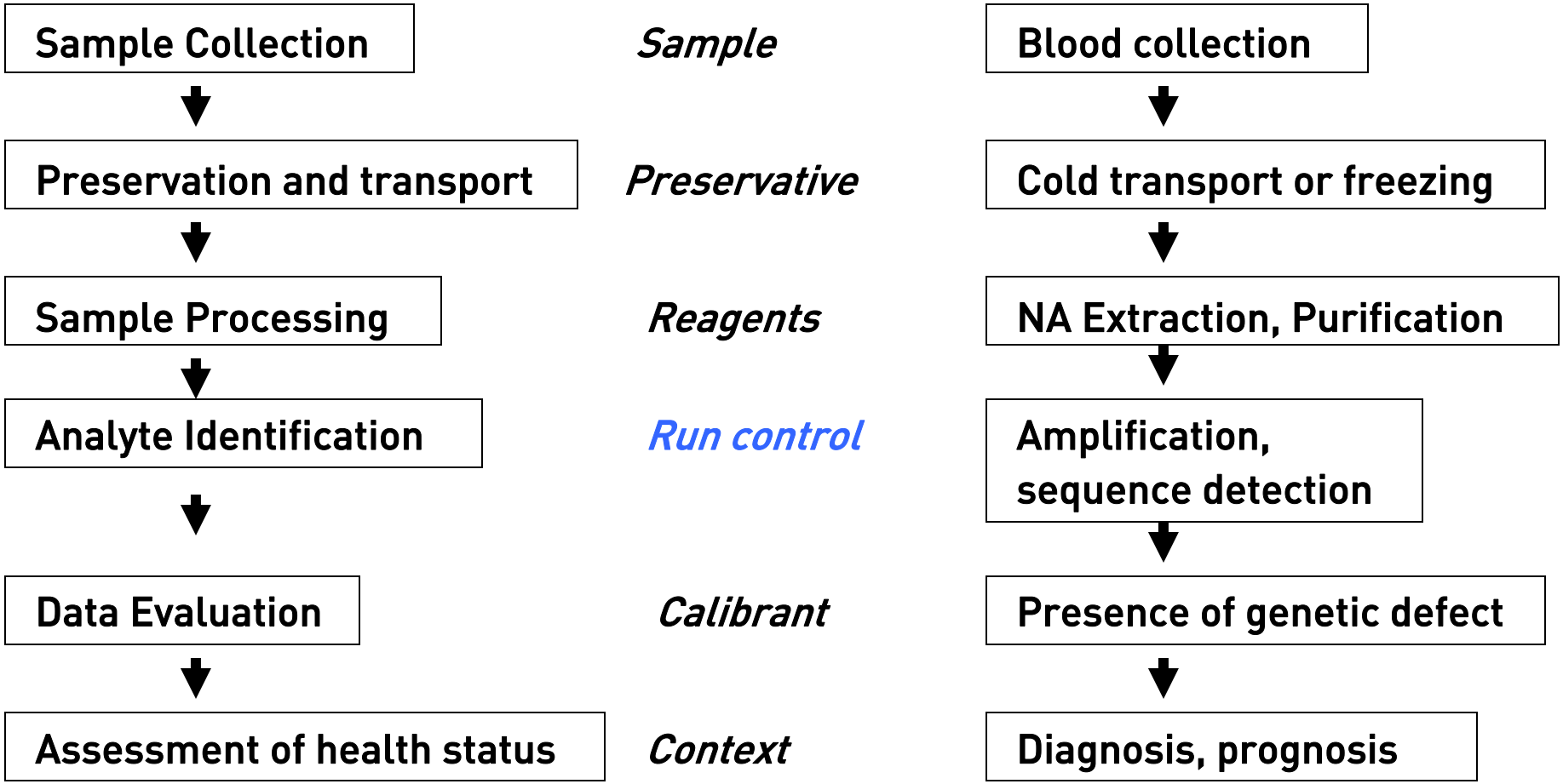
Quality Control Program

Analytical processes in Genetic testing



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Quality Control

Every mutation that you test for is a separate analyte.

- If you don't have a control for each one, how do you know that your test is detecting each one every time you run it?
- Will one control cover all test steps, or do you need an extraction control, an internal control and an amplification/detection control for each analyte?

Ideal Independent Controls in routine QC

- Act similarly to patient samples
- Are not biased to one specific test method.
- Can control the whole assay process
- Can allow continual comparison of site performance
  - Multiple labs within an organization
  - Across multiple labs offering the same tests.

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CALCULATION OF FOLD OVER ZERO VALUES

A fold over zero (FOZ) calculation to determine if the data is valid. Needs to be performed for both the FAM and Red dye readings.

$$\text{FOZ} = \frac{\text{Raw Signal of control or sample}}{\text{Raw Signal of No Target Blank}}$$

$$\text{Ratio} = \frac{\text{Net WT FOZ}}{\text{Net Mut FOZ}}$$

Analyte	FAM Dye	Red Dye
MTFHR A1298C	WT	Mut

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SAMPLE ACCEPTANCE CRITERIA-1298

Ratio	WT (FAM dye) FOZ	Mut (Red dye) FOZ	Genotype
≥5.0	≥2.0	≥0.2	WT
>2.0 to <5.0	(Invalid due to Ratio)	(Invalid due to Ratio)	Equivocal (EQ1)
≥0.5 to ≤2.0	≥2.0	≥2.0	Het
>0.2 to <0.5	(Invalid due to Ratio)	(Invalid due to Ratio)	Equivocal (EQ2)
≤0.2	≥0.2	≥2.0	Mut

When equivocal results are obtained, the sample must be repeated.
If the sample still generates an equivocal result, the concentration of the DNA sample should be verified.

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Effect of DNA concentration on genotype calling

Lot#	DNA (ng)	Invader® Genotype	F Signal FOZ	R Signal FOZ
100031	75ng	HET	2.07	2.16
100031		-	1.79	1.78
100031		-	1.79	1.85
100031	120	HET	2.00	2.09
100031		HET	2.31	2.43
100031		-	1.94	1.95
100031	150	HET	3.16	3.71
100031		HET	3.12	3.46
100031		HET	3.72	4.26
100031	225	HET	3.16	3.46
100031		HET	3.87	4.51
100031		HET	3.92	4.48

Lot#	DNA (ng)	Invader® Genotype	F Signal FOZ	R Signal FOZ
100032	75ng	HET	2.41	2.54
100032		-	1.89	1.94
100032		-	1.84	1.83
100032	120	HET	2.43	2.66
100032		HET	2.38	2.60
100032		HET	2.29	2.48
100032	150	HET	2.91	3.19
100032		HET	3.00	3.40
100032		HET	3.29	3.66
100032	225	HET	3.88	4.58
100032		HET	3.52	4.03
100032		HET	3.78	4.46

Lot#	DNA (ng)	Invader® Genotype	F Signal FOZ	R Signal FOZ
100033	75ng	HET	2.14	2.24
100033		-	1.93	2.08
100033		-	1.72	1.75
100033	120	HET	2.89	3.11
100033		HET	2.55	2.76
100033		HET	2.25	2.37
100033	150	HET	3.07	3.34
100033		HET	2.42	2.59
100033		HET	2.93	3.31
100033	225	HET	3.62	4.26
100033		HET	3.50	4.04
100033		HET	3.51	4.04

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Input of 150ng of DNA – two different assays

SampleID	Genotype	F Signal FOZ	R Signal FOZ	Result
MTHFR (C677T) WT (C1)	WT	5.63	1.57	VALID
MTHFR (C677T) HET (C2)	HET	4.03	3.96	VALID
MTHFR (C677T) MUT (C3)	MUT	1.05	5.98	VALID
No DNA Control (C4)				
100031	HET	4.23	3.84	VALID
100032	HET	3.71	3.67	VALID
100033	HET	3.39	3.43	VALID

SampleID	Genotype	F Signal FOZ	R Signal FOZ	Data
MTHFR (A1298C) WT (C1)	WT	5.41	0.98	VALID
MTHFR (A1298C) HET (C2)	HET	5.42	4.86	VALID
MTHFR (A1298C) MUT (C3)	MUT	1.10	8.84	VALID
No DNA Control (C4)				
100031	HET	2.01	2.01	VALID
100032	HET	2.09	2.38	VALID
100033	HET	2.61	2.96	VALID

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Design of Quality Controls

External run controls

Source of Run Control

- Patient Sample
- EBV Transformed Cell Line
- Recombinant Plasmid
- Oligonucleotide

External controls Usage:

- Method development and validation of assay
- Performance and qualification of instruments
- Proficiency testing/ training of the lab personnel
- Ensuring Lot to Lot and Run to Run Consistency

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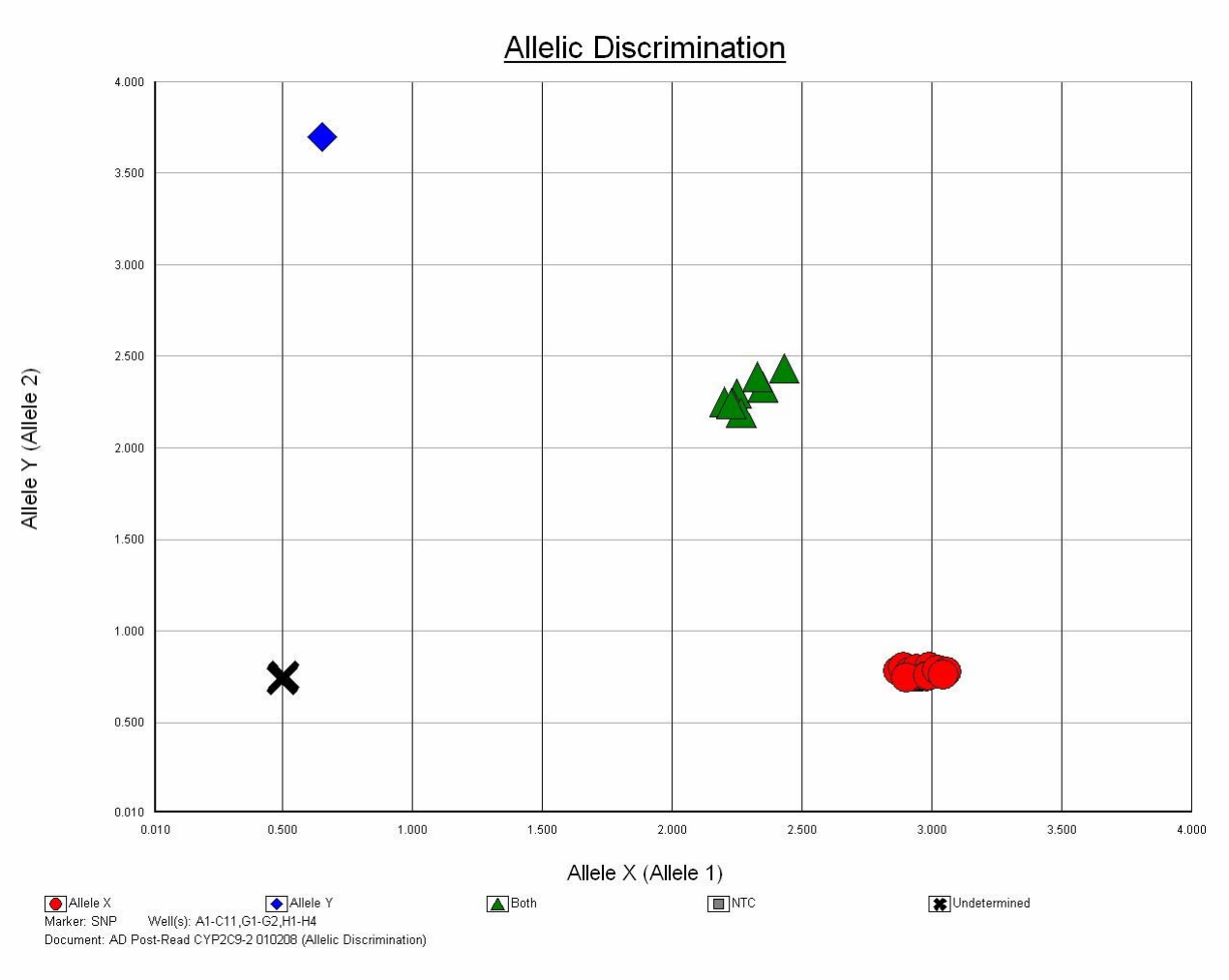
ABI Genotyping Assay on SDS7500: CYP2C9*2



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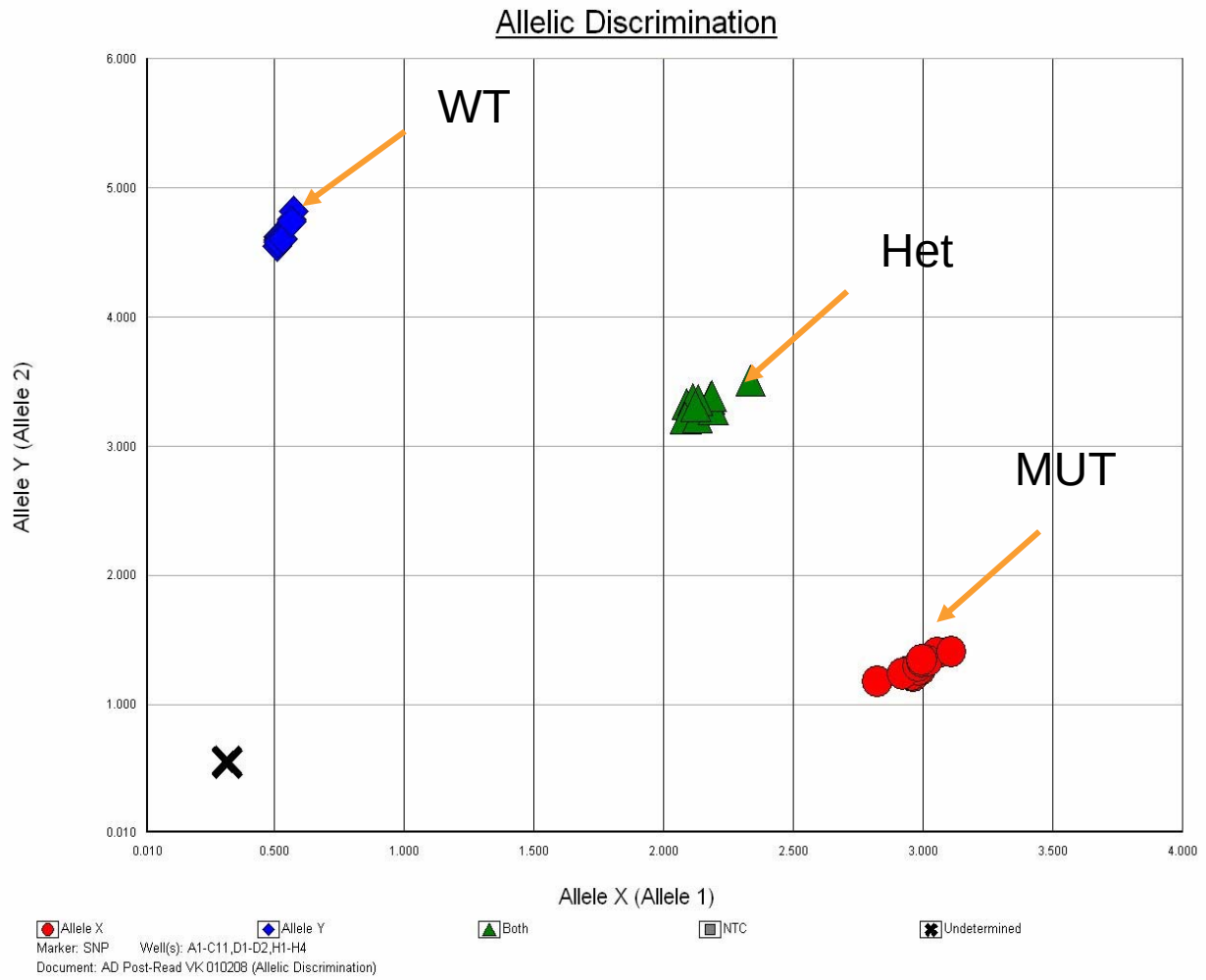
ABI Genotyping Assay on SDS7500: VKORC-1639



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Mutations in ACCURUN 601 Series 1000

Mutation	Vial 1	Vial 2
CYP2C9*2W	HET	WT
CYP2C9*3W	WT	HET
VKR3673G	WT	HET
VKR5808T	WT	HET
VKR6484C	WT	HET
VKR6853G	WT	HET
VKR7566C	WT	HET
VKR9041G	MUT	HET

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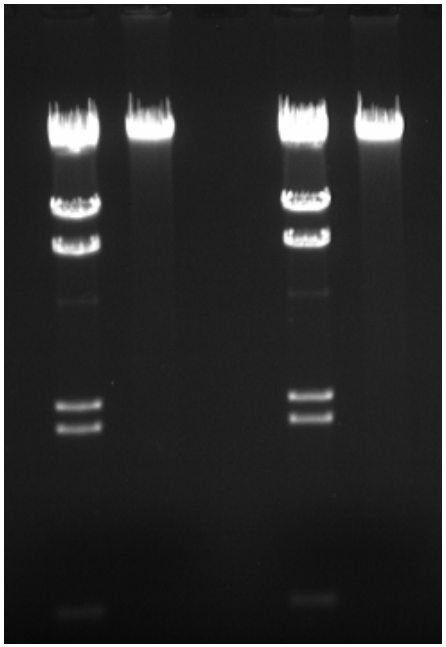
ACCURUN 601 Series 1000: Quality of DNA

Genomic DNA integrity assessed by EtBr stained agarose gel.

Lane 1&3: λ DNA Marker
Lane 2: PBMC lot 1001 gDNA
Lane 4: PBMC lot 1011 gDNA



Sample ID	260/280	DNA Conc. (ng/uL)
CYP2C9*2-Pilot1	1.88	30.16
CYP2C9*2-Pilot2	1.89	32.95
CYP2C9*2-Pilot3	1.88	31.50
CYP2C9*3 and VKORC1 -1639-Pilot1	1.90	35.01
CYP2C9*3 and VKORC1 -1639-Pilot2	1.90	33.06
CYP2C9*3 and VKORC1 -1639-Pilot3	1.92	35.10

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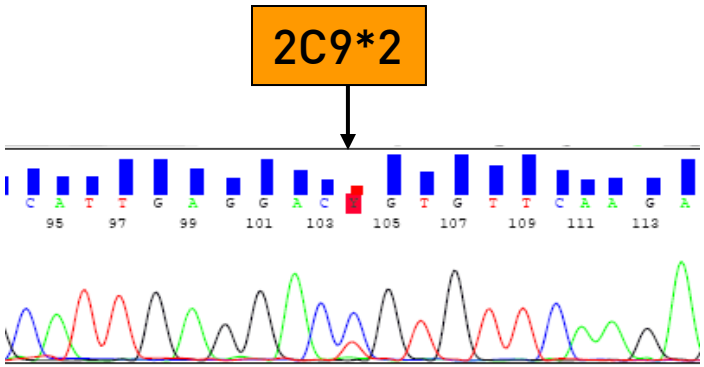
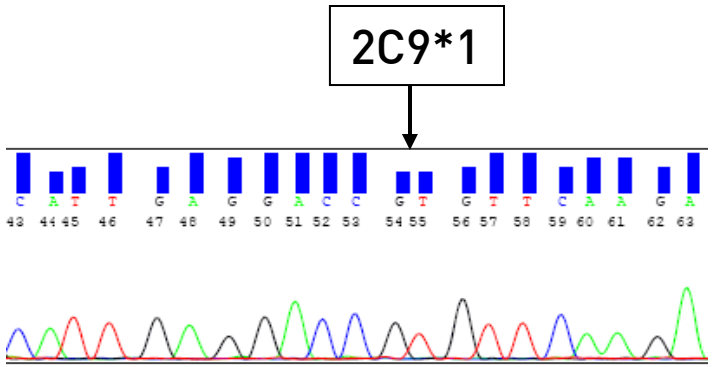
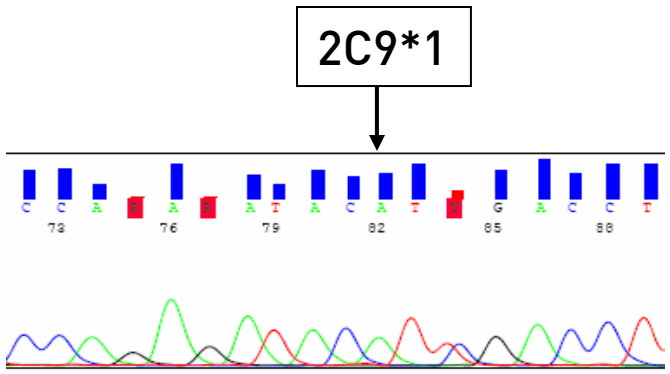
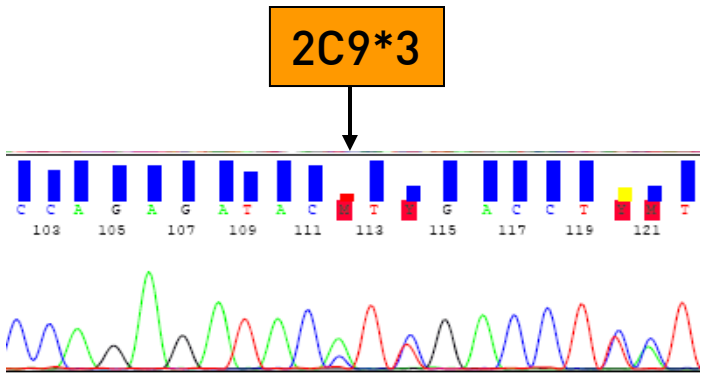
Sequence confirmation of mutations

	Vial 1	Vial 2
CYP2C9*2		
CYP2C9*3		
	PBMC1001	PBMC 1011

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Genotyping by Autogenomics Infinity Warfarin XP Assay

Vial 1

Analyte	Mean	Ratio	Analysis
2C9*2W	3161.88	1.03	Heterozygote
2C9*2M	3072.46	0	
2C9*3W	2062.09	25.17	Homozygote Wild
2C9*3M	81.93	0	
2C9*4W	1370.97	1370.97	Homozygote Wild
2C9*4M	1	0	
2C9*5W	921.62	921.62	Homozygote Wild
2C9*5M	1	0	
2C9*6W	1895.35	1895.35	Homozygote Wild
2C9*6M	1	0	
2C9*11W	1898.68	1898.68	Homozygote Wild
2C9*11M	1	0	
VKR3673G	527.76	8.61	Homozygote Wild
VKR3673A	61.27	0	
VKR5808T	932.05	932.05	Homozygote Wild
VKR5808G	1	0	
VKR6009C	3198.63	119.4	Homozygote Wild
VKR6009T	26.79	0	
VKR6484C	713.45	713.45	Homozygote Wild
VKR6484T	1	0	
VKR6853G	3071.9	6.12	Homozygote Wild
VKR6853C	501.96	0	
VKR7566C	2439.83	4.76	Homozygote Wild
VKR7566T	512.3	0	
VKR8773C	3119.88	3119.88	Homozygote Wild
VKR8773T	1	0	
VKR9041G	256.39	15.19	Homozygote Mutant
VKR9041A	3895.81	0	
POS	6000	68.78	-
NEG	87.23	0	-

Vial 2

Analyte	Mean	Ratio	Analysis
2C9*2W	5311.05	5311.05	Homozygote Wild
2C9*2M	1	0	
2C9*3W	1547.9	1.11	Heterozygote
2C9*3M	1715.05	0	
2C9*4W	2107.9	2107.9	Homozygote Wild
2C9*4M	1	0	
2C9*5W	1369.43	1369.43	Homozygote Wild
2C9*5M	1	0	
2C9*6W	2758.81	107.92	Homozygote Wild
2C9*6M	25.56	0	
2C9*11W	2682.15	2682.15	Homozygote Wild
2C9*11M	1	0	
VKR3673G	309.07	1.12	Heterozygote
VKR3673A	346.44	0	
VKR5808T	600.77	1.61	Heterozygote
VKR5808G	373.15	0	
VKR6009C	3579.35	127.56	Homozygote Wild
VKR6009T	28.06	0	
VKR6484C	588.66	1.35	Heterozygote
VKR6484T	793.11	0	
VKR6853G	2109.55	1.74	Heterozygote
VKR6853C	3670.54	0	
VKR7566C	1845.47	1.14	Heterozygote
VKR7566T	2097.49	0	
VKR8773C	3689.76	3689.76	Homozygote Wild
VKR8773T	1	0	
VKR9041G	2496.9	1.22	Heterozygote
VKR9041A	3044.08	0	
POS	6000	81.75	-
NEG	73.39	0	-

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Genotyping by TWT Invader assay

Sample ID	Genotype		
	CYP2C9*2	CYP2C9*3	VKORC1 (-1639)
Subtractive Component (SC) [Raw Signal]	N/A	N/A	N/A
DNA Target (HET)	**C/T**	**A/C**	**G/A**
No DNA Target (NDT) [Net Signal]	**N/A**	**N/A**	**N/A**
CYP2C9*2	**C/T**	**A/A**	**G/G**
CYP2C9*3 and VKORC1 -1639	**C/C**	**A/C**	**G/A**

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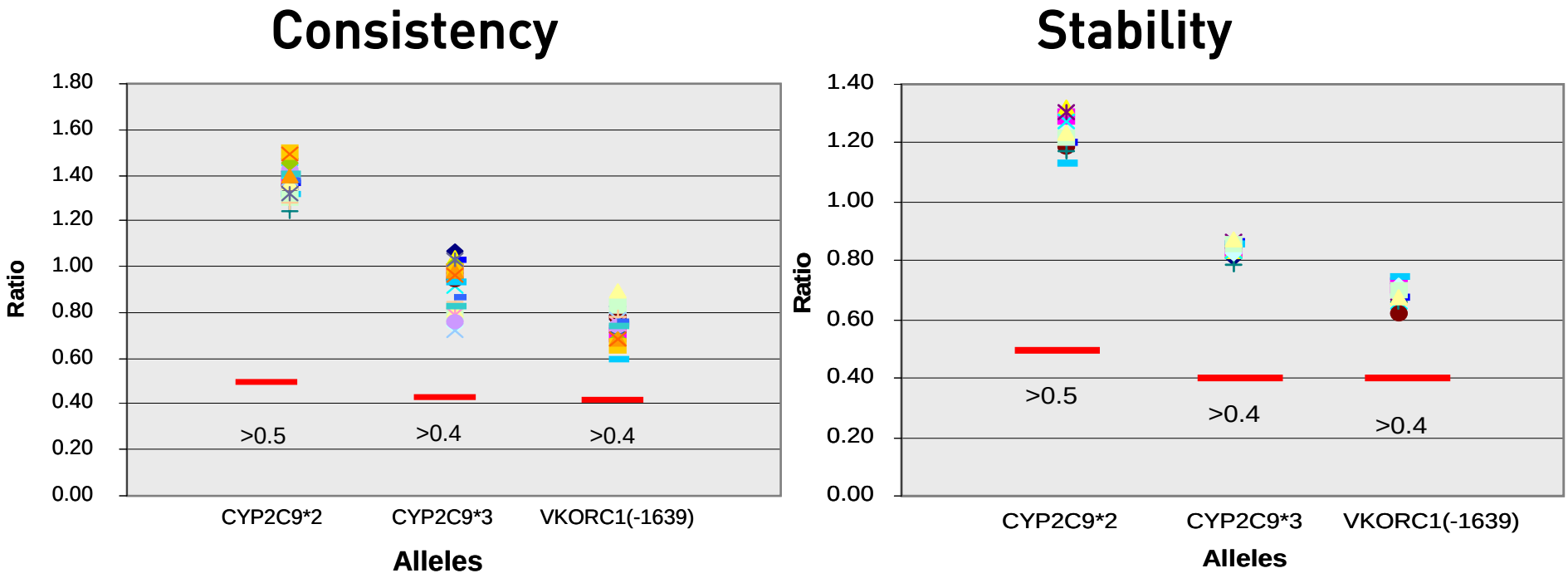
Stability of A610 Warfarin Controls



- Consistent performance over multiple runs
- Correct ratio and mutation identification
- Excellent stability

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