Co-Dx REGULATORY BULLETIN

Salt Lake City, August 10th, 2023

Re: Response to request for information on the impact of new SARS-CoV-2 variants of 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 and other agencies around the world are currently tracking the predominant circulating and emerging SARS-CoV-2 variants to proactively anticipate their possible impact on public health.

Table 1 shows a current list of the Variants of Concern (VOC) or Interest (VOI) identified by WHO, MHRA, ECDC, or CDC.

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

Variant of Concern / Interest Status by Agency	WHO Nomenclature	Pango Lineage	Nextstrain Clade	Designation, Date	First detected, Date	BEI Reference Material Number
VOC [MHRA, CDC]	Omicron	B.1.1.529, BA.1, BA.2, BA.4, BA.5	21K, 21L, 21M, 22A, 22B, 22C, 22D	MHRA: VOC NOV-2021 CDC: VOC 26NOV-2021	Multiple countries, Nov-2021	NR-56494 NR-56495 NR-56496 NR-56520 NR-56803 NR-58496 NR-58616
VOI [WHO, ECDC]	Omicron	BA.2.75, BQ.1, XBB, XBB.1.5, XBB.1.16	22D, 23A, 23B	ECDC: VOI SEP-2022 WHO: VOI JAN-2023, APR-2023	Multiple countries, May-2022, Jun-2022	NR-58706

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

The WHO has reported that PCR testing continues to effectively detect the B.1.1.529 (Omicron) variant, except for S gene target failure (SGTF) in some PCR assays. (WHO, 2022) SGTF in Omicron is not a concern for Co-Diagnostics assays, which instead target the Rdrp and E genes. The WHO also reports that studies are currently ongoing to assess the impact of Omicron on antigen-based rapid diagnostic tests (Ag-RDTs).

Since the initial design of CoPrimers targeting Gene RdRp and Gene E in Feb-2020, Co-Diagnostics has conducted monthly BLASTn queries of subsampled sequences in the Nextstrain database to monitor their homology against emerging strains. These in silico analyses confirm that none of the mutations related to the lineages B.1.1.529, B.1.1.529.XE, BA.1, BA1.1, BA.2, BA.3, BA.4 and BA.5 (Omicron), or Omicron sub-variants BA.2 + L452X, BA.2.12.1, XE, BA.4.6, BF.7, BF.14, BQ.1, BQ.1.1, or BA.5.2.35 occur in regions of RdRp or Gene E targeted by any COVID-19 tests designed and manufactured by Co-Diagnostics.

In April 2021, the WHO identified lineage B.1.167.2 (Delta) (PANGO lineages, 2021). This Delta lineage and related hybrid Omicron subvariants BA.2.75, BA.2.75.2, BN.1, CH.1.1, XBB, XBB.1.5, XBB.1.16, XBC, and XBF, contain one point mutation in the region targeted by the RdRp Fwd (Forward) CoPrimer. As shown in **Table 2**, this CoPrimer is used in all COVID-19 tests designed and manufactured by Co-Diagnostics.

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

According to WHO, from June 2021 through October 2021, Delta expanded to a peak of almost 90% of all viral sequences submitted to GISAID. More recently, Omicron has become the dominant variant circulating globally, accounting for >98% of all viral sequences submitted to GISAID after February 2022. This rise and fall in Delta and the later emergence of hybrid Delta/Omicron subvariants is reflected in the monthly homology statistics for RdRp and E Gene Targets of Co-Diagnostics COVID-19 tests shown in **Table 3** and **Table 4**.

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

Date of CoDx's Analysis for RdRp Marker	SARS-CoV-2 (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%)
04-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%)
Apr-20 to Apr-21 (Total)	78088	77648 (99.44%)	440 (0.56%)	0 (0%)	0 (0%)
07-Apr-21	4025	3962 (98.43%)	63 (1.57%)	0 (0%)	0 (0%)
06-May-21	3923	3847 (98.06%)	76 (1.94%)	0 (0%)	0 (0%)
01-Jun-21	3883	3000 (97.86%)	83 (2.14%)	0 (0%)	0 (0%)
06-Jul-21	3883	3566 (91.84%)	317 (8.17%)	0 (0%)	0 (0%)
02-Aug-21	3782	3172 (83.87%)	610 (16.13%)	0 (0%)	0 (0%)
01-Sep-21	3534	2894 (81.89%)	637 (18.02%)	3 (0.08%)	0 (0%)
01-Oct-21	3559	2882 (80.98%)	672 (18.88%)	5 (0.14%)	0 (0%)
01-Nov-21	3572	1648 (46.14%)	1919 (53.72%)	5 (0.14%)	0 (0%)
06-Dec-21	3386	1239 (36.59%)	2140 (63.20%)	7 (0.21%)	0 (0%)
03-Jan-22	3472	1293 (37.24%)	2171 (62.53%)	8 (0.23%)	0 (0%)
01-Feb-22	3201	1361 (42.52%)	1836 (57.36%)	4 (0.12%)	0 (0%)
01-Mar-22	3247	1831 (56.39%)	1411 (43.46%)	5 (0.15%)	0 (0%)
01-Apr-22	2967	1837 (62.01%)	1127 (37.89%)	3 (0.10%)	0 (0%)
01-May-22	2822	1938 (69.04%)	874 (30.61%)	10 (0.35%)	0 (0%)
01-Jun-22	2905	2202 (75.8%)	699 (24.06%)	4 (0.14%)	0 (0%)
01-Jul-22	2817	2570 (91.23%)	245 (8.70%)	2 (0.07%)	0 (0%)
01-Aug-22	2843	2652 (93.28%)	190 (6.68%)	1 (0.04%)	0 (0%)
01-Sep-22	2818	2611 (92.66%)	205 (7.27%)	2 (0.07%)	0 (0%)
03-Oct-22	1639	1520 (92.74%)	119 (7.26%)	0 (0%)	0 (0%)
01-Nov-22	2338	2046 (87.51%)	289 (12.36%)	3 (0.13%)	0 (0%)
01-Dec-22	1714	1448 (84.48%)	263 (15.34%)	3 (0.18%)	0 (0%)
01-Jan-23	1606	1116 (69.49%)	489 (30.45%)	1 (0.06%)	0 (0%)
01-Feb-23	1570	1016 (64.71%)	551 (35.10%)	3 (0.19%)	0 (0%)
01-Mar-23	2044	715 (34.98%)	1327 (64.92%)	2 (0.10%)	0 (0%)
03-Apr-23	2016	181 (8.97%)	1833 (90.92%)	2 (0.10%)	0 (0%)
01-May-23	1615	95 (5.88%)	1514 (93.75%)	6 (0.37%)	0 (0%)
01-Jun-23	1834	28 (1.53%)	1792 (97.71%)	12 (0.65%)	2 (0.11%)
03-Jul-23	2506	0 (0%)	2494 (99.52%)	12 (0.48%)	0 (0%)
01-Aug-23	1779	0 (0%)	1768 (98.77%)	11 (0.61%)	0 (0%)

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

Date of CoDx's Analysis for Gene E Marker	SARS-CoV-2 (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%)
04-Feb-20	53	53 (100%)	0 (0%)	0 (0%)	0 (0%)
Sep-20 to Apr-21 (Total)	32008	31865 (99.55%)	138 (0.43%)	2 (0.01%)	3 (0.01%)
06-May-21	3923	3870 (98.65%)	53 (1.35%)	0 (0%)	0 (0%)
01-Jun-21	3883	3820 (98.38%)	63 (1.62%)	0 (0%)	0 (0%)
06-Jul-21	3883	3823 (98.45%)	59 (1.52%)	0 (0%)	1 (0.03%)
02-Aug-21	3782	3723 (98.44%)	58 (1.53%)	0 (0%)	1 (0.03%)
01-Sep-21	3534	3476 (98.36%)	56 (1.58%)	2 (0.06%)	0 (0%)
01-Oct-21	3559	2882 (98.76%)	44 (1.24%)	0 (0%)	0 (0%)
01-Nov-21	3572	3527 (98.74%)	44 (1.23%)	1 (0.03%)	0 (0%)
06-Dec-21	3386	3527 (99.03%)	33 (0.97%)	0 (0%)	0 (0%)
03-Jan-22	3472	3442 (99.14%)	30 (0.86%)	0 (0%)	0 (0%)
01-Feb-22	3201	3170 (99.03%)	30 (0.94%)	0 (0%)	1 (0.03%)
01-Mar-22	3247	3222 (99.23%)	24 (0.74%)	0 (0%)	1 (0.03%)
01-Apr-22	2967	2947 (99.33%)	20 (0.67%)	0 (0%)	0 (0%)
01-May-22	2822	2802 (99.29%)	19 (0.67%)	1 (0.04%)	0 (0%)
01-Jun-22	2905	2883 (99.24%)	21 (0.72%)	1 (0.04%)	0 (0%)
01-Jul-22	2817	2793 (99.15%)	24 (0.85%)	0 (0.00%)	0 (0%)
01-Aug-22	2843	2831 (99.58%)	12 (0.42%)	0 (0.00%)	0 (0%)
01-Sep-22	2818	2794 (99.18%)	22 (0.78%)	1 (0.04%)	0 (0%)
03-Oct-22	1639	1627 (99.27%)	12 (0.73%)	0 (0%)	0 (0%)
01-Nov-22	2338	2323 (99.36%)	15 (0.64%)	0 (0%)	0 (0%)
01-Dec-22	1714	1701 (99.24%)	13 (0.76%)	0 (0%)	0 (0%)
01-Jan-23	1606	1594 (99.25%)	11 (0.68%)	1 (0.06%)	0 (0%)
01-Feb-23	1570	1553 (98.92%)	16 (1.02%)	1 (0.06%)	0 (0%)
01-Mar-23	2044	2016 (98.63%)	28 (1.37%)	0 (0%)	0 (0%)
03-Apr-23	2016	2005 (99.45%)	11 (0.55%)	0 (0%)	0 (0%)
01-May-23	1615	1607 (99.50%)	8 (0.50%)	0 (0%)	0 (0%)
01-Jun-23	1834	1823 (99.40%)	10 (0.55%)	1 (0.05%)	0 (0%)
03-Jul-23	2506	2489 (99.32%)	16 (0.64%)	1 (0.04%)	0 (0%)
01-Aug-23	1779	1764 (99.16%)	13 (0.73%)	2 (0.11%)	0 (0%)

As in any other primer used in qPCR techniques, the single mismatch caused by the Delta point mutation is not expected to prevent primer binding or functionality toward the SARS-CoV-2 genome. In theory, a single CoPrimer with 2 mismatches is expected to retain sensitivity with marked Ct delay and with 3+ mismatches, a CoPrimer is expected to have serious impairment (U.S. FDA, 2021) (FDA, 2021).

In addition, the qualitative detection of SARS-CoV-2 by more than 1 redundant marker minimizes the expected impact of a point mutation in any single CoPrimer or assay. 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.

As discussed previously, 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 in lineage Delta 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 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. Also, (King, et al., 2022) reportedly used the Logix Smart COVID-K-001 assay to accurately quantify COVID strains throughout the period from January 2021 through November 2021 as Delta became the predominant circulating strain.

Based on these evaluations, Co-Diagnostics remains confident that all Logix Smart SARS-CoV-2 assays, namely COVID-K-001, COVID-K-002, ABC-K-001, and COVDS-K-003/COVDS-K-004, retain full sensitivity for lineages classified as Variants of Concern (VOC) or Variants of Interest (VOI) at this time, including Omicron and all of its subvariants (B.1.1.529, B.1.1.529.XE, BA.1, BA.1.1, BA.2, BA.3, BA.4, BA.5, B.A.2 + L452X, BA.2.12.1, B.A.2.75, BA.2.75.2, BA.4.6, BA.5.2.35, BF.7, BF.14, BN.1, BQ.1, BQ.1.1, BQ.1.1.20, CH.1.1, CH.1.1.1, CH.1.1.2, XBB, XBB.1.5, XBB.1.16, XBC, or XE), Delta, as well as other emerging variant sequences reflected in monthly analyses of the Nextstrain database thus far.

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In case of additional questions, please contact support@codiagnostics.com.

Kenneth K.C. Bramwell, Ph.D. Deputy Chief Science Officer

k.bramwell@co-dx.com
Co-Diagnostics, Inc.
2401 S. Foothill Dr, Ste. D, SLC, Utah 84109
co-dx.com