The laboratory in collaboration with CDPH and the operator, PerkinElmer, had finalized the analysis and reporting SOP wherein up to 37 is reported positive, 37-42 as presumptive positive and >42 as negative. The laboratory performs COVID virus sequencing on de-identified positive samples previously tested by rtPCR, for CDPH for tracking and surveillance purposes to understand the prevalence of Sars-CoV2-2019 variants, such as the delta variant in the state of California. This activity is not validated for diagnostic use and patient reports are not generated. This activity is not regulated by CMS.

Sequencing of High Ct (>37 - ≤42) Samples

We have performed retrospective data analysis of the positives samples identified in the period of April 14, 2021 –August 17, 2021 (72, 924 samples), within a Ct range of > 37 to ≤ 42 . Of all samples with *N* or *ORF1ab* detected with Ct > 37 - < 42, the sequencing success rate ranged from 33.5% - 63.3%, depending on whether detection of one or both genes fell within this range, indicating that a significant percentage of samples with one or both targets in the Ct > 37 - < 42 range had virus that was sequenced in agreement with previously published data [1] (Table I) Please note this demonstrates the analytical sensitivity and specificity of assay and may not correlate with the clinical sensitivity and specificity which is still not clearly understood for the SARS-CoV-2 disease [2].

		N gene Ct value			
		≤ 37	>37 - ≤ 42	Not detected	**Total**
ORF1ab Gene Ct value	≤ 37	53,793 (93.4%)	1,387 (63.3%)	1,135 (42.7%)	56,315 (90.3%)
	>37 - ≤ 42	1,287 (61.0%)	416 (53.8%)	452 (51.8%)	2,155 (51.8%)
	Not detected	1,502 (40.2%)	884 (33.5%)	0*	2,386 (37.4%)
	Total	56,582 (89.3%)	2,687 (47.9%)	1,587 (40.3%)	60,856

Table 1. *Samples with no detected virus were not included in this study

Sequencing of Sample At, Above, and Below Ct 37

To match our clinical interpretation protocol, we applied Ct cutoffs to both the *N* and *ORF1ab* genes, in the sequencing analysis. The probability of detecting virus was approximately 95% at Ct ≤ 35, 70% at Ct > 35 - ≤ 36 and 50% at Ct >36. (Table II, yellow highlights) These data indicate an appropriate cutoff of either >36 or >37 in differentiating detected versus presumptive positive results based on the probability of detecting virus in an orthogonal assay.

		N Gene Ct value									
		≤ 35	**>35 - ≤36**	**>36 - ≤37**	**>37- ≤38**	**>38- ≤39**	**>39- ≤40**	**>40- ≤41**	**>41- ≤42**	**Not detected**	**Total**
ORF1ab Gene Ct value	**≤ 35**	**49,340 (95.8%)**	805 (85.5%)	414 (85.5%)	185 (84.9%)	38 (80.9%)	14 (87.9%)	*7 (100%)*	*2 (66.7%)*	272 (66.8%)	**51,077 (95.3%)**
	>35- ≤36	844 (78.0%)	528 (73.3%)	410 (71.7%)	299 (66.7%)	75 (66.8%)	19 (48.7%)	9 (90.0%)	*2 (50.0%)*	184 (51.4%)	**2,370 (70.8%)**
	>36- ≤37	460 (72.8%)	487 (69.0%)	505 (55.7%)	549 (58.3%)	137 (53.1%)	34 (57.6%)	12 (57.1%)	*5 (83.3%)*	679 (35.9%)	**2,868 (52.9%)**
	>37- ≤38	268 (70.7%)	351 (65.5%)	408 (53.4%)	233 (52.6%)	54 (61.4%)	16 (55.2%)	*1 (50.0%)*	*1 (100%)*	329 (35.2%)	**1,661 (52.3%)**
	>38- ≤39	46 (75.4%)	61 (59.2%)	97 (57.7%)	57 (48.3%)	29 (58.0%)	8 (53.3%)	*0*	*0*	105 (35.1%)	**403 (49.5%)**
	>39- ≤40	10 (47.6%)	12 (60.0%)	16 (57.1%)	*4 (44.4%)*	*7 (87.5%)*	*0*	*0*	*0*	12 (32.4%)	**63 (57.1%)**
	>40- ≤41	6 (60.0%)	*1 (100%)*	*2 (66.7%)*	*2 (50.0%)*	*2 (100%)*	*0*	*0*	*0*	*3 (60.0%)*	**16 (66.7%)**
	>41- ≤42	*3 (54.8%)*	*4 (46.0%)*	*2 (35.8%)*	*0*	*0*	*0*	*0 (0%)*	*0*	*3 (75.0%)*	**12 (37.4%)**
	Not detected	217 (54.8%)	399 (46.0%)	886 (35.8%)	637 (32.9%)	167 (34.5%)	59 (34.9%)	14 (36.8%)	7 (46.7%)	0*	**2,386 (37.4%)**
	Total	**51,194 (94.7%)**	**2648 (67.9%)**	**2,740 (50.7%)**	**1,966 (47.7%)**	**509 (48.5%)**	**152 (46.2%)**	**43 (53.8%)**	**17 (58.6)**	**1,587 (40.3%)**	**60,856**

Table 2. Total number of samples successfully sequenced by Ct value. Samples shaded in green were reported as "detected" using a Ct cut-off of ≤ 37. Samples shaded yellow would have been reported "detected" using a Ct cut-off of ≤ 38. Samples in yellow and white were reported as "presumptive positive" using a Ct range of >37 - ≤42.

Agreement between RT-PCR and NGS assays: false-positive rates, false-negative rates and positive predictive agreement (PPA): The NGS assay used for identifying Sars-CoV2-2019 at VBL is intended for genotyping the virus and identifying strains for public health uses. Since the RT-PCR assay is more sensitive than the NGS assay for detection of Sars-CoV2-2019, we are not able to get a true estimate of sensitivity and specificity (including false-negative and false positive rates). In addition, since we do not sequence samples that are negative by RT-PCR, we are unable to estimate a false-negative rate. However, with reference to table 2, the PPA between RT-PCR and NGS when both *N* and *ORF1ab* gene targets are detected in the Ct range >37- ≤ 42, by RT-PCR is 53.8%, the PPA when only *N* is detected in the range >37- ≤ 42 is 33.5%, and the PPA when only the *ORF1ab* gene is detected in the range >37- ≤ 42 is 51.8%. It should be noted that the PPA when both genes are detected <37 Ct is 93.5%. This data strongly indicates that a cutoff of 37 is an appropriate threshold in determining a "detected" versus a "presumptive positive" result.

Clinical and epidemiological implications

This data supports the decision to interpret tests with Ct values for one or both gene targets >37- ≤ 42 as "presumptive positive", with a different clinical recommendation in the test report, because this result indicates a lower likelihood of infectivity than a "detected" result, supported by in-vitro data [3,4].

Furthermore, it is not known whether low-viral load SARS-COV-2 patients are infectious to others [5]. Furthermore, the NGS data supports the test report language in the interpretation which states that “Presumptive positive" means that the test was not able to confirm with adequate certainty that the patient’s sample collected on <date> had SARS-CoV-2 RNA present. Both the laboratory and CDPH agree that interpreting results with Ct values >37<42 as “not detected”, particularly during periods where the prevalence of infection is high, would miss a significant fraction of true positive cases and pose a patient and public health risk.

1. Zehnbauer et al., J Mol Diag 23: 2021
2. Sahahjpal et al., medRxiv: https://doi.org/10.1101/2021.03.24.21254271
3. Jefferson et.al. Clin Infect. Dis. 2020, online ahead of print
2. Basile et. al Clin Infect. Dis. 2020, online ahead of print
3. Levine-Tiefenbrun. *et al. Nat* Med 2021, online ahead of print