

Dual-labeled Hydrolysis Probe Based Real-Time PCR Genotyping for Rapid Detection of Mutations of Interest from SARS-CoV-2 Variants

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INTRODUCTION

Real-time PCR is a rapid method of mutation detection for tracking the emergences of SARS-CoV-2 variants which is critical for public health decision making. Here, we present a series of PerkinElmer's PKamp™ VariantDetect™ assays/kits using mutation-specific dual-labeled hydrolysis probe method to detect mutations which have been identified in certain SARS-CoV-2 variants with high sensitivity and specificity in around 2 hours. Thirteen combinations (combos) with total fourteen mutations of interest (MOIs) including 69-70 Del, V213G, R408S, K417N/T, L452R/Q, E484K/A/Q, Q493R, N501Y, and P681H/R from Spike (*S*) gene were developed. Each assay provides up to three MOIs together with internal control nucleocapsid (*N*) and *ORF1ab* genes in a single reaction. All assays were evaluated with quantified synthetic SARS-CoV-2 RNAs (Twist Bioscience) including variant of concern (VOC) strains. Subsets of assays were also evaluated with SARS-CoV-2 confirmed positive samples. One universal thermocycling program with FAM/VIC/ROX/Cy5 detectors is adapted to all assays, and result interpretation is qualitative decision by Ct cutoff at 42 cycles.

APPLICATION WORKFLOW

First-tier SARS-CoV-2 screening



Second-tier SARS-CoV-2 mutation detection

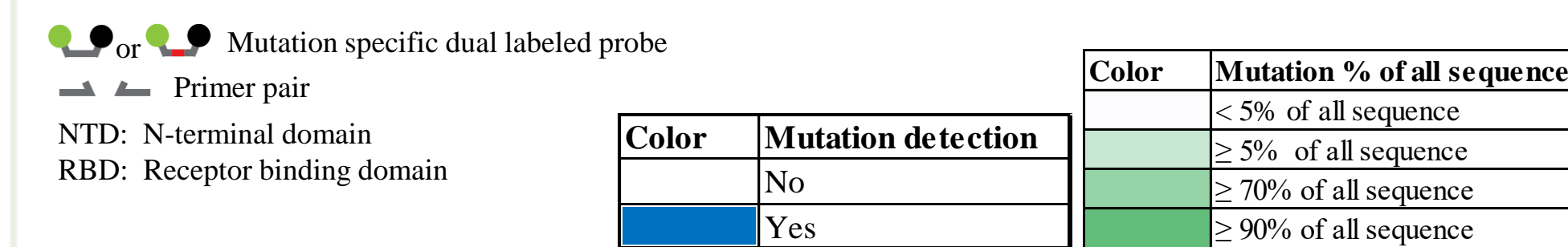
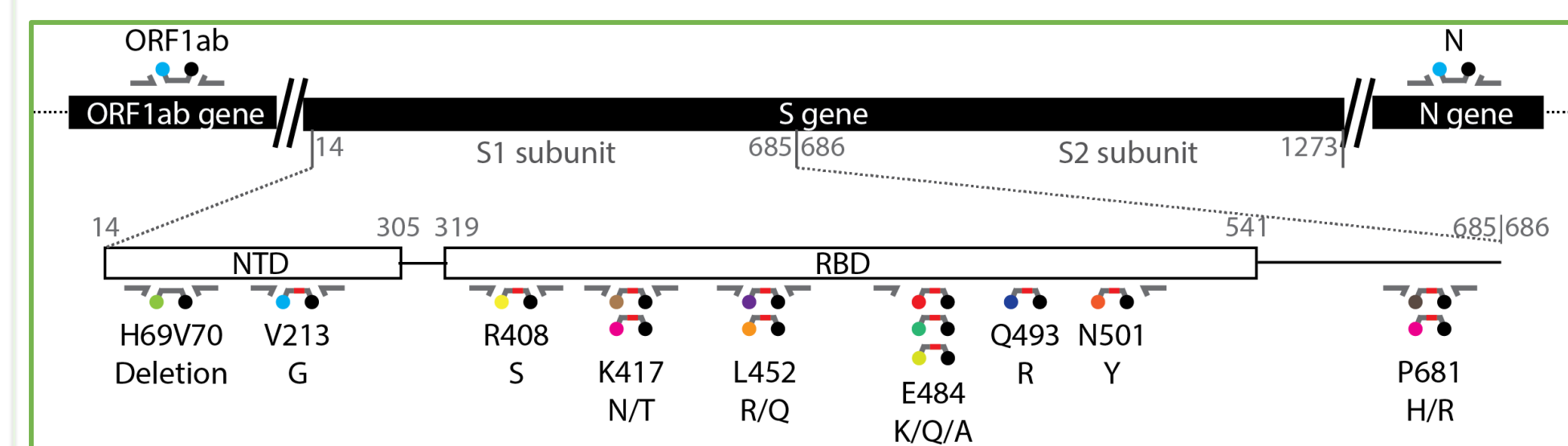
SARS-CoV-2 confirmed positive sample (leftover extracted RNA / new extraction)
PKamp™ VariantDetect™ assays



Compatible qPCR instruments

- Applied Biosystems™ 7500 Fast systems
- CFX Touch™ systems
- EONIS™ Q systems
- QuantStudio™ systems
- qTower3/3G systems

ASSAY DESIGN



Classification	WHO Name	S gene mutation													
		69-70 Del	V213 G	R408 S	K417 N	K417 T	L452 R	L452 Q	E484 K	E484 A	E484 Q	Q493 R	N501 Y	P681 H	P681 R
VOC	Alpha (B.1.1.7)														
	Beta (B.1.351)														
	Gamma (P.1)														
	Delta (B.1.617.2)														
	Delta (AY.1.2)														
VOI	Omicron (BA.1/3)														
	Omicron (BA.2)														
VUM	Lambda (C.37)														
	Mu (B.1.621)														
	Kappa (B.1.617.1)														
	Combination 3														
	Combination 4														
	Combination 5														
	Combination 6														
	Combination 7														
	Combination 8														
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Combination 13															
Combination 14															
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Combination 16															

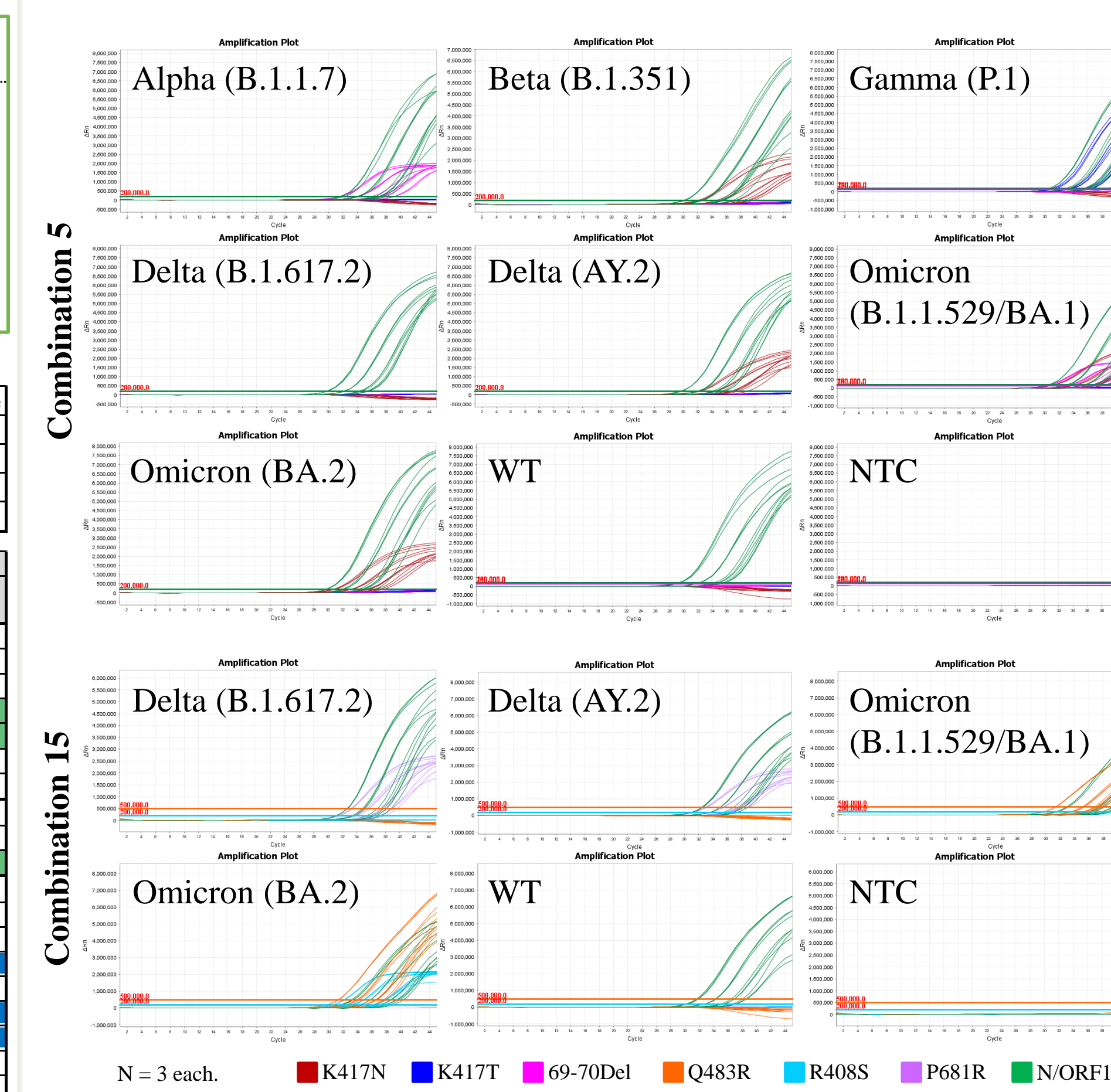
RESULTS

Combination sensitivity evaluation with different synthetic RNAs of SARS-CoV-2 variants

Combo	Sensitivity	WHO classification	Alpha	Beta	Gamma	Delta	Delta	Omicron	Omicron	Kappa	L452Q (synthetic plasmid)	WT
		Pango lineage	B.1.1.7	B.1.351	P.1	B.1.617.2	AY.2	BA.1.1.529/BA.1	BA.2	B.1.617.1	Wuhan-Hu-1	
3	40 cp/rxn	N501Y	Det	Det	Det	ND	ND	Det	Det		ND	
		E484K	ND	Det	Det	ND	ND	ND	ND		ND	
		69-70 Del	ND	ND	ND	ND	ND	Det	Det		ND	
4	40 cp/rxn	N501Y		Det	ND	ND	ND	Det	Det	ND	ND	
		E484K	Det	Det	ND	ND	ND	ND	ND	ND	ND	
		E484Q	ND	ND	ND	ND	ND	ND	Det	Det	ND	
5	40 cp/rxn	K417N	ND	Det	ND	ND	Det	Det	Det		ND	
		K417T	ND	ND	Det	ND	ND	ND	ND	ND	ND	
		69-70 Del	Det	ND	ND	ND	ND	Det	Det		ND	
6	200 cp/rxn	L452R	ND	ND	ND	Det	Det	ND	ND	Det	ND	
		P681H	Det	ND	ND	ND	ND	ND	Det	Det	ND	
		P681R	ND	ND	ND	Det	Det	ND	Det	Det	ND	
7	1000 cp/rxn	L452R	ND					Det	Det		ND	
		E484K	Det							ND	ND	
		E484Q	ND							Det	ND	
8	40 cp/rxn	K417N	Det	ND	ND	Det	Det	Det			ND	
		K417T	ND	Det	ND	ND	ND	ND			ND	
		P681R	ND	ND	ND	Det	Det	ND			ND	
9	200 cp/rxn	L452Q	ND							ND	Det	ND
		P681H	Det							ND	N/A	ND
		P681R	ND							Det	N/A	ND
10	200 cp/rxn	L452Q		ND						ND	Det	ND
		E484K	Det							ND	N/A	ND
		E484Q	ND							Det	N/A	ND
12	400 cp/rxn	L452R	ND	ND			Det	ND			ND	ND
		E484K	ND	Det			ND	ND				ND
		69-70 Del	Det	ND			ND	Det				ND
13	500 cp/rxn	L452R	ND				Det	ND			ND	ND
		E484K	Det				ND	ND				ND
		Q493R	ND				ND	Det				ND
14	20 cp/rxn	E484A	ND	ND			ND	Det		ND	ND	ND
		E484K	ND	Det	Det	ND	ND	ND	ND	ND		ND
		E484Q	ND	ND	ND	ND	ND	ND	Det			ND
15	40 cp/rxn	Q493R	ND	ND			ND	Det			ND	ND
		R408S	ND	ND			ND	ND	Det			ND
		P681R	ND	ND			ND	ND	Det			ND
16	40 cp/rxn	Q493R					Det	Det			ND	ND
		R408S					ND	Det				ND
		V213G					ND	Det				ND

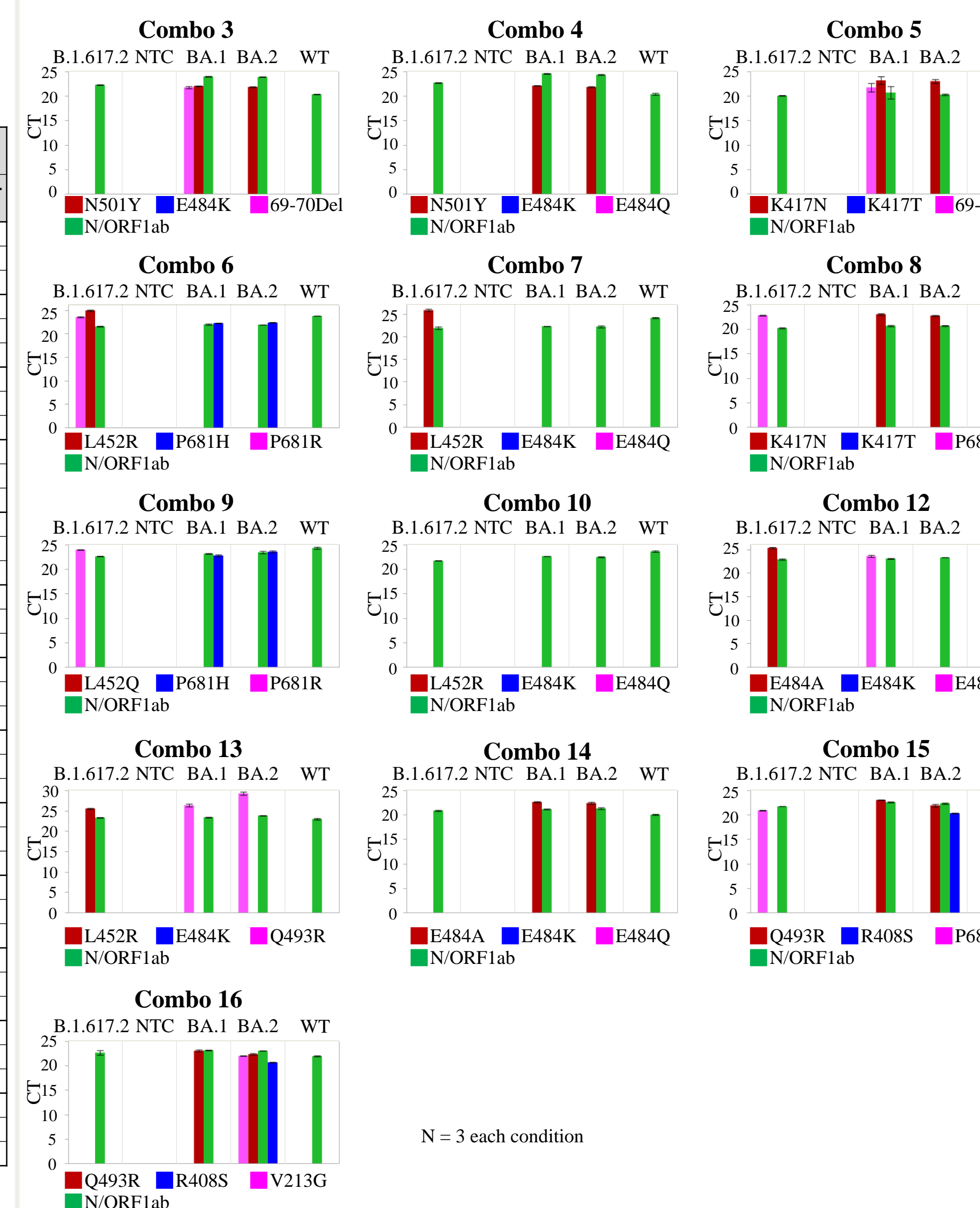
Det: detected ND: not detected Blank: not tested N/A not applicable

VOC-like typing with two combinations (example)



MOI specificity study with Delta (B.1.617.2), Omicron (BA.1, BA.2) and WT strains at 5E5 cp/rxn

-- MOIs were detected in Omicron or Delta strains as expected while remain absence in WT strain.



High concordance between PCR and NGS results using SARS-CoV-2 confirmed positive sample

Target	# of Samples	Test Results				Agreement Statistics		
		Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Agreement %	95% CI LCL, UCL
K417N	23	23	0	0	0	PPA	100%	85.7%, 100%
						NPA	N/A	N/A
K417T	23	0	0	23	0	PPA	N/A	N/A
						NPA	100%	85.7%, 100%
69-70 Del	23	11	0	12	0	PPA	100%	74.1%, 100%
						NPA	100%	75.8%, 100%

Target	# of Samples	Test Results				Agreement Statistics		
		Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Agreement %	95% CI LCL, UCL
L452R	35	0	0	35	0	PPA	N/A	N/A
						NPA	100%	90.1%, 100%
P681H	35	35	0	0	0	PPA	100%	90.4%, 100%
						NPA	N/A	N/A
P681R	35	0	0	35	0	PPA	N/A	N/A
						NPA	100%	90.1%, 100%

Target	# of Samples	Test Results				Agreement Statistics		
		Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Agreement %	95% CI LCL, UCL
E484K	104	68	1	35	0	PPA	98.6%	92.4%, 99.7%
						NPA	100%	90.1%, 100%
L452R	104	36	0	68	0	PPA	100%	90.4%, 100%
						NPA	100%	94.7%, 100%
E484Q	104	0	0	104	0	PPA	N/A	N/A
						NPA	100%	96.4%, 100%

Target	# of Samples	Test Results				Agreement Statistics		
		Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Agreement %	95% CI LCL, UCL
K417N	104	17	0	87	0	PPA	100%	81.6%, 100%
						NPA	100%	95.8%, 100%
K417T	104	52	0	52	0	PPA	100%	93.1%, 100%
						NPA	100%	93.1%, 100%
P681R	104	36	0	68	0	PPA	100%	90.4%, 100%
						NPA	100%	94.7%, 100%

Target	# of Samples	Test Results				Agreement Statistics		
		Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Agreement %	95% CI LCL, UCL
Q493R	44	41	2	1	0	PPA	95.3%	84.5%, 98.7%
						NPA	100%	20.7%, 100%
R408S	44	24	0	20	0	PPA	100%	86.2%, 100%
						NPA	100%	83.9%, 100%
P681R	44	1	0	43	0	PPA	100%	20.7%, 100%
						NPA	100%	91.8%, 100%

* Combo 8preO2 and 15preO2 were early formulations that now have been improved and named as combo 8 and 15. N/A: not applicable.

SUMMARY

- Assay-dependent sensitivity ranges from 20 to 1000 copies per PCR reaction (cp/rxn).
- High MOI specificity even at 500,000 cp/rxn among Omicron, Delta, and wild-type (WT) strains.
- Result interpretation is qualitative decision by Ct cutoff at 42 cycles.
- High concordance rate between VariantDetect™ and NGS results.
- Cost-efficient fast solution for monitoring circulating SARS-CoV-2 variants with custom choices of 2-3 combinations (e.g. 6-9 MOI targets).

ACKNOWLEDGEMENT

We thank CDPH Valencia Branch Laboratory for providing SARS-CoV-2 confirmed positive pre-extracted samples and NGS data for assays evaluation.