***Methods.*** Retrospective analysis of 15,314 inpatients within the Mass General Brigham healthcare system who had two tests within a 36-hour period between May 1 2020 and May 29 2021. Early infection was defined as having a negative test followed by a positive test. Patients with prior positive tests were excluded. The primary outcome was the proportion of patients in early infection over the total number tested serially, stratified by 4-hour testing intervals from the timestamp of the first test. Multivariate modeling was used to identify features predictive of early infection. Covariates included demographics, body site, PCR assay, location, community incidence, percent positivity, and median / skew of Ct value distributions.

***Results.*** Of 19,971 test pairs, 193 (0.97%) were characterized as a negative followed by a positive within 36 hours. Bivariate analysis showed a close association between negative to positive test pairs during the first surge in spring 2020 that was not present during the winter surge. Negative to positive test pairs were most common in the 12 to 16 hour time interval (51/193, 26%, Figure 1). After controlling for covariates, the Roche cobas assay was more likely to identify patients with a negative to positive test pair relative to the Cepheid Xpert, Hologic Panther Fusion and Roche Liat assays. A second specimen from the lower respiratory tract was more likely to lead to a positive relative to other body sites. Community incidence and Ct value distributions were not predictive and there were no differences between nasal and nasopharyngeal swabs. All 4-hour time intervals from 16 to 36 hours were significant for predicting a negative to positive test pair (Table 1).

Figure 1. Distribution of negative to positive test pairs by 4 hour time intervals

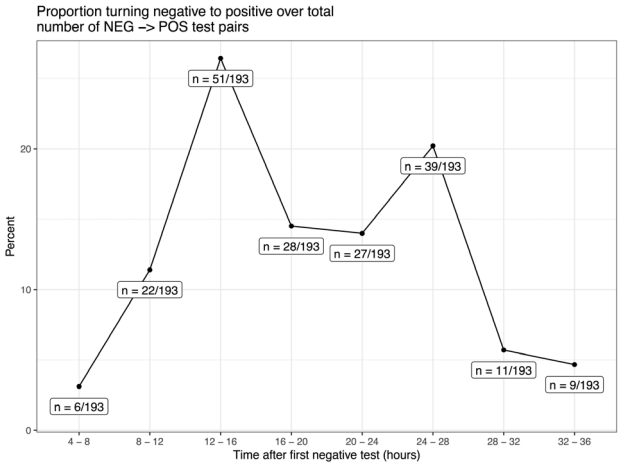


Table 1. Multivariate regression predicting a negative to positive test pair

**Multivariate regression predicting a negative to positive test pair**

| Variable | Subgroup | OR | Lower 95% CI | Upper 95% CI | p value |
|---|---|---|---|---|---|
| Age | | 0.99 | 0.98 | 1.00 | 0.02 |
| Gender (reference: Female) | Male | 1.04 | 0.77 | 1.39 | 0.81 |
| Month | | 1.00 | 1.00 | 1.00 | 0.08 |
| Incidence in Boston | | 1.00 | 1.00 | 1.00 | 0.88 |
| Percent positive across Mass General Brigham | | 1.10 | 0.97 | 1.25 | 0.15 |
| Median Ct for Mass General Brigham | | 1.14 | 0.98 | 1.33 | 0.09 |
| Skew of Ct distribution for Mass General Brigham | | 4.31 | 0.81 | 23.09 | 0.09 |
| Assay for specimen 1 (reference: Cepheid Xpert) | Hologic Panther | 1.33 | 0.88 | 2.01 | 0.17 |
| | Roche cobas | 1.92 | 1.13 | 3.24 | 0.01 |
| | Roche Liat | 0.84 | 0.15 | 3.52 | 0.83 |
| Assay for specimen 2 (reference: Cepheid Xpert) | Hologic Panther | 0.99 | 0.66 | 1.48 | 0.95 |
| | Roche cobas | 0.20 | 0.11 | 0.37 | 0.00 |
| | Roche Liat | 3.08 | 0.53 | 13.12 | 0.17 |
| Body site for specimen 1 (reference: Nasopharynx) | Nasal | 0.00 | 0.00 | 0.00 | 0.97 |
| | Lower respiratory tract | 0.67 | 0.16 | 1.94 | 0.52 |
| | Other | 3.73 | 0.21 | 18.45 | 0.20 |
| Body site for specimen 2 (reference: Nasopharynx) | Nasal | 1.78 | 0.29 | 6.00 | 0.43 |
| | Lower respiratory tract | 2.38 | 1.43 | 3.92 | 0.00 |
| | Other | 0.00 | 0.00 | 86067 | 0.98 |
| Location category for specimen 1 (reference: ER) | Inpatient | 1.19 | 0.80 | 1.76 | 0.39 |
| Location category for specimen 2 (reference: ER) | Inpatient | 0.86 | 0.55 | 1.38 | 0.52 |
| Time interval between specimens (reference 4 - 8 hours) | 8 - 12 | 1.22 | 0.51 | 3.37 | 0.68 |
| | 12 - 16 | 1.61 | 0.72 | 4.28 | 0.29 |
| | 16 - 20 | 2.71 | 1.16 | 7.40 | 0.03 |
| | 20 - 24 | 3.17 | 1.36 | 8.65 | 0.01 |
| | 24 - 28 | 4.76 | 2.10 | 12.85 | 0.00 |
| | 28 - 32 | 3.24 | 1.20 | 9.60 | 0.02 |
| | 32 - 36 | 3.72 | 1.31 | 11.31 | 0.01 |

***Conclusion.*** The likelihood of detecting early infection is dependent on PCR platform and body site of sampling. A range of time intervals between 16 to 36 hours after the initial test were likely to identify positive cases.

***Disclosures.*** **Sanjat Kanjilal, MD, MPH**, **GlaskoSmithKline** (Advisor or Review Panel member)

### 148. Single-amplicon, Multiplex Real-time RT-PCR with Tiled Probes to Detect SARS-CoV-2 *spike* Mutations Associated with Variants of Concern

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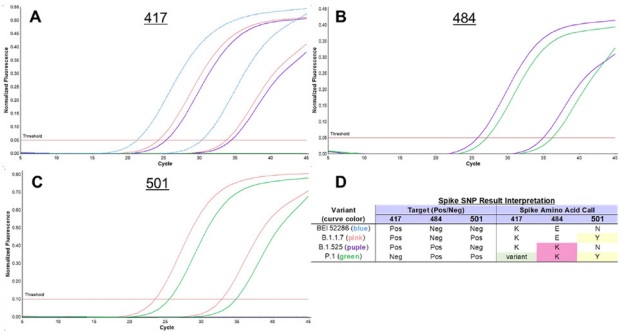
***Background.*** Detection and surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants is of great public health importance. Broadly accessible and inexpensive assays are needed to enhance variant surveillance and detection globally. We developed and validated a single-reaction multiplex real-time RT-PCR (the Spike SNP assay) to detect specific mutations associated with variants of concern (VOC).

***Methods.*** A single primer pair was designed to amplify a 348 bp region of *spike*. Probes were initially designed with locked nucleic acids (LNAs) to increase probe melting temperature, shorten probe length, and specifically detect 417K, E484K, and N501Y (**Figure**). The assay was optimized and evaluated using characterized variant sample pools. Clinical evaluation was performed on a convenience set of residual nasopharyngeal swabs, and variant calls were confirmed by SARS-CoV-2 genomic sequencing in a subset of samples. Following the initial evaluation, unmodified probes (without LNAs) were designed to detect L452R, L452Q, and E484Q.

Figure. Spike SNP distinguishes mutations occurring in different lineages (A-C).




Representative results of variant detection a single Spike SNP run are shown for mutations in the codons for 4177K (A) and mutations that encode 484K (B) and 501Y (C). Curves show dilutions of the following variants: blue, BEI 52286 (wild type); pink B.1.1.7; purple, B1.525; and green, P.1. Variant pools were used for B.1.17, B.1.525, and P.1 strains. Curves are displayed for a given dilution in each channel and result interpretation is shown (D).

***Results.*** The lower limit of 95% detection was 2.46 to 2.48 $\log_{10}$ GE/mL for the three targets (~1-2 GE/reaction). Among 253 nasopharyngeal swabs with detectable SARS-CoV-2 RNA, the Spike SNP assay was positive in 238 (94.1%), including all samples with Ct values < 30 (220/220) for the N2 target and 18/33 samples with N2 Ct values ≥ 30. Results were confirmed by SARS-CoV-2 genomic sequencing in 50/50 samples (100%). Subsequent addition of the 452R probe did not affect performance for the original targets, and probes for 452Q and 484Q performed similarly to LNA-modified probes.

***Conclusion.*** The Spike SNP assay provides fast, inexpensive and sensitive detection of specific mutations associated with SARS-CoV-2 VOCs, and the assay can be quickly modified to detect new mutations in the receptor binding domain. Similar analytical performance of LNA-modified and unmodified probes presents options for future assay customization that balance the shorter probe length (LNAs) and increased accessibility (unmodified). The Spike SNP assay, if implemented across laboratories offering SARS-CoV-2 testing, could greatly increase capacity for variant detection and surveillance globally.

***Disclosures.*** **Colleen S. Kraft, MD, MSc**, Rebiotix (Individual(s) Involved: Self): Advisor or Review Panel member

### 149. Extraction-free RT-PCR to Detect SARS-CoV-2 Variants of Concern

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