CO-DIAGNOSTICS INC.

CoDx - REGULATORY BULLETIN

Salt Lake City, June 23rd, 2021

Re: Response to request for information on the impact of new SARS-CoV-2 variants of epidemiological interest and variants of concern on the performance of SARS-CoV-2 molecular IVD test kits designed, developed, and manufactured by Co-Diagnostics, Inc. (Salt Lake City, USA)

The World Health Organization, as well as other agencies around the world are currently tracking the SARS-CoV-2 variants to proactively anticipate the possible impact of the mutations in the public health.

According to the WHO, the SARS-CoV-2 variant B.1.617.2 has emerged in India in Oct-2020. The variant received the designation of Variant of Interest (V.O.I.) by the WHO in 4-Apr-2021 and Variant of Concern (V.O.C.) in 11-May-2021 (WHO, 2021).

Since last reviews in Jun-2021, the US CDC has also classified the B.1.167.2 as a Variant of Concern due to increased transmissibility, potential reduction in neutralization by some EUA monoclonal antibody treatments, and potential reduction in neutralization by post-vaccination sera (CDC, 2021).

Table 1 shows a list of the Variants of Concern listed by WHO and CDC.

Table 1 SARS-CoV-2 Genomic Variants Currently Classified as Variants of Concern

Variant of Concern Status by Agency	Pango Lineage	Nextstrain Clade	Designation	First detected, Date	WHO nomenclature	BEI Reference Material Number
VOC [WHO, MHRA (UK), CDC (US)]	B.1.1.7	201/S:501Y.V1	WHO: 18-Dec-20 MHRA: VOC-20DEC-01 CDC: Date not available	UK, Sep-2020	Alpha	NR-54000
VOC [WHO, MHRA (UK), CDC (US)]	B.1.351	20H/S:501YV2	WHO: 18-Dec-20 UK: VOC-20DEC-02 CDC: Date not available	South Africa, May-2020	Beta	NR-55282
VOC [CDC (US)]	B.1.427	20C/S:452R	CDC: Date not available	Unites States (California)	Epsilon	Not yet available
VOC [CDC (US)]	B.1.429	20C/S:452R	CDC: Date not available	Unites States (California)	Epsilon	Not yet available
VOC [WHO, MHRA (UK), CDC (US)]	P.1	20J/S:501Y.V3	WHO: 11-Jan-2021 UK: VOC-21JAN-02	Japan/Brazil, Nov-2020	Gamma	NR-54982
VOC [WHO, MHRA (UK), CDC (US)]	B.1.167.2	21A/S:478K	WHO: VOI-4-Apr-21/ VOC-11-May-21 UK: VOC-21APR-02 CDC: 15-Jun-2021	India, Oct-2020	Delta	NR-55611

Note: VOC – Variant of Concern, VOI – Variant of Interest, WHO – World Health Organization, MHRA – Medicines and health Products Regulatory Agency (UK), CDC – Centers for Disease Control and Prevention

Even though until Jun/2021 no variant had a mutation occurring on a region targeted by the CoPrimers used to target Gene RdRp and Gene E, the variant B.1.167.2 (Delta) has shown one point mutation in the region targeted by the RdRp Fwd (Forward) CoPrimer. This CoPrimer is used in all COVID-19 tests designed and manufactured by Co-Diagnostics, Inc.

Table 2 COVID-19 Tests Designed and Manufactured by Co-Diagnostics, Inc.

Test Name	Product Code	Gene RdRp (CoPrimer RdRp Fwd / CoPrimer RdRp Rev)	Gene E (CoPrimer E Fwd / CoPrimer E Rev)
Logix Smart Coronavirus Disease 2019 (COVID-19)	COVID-K-001	Yes	No
Logix Smart SARS-CoV-2 (genes RdRp/E)	COVID-K-002	Yes	Yes
Logix Smart ABC (Influenza A/B, SARS-CoV-2)	ABC-K-001	Yes	Yes
	ABC-K-002	Yes	Yes
Logix Smart SARS-CoV-2 DS	COVDS-K-003	Yes	Yes
	COVDS-K-004	Yes	Yes

Previous in silico analysis performed by Co-Diagnostics since initial design in Feb-2020 of CoPrimers targeting Gene RdRp and Gene E observed that none of the mutations related to the lineages B.1.1.7 (Alpha) (Public Health England, 2020) (Rambaut, et al., 2020), B.1.351 (Beta), B.1.427 (Epsilon), B.1.429 (Epsilon), and P.1 (Gamma) had any predicted impact in the performance of any of the COVID-19 tests listed in **Table 2** designed and manufactured by Co-Diagnostics.

The most recent in silico analysis performed in Jun/2021 found that variants of concern lineages B.1.1.7 (Alpha) (GISAID, 2020), B.1.351 (Beta), B.1.427 (Epsilon), B.1.429 (Epsilon), and P.1 (Gamma) still does not impact the performance of any of the listed tests designed and manufactured by Co-Diagnostics. The most relevant rationale for this conclusion is that none of the mutations on the lineages in the Variants of Concern occur in a region targeted by the sets of RdRp or Gene E CoPrimers.

As mentioned in previous communications, Co-Diagnostics has conducted monthly BLASTn queries of a subsampling from the Nextstrain database to monitor the homology of the RdRp and Gene E sets of CoPrimers.

The results obtained from the monthly investigation of the homology status of Co-Diagnostics COVID-19 tests is provided in **Table 3** and **Table 4**.

Table 3 In Silico Analysis Performed Over Time for RdRp Gene Target

Date of CoDx's Analysis for RdRp Marker	SARS-CoV-2 (number of sequences in analyzed subsample)	Sequences in the pool with 100% homology	Single nucleotide mutation events: Sequences with 1 mismatch on CoDx target (98% homology)	Double nucleotide mutation events: Sequences with 2+ mismatches on CoDx target (95% homology)	Multiple nucleotide mutation events: Sequences with 3+ mismatches on CoDx target <95% homology)
27-Jan-20	14	14 (100%)	0 (0%)	0 (0%)	0 (0%)
4-Feb-20	53	53 (100%)	0 (0%)	0 (0%)	0 (0%)

17-Mar-20	571	570 (99.8%)	1 (0.2%)	0 (0%)	0 (0%)
6-Apr-20	3639	3634 (99.86%)	5 (0.14%)	0 (0%)	0 (0%)
4-May-20	4468	4459 (99.80%)	9 (0.2%)	0 (0%)	0 (0%)
3-Jun-20	4558	4537 (99.54%)	21 (0.46%)	0 (0%)	0 (0%)
6-Jul-20	11361	11328 (99.71%)	33 (0.29%)	0 (0%)	0 (0%)
10-Aug-20	22054	22012 (99.81%)	42 (0.19%)	0 (0%)	0 (0%)
9-Sep-20	4417	4394 (99.48%)	23 (0.52%)	0 (0%)	0 (0%)
12-Oct-20	5139	5114 (99.51%)	25 (0.49%)	0 (0%)	0 (0%)
5-Nov-20	3494	3463 (99.11%)	31 (0.89%)	0 (0%)	0 (0%)
4-Dec-20	3407	3371 (98.94%)	36 (1.06%)	0 (0%)	0 (0%)
4-Jan-21	3540	3505 (99.01%)	35 (0.99%)	0 (0%)	0 (0%)
4-Feb-21	3962	3909 (98.66%)	53 (1.34%)	0 (0%)	0 (0%)
1-Mar-21	4024	3960 (98.41%)	64 (1.59%)	0 (0%)	0 (0%)
7-Apr-21	4025	3962 (98.43%)	63 (1.57%)	0 (0%)	0 (0%)
6-May-21	3923	3847 (98.06%)	76 (1.94%)	0 (0%)	0 (0%)
1-Jun-21	3883	3000 (97.86%)	83 (2.14%)	0 (0%)	0 (0%)

Table 4 In Silico Analysis Performed Over Time for Gene E Target

Date of CoDx's Analysis for Gene E Marker	**SARS-CoV-2 (number of sequences in analyzed subsample)**	**Sequences in the pool with 100% homology**	**Single nucleotide mutation events: Sequences with 1 mismatch on CoDx target (98% homology)**	**Double nucleotide mutation events: Sequences with 2+ mismatches on CoDx target (95% homology)**	**Multiple nucleotide mutation events: Sequences with 3+ mismatches on CoDx target <95% homology)**
27-Jan-20	14	14 (100%)	0 (0%)	0 (0%)	0 (0%)
4-Feb-20	53	53 (100%)	0 (0%)	0 (0%)	0 (0%)
9-Sep-20	4417	4400 (99.62%)	14 (0.32%)	2 (0.05%)	1 (0.02%)
12-Oct-20	5139	5126 (99.96%)	11 (0.21%)	0 (0%)	2 (0.04%)
5-Nov-20	3494	3478 (99.54%)	16 (0.46%)	0 (0%)	0 (0%)
4-Dec-20	3407	3397 (99.71%)	10 (0.29%)	0 (0%)	0 (0%)
4-Jan-21	3540	3530 (99.72%)	10 (0.28%)	0 (0%)	0 (0%)

4-Feb-21	3962	3944 (99.55%)	18 (0.45%)	0 (0%)	0 (0%)
1-Mar-21	4024	3999 (99.38%)	25 (0.62%)	0 (0%)	0 (0%)
7-Apr-21	4025	3991 (99.16%)	34 (0.84%)	0 (0%)	0 (0%)
6-May-21	3923	3870 (98.65%)	53 (1.35%)	0 (0%)	0 (0%)
1-Jun-21	3883	3820 (98.38%)	63 (1.62%)	0 (0%)	0 (0%)

From the investigation performed in Jun-2021, it has been found that the lineage B.1.167.2 (Delta) (PANGO lineages, 2021) contains a single point mutation within the binding site of one of the two RdRp CoPrimers.

As in any other primer used in qPCR techniques, the single mismatch caused by the mutation is not expected to prevent the primer to bind to the SARS-CoV-2 genome. In case of a potential 2 mismatches, sensitivity is expected to be retained with marked Ct delay. And with 3+ mismatches, a CoPrimer is expected to have serious impairment (U.S. FDA, 2021) (FDA, 2021).

Another observation highlights that assays for qualitative detection of SARS-CoV-2 targeting more than 1 marker minimizes the impact of a mutation leading to mismatches of the CoPrimer to its targeted site. As demonstrated by **Table 2**, only COVID-K-001 has a single marker, all the other products, COVID-K-002, ABC-K-001, and COVDS-K-003 / COVDS-K-004 target at least 2 markers.

Although the Co-Diagnostics risk analysis did not indicate that loss of sensitivity was likely with any of the products listed in **Table 2**. The impact of the single point mutation was analyzed for Tm impact on COVID-K-001. The impact on Tm was determined to be modest, and the affected portion of the CoPrimer retained a predicted annealing temperature above that used in the validated thermocycling protocols. Therefore, the predicted Tm impact analysis corroborated with the previous risk determination that sensitivity toward Delta was unlikely to be affected.

As a final analysis, synthetic RNAs were obtained with the Wild Type sequence and with the single point mutation present in the Delta lineage. The wet testing analysis confirmed that both RNAs exhibited estimated and confirmed Limits of Detection (LoD) within the 3-fold limit set as the acceptance criterion, further corroborating that all Co-Diagnostics SARS-CoV-2 assays retain full sensitivity for lineages classified as Variants of Concern (VOC) at this time, including Alpha, Beta, Gamma, Delta, and Epsilon, and other emerging variant sequences analyzed in the Co-Diagnostics' monthly subsampling from the Nextstrain database.

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