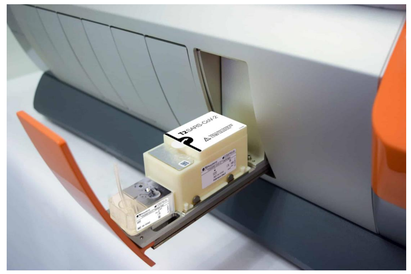
T2SARS-CoV-2 Panel is able to detect all SARS-CoV-2 variants as confirmed by genomic surveillance

M. Nabuan Naufer, Christopher A. Steele, Jack D. Spicer, Jessica L. Snyder
T2 Biosystems, 101 Hartwell Ave. Lexington, MA USA

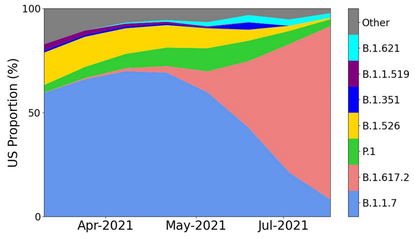
Background

Overview: SARS-CoV-2, the causative virus of the COVID-19 pandemic, can result in severe respiratory infection and death. Over the course of the pandemic, mutated variants of the virus have been documented globally. As the virus continues to evolve, it is critical that diagnostics be able to still detect the virus. To this end, we streamlined the routine genomic surveillance of SARS-CoV-2 sequences to confirm that the T2SARS-CoV-2 Panel detects the prevalent variants and proactively identify emerging mutations that may affect the assay sensitivity.



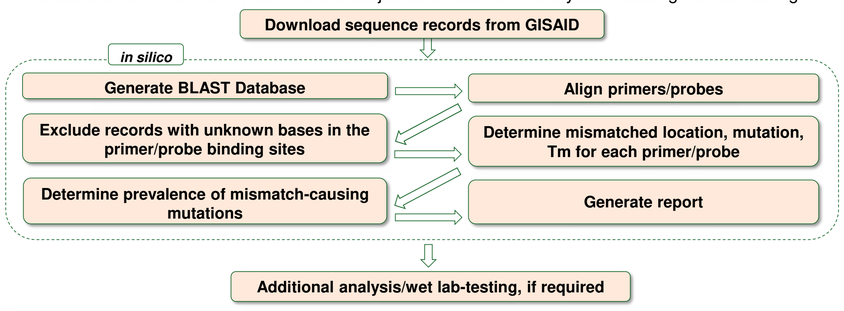
T2SARS-CoV-2 Panel: The T2SARS-CoV-2 Panel run on the T2Dx® Instrument, is an RT-PCR and T2 Magnetic Resonance (T2MR®) test. This test is designed for the direct detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens. The Panel is commercially available and was awarded EUA by the FDA on August 31, 2020. The T2SARS-CoV-2 Panel was validated in accordance with the EUA requirements from the FDA and is being distributed in accordance with the FDA guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency.

SARS-CoV-2 variants: Multiple genetic variants of SARS-CoV-2 have been emerging and circulating globally with the potential of affecting diagnostics and therapeutics. The estimated biweekly proportions of the most common SARS-CoV-2 lineages circulating in the US including B.1.1.7 (Alpha), P.1 (Gamma), B.1.526 (Iota), B.1.351 (Beta), B.1.1.519, and B.1.617.2 (Delta) is shown as a function of time. (Data source: www.cdc.gov)



Methods

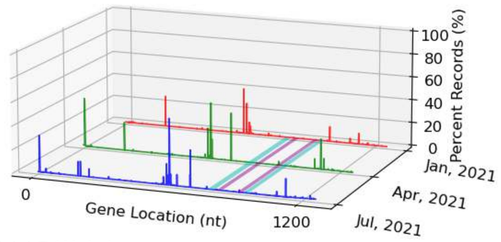
Sequence records from GISAID are routinely monitored to identify prevalent mutations that may result in mismatches with T2SARS-CoV-2 primers/probes. The *in silico* procedure is outlined within the dashed box. Prevalent mutations are subjected to additional analyses including wet-lab testing.



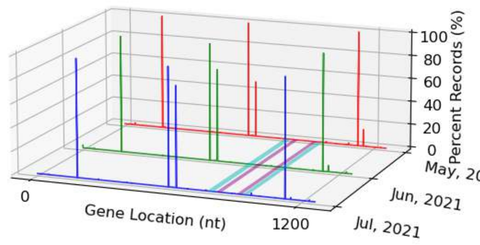
Routine surveillance of *N* gene mutations

Contemporary records of US submissions (all variants), and most common lineages in the US (www.cdc.gov) are analyzed biweekly. The mutational trends with respect to the reference sequence (NC_045512) in the nucleocapsid (*N*) gene on representative sequence records are shown as a function of time. Cyan and magenta shades denote the T2SARS-CoV-2 primer/probe binding sites. No prevalent mutations resulting in mismatches were identified except with the B.1.1.519 variant, which only constitutes <0.2% of US cases. These mutations were subjected to wet-lab testing and were shown not to affect the T2SARS-CoV-2 Panel's sensitivity.

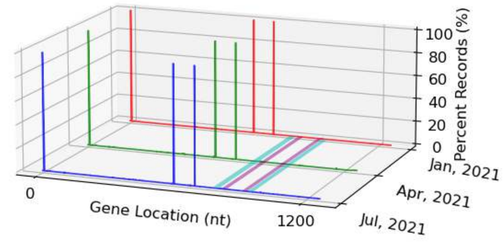
US (all variants)



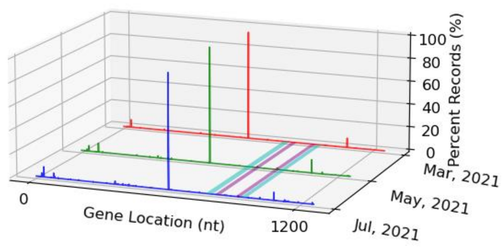
B.1.617.2 (Delta)



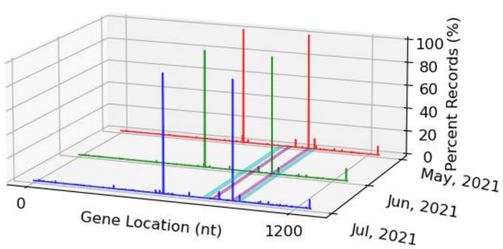
B.1.1.7 (Alpha)



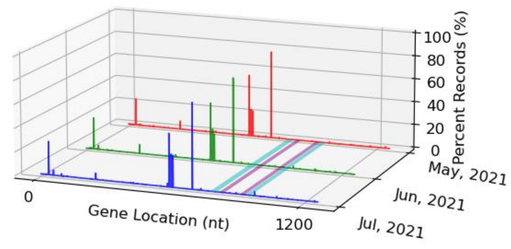
B.1.351 (Beta)



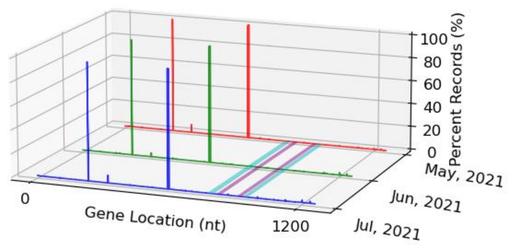
B.1.1.519



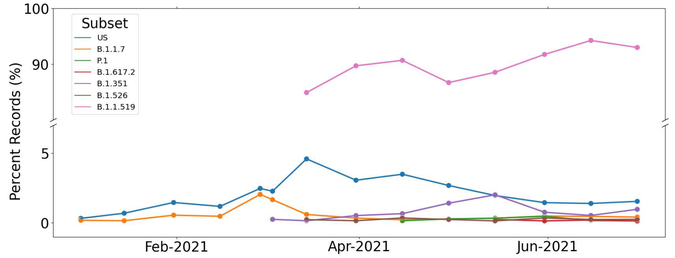
B.1.526 (Iota)



P.1 (Gamma)

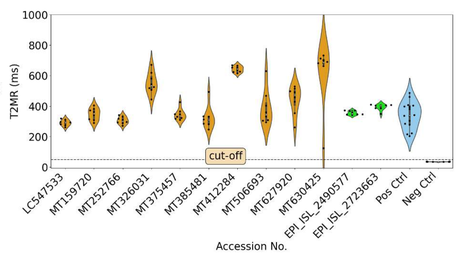


Mismatch-causing mutations



The highest percentage of records with mismatch-causing mutations for each subset is shown as a function of time. Only B.1.1.519 variant contains prevalent (>5%) mutations that result in a mismatch with a T2SARS-CoV-2 probe.

Wet Lab testing



Primer/ Probe	Terminal mismatch	Mismatch Type	Representative Accession No.
primer	No	C/A	MT506693.1
primer	No	G/A	MT627920.1
primer	No	C/A	MT252766.1
primer	No	G/T	MT159720.2
primer	No	C/A	LC547533.1
primer	No	G/T	MT385481.1
probe	N/A	A/A	MT375457.1
probe	N/A	T/G	MT326031.1
probe	N/A	C/A	MT412284.1
probe	N/A	C/A	MT630425.1
Probe (B.1.1.519)	No	C/A	EPI_ISL_2723663
Primer & probe (B.1.1.519)	No	C/T &C/A	EPI_ISL_2490577

Several mutations that result in mismatches were wet lab tested for FDA-EUA (orange). Genomic surveillance identified that B.1.1.519 contains a mutation that produces a mismatch with a probe (<90% of records) and mutations that produce mismatches with both a primer and a probe (4.8% of records). All the mutations were wet lab tested at 5000 copies/mL of target DNA, and yielded 100% hit rates.

Summary

- An analysis pipeline to execute routine surveillance of SARS-CoV-2 genome was developed to verify that the T2SARS-CoV-2 Panel detects the most common SARS-CoV-2 variants, as well as to identify emerging mutational trends that may affect the sensitivity of the T2SARS-CoV-2 Panel.
- No prevalent mutations that result in T2SARS-CoV-2 primers or probes were identified in the most common variants, such as B.1.1.7 (Alpha), B.1.617.2 (Delta), except for B.1.1.519 that currently constitutes <2% of the US cases.
- The mutations observed in B.1.1.519 were subjected to wet lab testing and were verified not to affect the T2SARS-CoV-2 panel's sensitivity.
- To date, T2SARS-CoV-2 Panel is able to detect all known SARS-CoV-2 variants, and routine surveillance of the SARS-CoV-2 genome records will be conducted throughout the course of EUA.