Host, Viral, and Environmental Transcriptome Profiles of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

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Abstract

A global pandemic of the 2019 coronavirus disease (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is responsible for over 150,000 deaths worldwide, including >12,000 deaths in New York City (NYC) alone. There is a pressing clinical and public health need for scalable molecular technologies that can rapidly detect SARS-CoV-2 infection and robustly interrogate strain evolution and host response in patients. To address these challenges, we designed a loop-mediated isothermal amplification (LAMP) assay to identify SARS-CoV-2 infection within 30 minutes of application, including directly from lysed cells. Simultaneously, we developed a large-scale host and viral transcriptomic profiling platform that employs total RNA sequencing of nasopharyngeal swab specimens. Applying both technologies to 442 samples, spanning 338 clinical samples tested for SARS-CoV-2, 86 environmental samples from the NYC subway, and 14 controls, we assembled a broad molecular picture of the COVID-19 epidemic in NYC. We found close concordance between viral titers measured with our rapid LAMP assay, RNA-seq, and the state-of-the-art RT-PCR. Full transcriptomic analyses revealed a distinct subset of the Western European clade A2a as the predominant genomic subtype in NYC cases, which was defined by two single nucleotide variants in nsp2 and ORF 3a and a clade-specific, 9-bp in-frame deletion in the virulence factor nsp1. High SARS-Cov2 viral titers were associated with distinct host transcriptional responses, including activation of the *ACE2* gene and interferon response genes (e.g., *IFIT1*). Since ACE inhibitors (ACEIs) can also increase *ACE2* expression, outcomes for patients taking ACEIs were examined, and showed a significantly increased risk of intubation and death. Our results demonstrate the utility of two molecular diagnostic platforms in defining the genetic features of an evolving global pandemic and provide insights that can aid future COVID-19 diagnostics, public health monitoring, and therapeutic options.

Keywords: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), 2019 coronavirus disease (COVID-19), loop-mediated isothermal amplification (LAMP), real-time polymerase chain reaction (RT-PCR), next-generation sequencing (NGS), RNA-seq, global health

Introduction

In March 2020, a novel viral pandemic was declared by the World Health Organization (WHO) for the 2019 coronavirus disease (COVID-19), an infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (He *et al*., 2020). New York City (NYC) emerged as a global hotspot of this pandemic, with over 120,000 cases, 32,000 hospitalizations, and 12,000 deaths attributed to COVID-19 as of April 17, 2020, according to John's Hopkins University (JHU) Coronavirus Resource Center (https://coronavirus.jhu.edu).

The presenting symptoms of COVID-19 resemble those of common viral respiratory infections, requiring molecular diagnosis to reliably distinguish SARS-Cov-2 infection from influenza and the common cold (Guan *et al.,* 2020, Zhou *et al*., 2020). Current approaches to molecular testing are mostly limited to hospital labs and are largely reserved for the most severe cases, with limited accessibility to the general population. As a result, the prevalence of SARS-Cov-2 in the population is mostly unknown, particularly among mild or asymptomatic cases. Though several novel, scalable biotechnological innovations for viral testing have begun to emerge (e.g. CRISPR-Cas12a (Broughton *et al*., 2020) or CRISPR-Cas13 (Metsky *et al*., 2020) on paper-based detection systems, or loop-mediated isothermal amplification (LAMP, Tanner *et al*., 2015,

Zhang *et al.*, 2020, Yu *et al*., 2020, Schmid-Burgk *et. al*., 2020) these have not been validated against gold-standard clinical assays or next-generation sequencing (NGS).

The lack of rapid and widely available SARS-CoV-2 diagnostics has fundamentally limited the public health approach to COVID-19, including the implementation of contact tracing and estimation of infection fatality rates. In addition, the persistence of SARS-CoV-2 across a range of surfaces (e.g. glass, metal) for up to nine days in experimental conditions (van Doremalen *et al*., 2020) and hospital areas (Ong *et al.*, 2020) raises the possibility that fomite transmission may be playing a role in COVID-19 spread. A key question is whether the environmental surface distribution of SARS-CoV-2 in high-traffic areas (e.g. subways) may have driven its rapid emergence in certain regions (e.g. NYC).

Modern viral surveillance combines large-scale molecular diagnostics with networks of data sharing (Gardy *et al*., 2015). Resources such as Global Initiative on Sharing All Influenza Data (GISAID), the JHU Dashboard, and NextStrain (https://nextstrain.org/ncov), have enabled dynamic tracking of the evolution of the COVID-19 pandemic (Dong *et al*., 2020, Meyers *et al*., 2020, Hadfield *et al*., 2018). Nevertheless, the fraction of cases that have undergone full-length viral sequencing are still low (<0.5% of documented cases), underscoring the need for additional profiling, particularly in global infection epicenters such as NYC. Full-length viral sequences are required to chart the dynamics of strain evolution, infer the temporal and geographic trajectories of spread, and correlate clinical features (e.g. disease severity, comorbidities, viral load) with specific genotypes. Indeed, such genomic epidemiology efforts have shown trajectories of the spread of SARS-CoV-2 in Washington, California, and Connecticut (Zhao *et al*., 2020, Gonzalez-Reiche *et al*., 2020, Fauver *et al*., 2020) as well as in China (Lu *et al*., 2020). However current approaches to viral profiling (targeted sequencing of SARS-CoV-2) fail to provide information on host immune response or microbial co-infections, whose associations could exacerbate or modify presentation of COVID-19.

To address these gaps in technology and disease knowledge, we designed and optimized a rapid LAMP assay to detect SARS-Cov-2 infection from nasopharyngeal swabs and saliva cell lysates. We then developed a large-scale shotgun metatranscriptomics platform to comprehensively profile nasopharyngeal swabs from patient samples with total RNA sequencing (RNA-seq). Both technologies were applied to 338 COVID-19 patients at Weill Cornell Medicine / New York Presbyterian Hospital (WCM/NYPH) and 86 environmental samples collected from high-transit areas in the NYC subway in early March. Using these data, we validated the rapid LAMP assay against viral loads obtained with total RNA-seq and gold standard qPCR. Host and viral expression data were then compared to clinical metadata from NYPH, specifically with a focus on angiotensin-converting enzyme inhibitors (ACEI) and clinical outcomes. Our results propose novel molecular approaches for detecting and tracking SARS-CoV-2 infection, provide new molecular insights into the evolution of the COVID-19 outbreak in NYC, and implicate specific host factors and drug interactions in the biology of this disease.

Results

Rapid single tube detection of SARS-CoV-2

We developed a colorimetric assay to quickly detect and quantify SARS-CoV-2 viral load in patient and environmental samples, utilizing a set of six LAMP primers and simple single tube protocol (**Figure 1b**). Primers were designed to create two nested loops and amplify within the SARS-CoV-2 nucleocapsid gene (N gene), which enabled a 30-minute reaction workflow. Related pathogens, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimens were evaluated to identify the % homology between the primer sequences and these other organisms, and the probes were also designed to avoid known polymorphisms (see Methods).

To validate the assay, we first evaluated two synthetic RNAs (see Methods) whose sequences matched the viral sequences of patients from Wuhan, China and Melbourne, Australia (**Supp. Fig. 1**). The first control (MT007544.1) was used to test the analytical sensitivity via the limit of detection (LoD), titrated from 1 million molecules of virus (10^6) down to a single copy, using serial log-10 dilutions. The reaction output was measured at 0-, 20-, and 30-minute intervals (**Supp. Figure 2**) before the samples were heated to 95ºC for inactivation. The LoD was found to be between 10–100 viral total copies, and this was then replicated to show a similar LoD of 10–100 viral copies on the second synthetic control from the patient from Wuhan (**Supp. Figure 2b**).

We evaluated the reproducibility, sensitivity, and specificity for the LAMP assay across a set of serial titrations. Across these experiments, LAMP fluorescence correlated closely with SARS-CoV-2 RNA viral copies (**Figure 1d**), with an overlap of the median signal from negative controls at lower levels (25 total copies) of viral RNA (n=10). This translated to a 100% reproducibility at 1,000 and 500 total copies of viral RNA, 90% reproducibility at 100 copies, and 80% reproducibility at 50 copies (**Supp. Figure 3a**). This indicates an LoD threshold (95% reproducibility at two times the LoD) that is likely near 200 copies of RNA, with a maximum sensitivity of 10-50 copies of the viral RNA.

To optimize the LAMP assay for clinical samples, we compared replicates of XX clinically SARS-CoV-2+ positive and XX negative nasopharyngeal swab samples (as determined by a clinically validated quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay) across a reaction matrix of samples, dilutions, and reaction volumes. As expected, clinically positive samples showed a much higher fluorescence than negative samples, with superior performance observed with higher (10µL vs 5µL) input volumes (**Supp. Figure 3b**). Clinically positive samples that failed to generate LAMP fluorescence were associated with lower viral genome abundance (high cycle threshold [Ct] value qRT-PCR). We obtained similar performance on bulk saliva lysate (**Supp. Figure 3c-e**), including increasing reaction sensitivity as a function of viral copy number. These results indicate robust performance of the LAMP assay across a broad range of cellular lysates (see Methods).

Having optimized the LAMP assay, we evaluated its efficacy as a diagnostic across 133 samples that tested positive (clinical positives) and 205 samples that tested negative (clinical negatives) for SARS-CoV-2 by qRT-PCR (see Methods). Among these, 182 had sufficient total nucleic acids for testing. Quantitative LAMP fluorescence data were used to build a Receiver Operator Characteristic (ROC) plot and evaluate the clinical sensitivity and specificity of the assay. At the optimal threshold, we observed an overall sensitivity of 94% and specificity of 100% (**Figure**

1e) as well as a positive predictive value (PPV) of 95% and negative predictive value (NPV) of 100%. With increasing viral load, as measured by Ct values, the LAMP assay showed an increased diagnostic sensitivity, with the lowest sensitivity (78%) up to the highest (100%), especially for those Ct values showing the highest level of virus (15-25 Ct values, **Supp. Fig. 4**). These same LAMP assay thresholds yielded consistent test positivity for synthetic RNA positive controls (Twist Biosciences) as well as clinical spike-in carrier RNAs (20/20) 12/12) (**Supp. Fig. 4).**

Shotgun metatranscriptomics platform for viral and host genomics

To provide orthogonal validation of our LAMP assay and further investigate the biological characteristics of clinical SARS-Cov2+ positive and negative samples, we developed a shotgun metatranscriptomics platform utilizing total RNA-seq (RNA-sequencing with ribosomal RNA depletion) comprehensively profile human, microbial, and viral RNAs. We aligned total RNA-seq reads to several NCBI databases and classified those of non-human origin using Kraken2 (Wood *et al*., 2019).

Applying this platform to 338 clinical specimens, we obtained an average of 63.2M read pairs per total RNA-seq library. All libraries were dominated by reads that mapped to either human, bacterial, or SARS-CoV-2 genomes, with rare reads mapping to viruses, fungi, and archaea (**Figure 2c, 2d**). The reads that mapped to SARS-CoV-2 from total RNA-seq were sufficient to provide >10x coverage of the viral genomes for 118/133 (88%) of patient samples (**Figure 3a**), with robust (>1000X) sequencing depth for the high viral titer samples. Indeed, when downsampling the sets of reads/patient (50M down to 0.5M), we observed consistent detection of the viral genome across RNA-libraries (**Figure 3b**). While we observed a low level of index hopping with our initial single-index libraries (n = XX), leading to a non-negligible background of viral loads in clinical negative samples, this effect was eliminated in subsequent libraries (n = XXX), where we used a dual-indexing approach.

Clinical positives showed higher viral content (0.1%-62.2% of total RNA) than clinical negative samples (P = XXXX, wilcoxon test). Comparing quantitative estimates of viral load from all three assays (LAMP, total-RNA Seq, qRT-PCR), we observed a high overall correlation (R^2= 0.78) between the total RNA-seq viral abundance and the quantification by RT-PCR and LAMP (**Figure 2b, Supp. Figure 5**). Indeed, 97% of clinical negative samples (199/205) demonstrated fewer than 0.01% of total RNA-seq reads mapping to the SARS-CoV-2 reference. Three of the remaining six demonstrated high SARS-CoV-2 read proportion (>0.01%) , high SARS-CoV-2 genome coverage (>99%), and high LAMP fluorescence, suggesting that these were RT-qPCR false negatives (**Supp. Figure 6**). The remaining three showed lower coverage (97%-99%) of the SARS-CoV-2 genome and XXX signal with LAMP. These data provide evidence that false negatives from qRT-PCR are likely rare (3-6/205, or 1.4-2.8%), but may require orthogonal assays to reliably detect.

Analysis of total RNA-seq sequences enabled mapping of co-infections and colonization with commensal species across both clinical positives and negatives in our sample set. We identified additional RNA viruses and organisms that distinguished high, medium, and lower viral titer patients (**Supp Table 1**), with overall similarity seeing across patients in the bacterial RNA

fractions (**Figure 5a)**. However, there were distinct variations in known respiratory viruses, including human coronaviruses 229E, NL63, and HKU1, as well as influenza A, rhinovirus (A and C), and respiratory syncytial virus (**Figure 5a)**. As expected, most patients (129/133) that presented with the SARS-CoV-2 virus lacked reads associated with other respiratory viruses. A subset of patients (4/133, 3.0%) also carried Influenza A These cases were enriched in the lower titer (bottom third) of COVID-POS (3/4) patients. One patient carried SARS-CoV-2, metapneumovirus, and coronavirus NL63 at the same time (**Figure 5a)**. These result indicate that a positive test for common respiratory viruses does not rule out the presence of SARS-Cov-2.

Environmental sampling of SARS-CoV-2 in the NYC subway

Having validated a rapid LAMP assay and shotgun metratranscriptomics platform, we deployed both to investigate the environmental distribution of SARS-Cov-2 in high-transit areas of the NYC subway at the dawn of the NYC pandemic. We collected 86 samples from handrails, kiosks, and the floor of Grand Central and Times Square subway stations between March 6 and 13th, 2020. Each sample was collected using a sterile and DNA/RNA free swab, following the MetaSUB sampling protocols for nucleic acid stabilization (Danko *et al*., 2020). None of these 86 samples reached appreciable levels of LAMP fluorescence, suggesting that these high transit surfaces were free of SARS-Cov-2 virions.

To further investigate these environmental samples for the presence of SARS-Cov-2, we investigated total RNA-seq. These samples demonstrated a mix of fungal and archaeal species that was consistent with underground subway origin (Danko *et al*., 2020) (**Supp. Table 1**). However, we did not observe significant read counts or proportions (**Figure 2a**) of SARS-Cov-2 reads, which was particularly clear with dual-index library preparations (**Supp Figure 7**). However, a broad range of other bacterial and viral species was found, including a large set of phages (e.g. *Streptomyces* phage VWB), desiccation-tolerant bacteria (e.g *Deinococcus radiodurans*), and more abundant bacterial and archaeal RNA than the clinical samples (**Supp. Table 1**). Of note, these were checked against a database of putative false positives (**Supp. Table 2**), which was created from *in silico* fragmentation of the SARS-CoV-2 genome and mapping against the same database. Taken together, these results indicate that high transit surfaces were not likely to harbor significant levels of SARS-Cov-2 in the early phases of the NYC epidemic.

SARS-CoV-2 genotypes nominate an NYC- enriched subclade

We then investigated samples with sufficient coverage across the 30kb SARS-CoV-2 genome (>10x) to identify genetic variants and place NYC strains onto phylogenetic tree of the global outbreak. We identified variants both through alignment and *de novo* assembly of Kraken-classified SARS-CoV-2 reads. In both cases, single nucleotide variants (SNVs) and indels (insertions/deletions) were called relative to the SARS-CoV-2 reference sequence (GCF_009858895.2) and then compared with 4,964 sequenced samples in GISAID (build XXX) using the NextStrain database (Hadfield *et al*., 2018). Among our data, we found XXX SNV and XX indel variants across 93 samples with sufficient quality assemblies for placement into the

NextStrain-derived maximum likelihood phylogenetic tree. Though the vast majority (XX) of variants were present across all SARS-CoV-2 reads aligning to a given reference position, a small subset (XX / XX) were polymorphic with robust read coverage (>XX reads), suggesting that microdiversity may play some role in SARS-Cov-2 evolution.

Analyzing groupings obtained from the NextStrain-derived maximum likelihood phylogenetic tree, we found a high proportion (>XX%) of our samples were associated with A2a, a large Western European derived clade that comprises nearly half of the sequences in GISAID. These results validate recently published findings (Gonzalez-Reiche *et al*., 2020). In addition, we found additional samples from clades with mixed Asian and European sources (XX, XX% of cases; XX, XX% of cases). Among these, we identified a predominance (90%) of the "L" strain, which is defined by an SNV (XX X>X) in ORF8 and previously associated with severe cases in Wuhan (Tang *et al*., 2020) (**Figure 4b).**

We then asked whether specific variants were enriched in NYC A2a samples relative to A2a samples from other regions. Comparing variant frequencies between A2a cases in our data and non-NYC cases in GISAID, we identified five enriched loci with FDR<0.1 (Fisher's exact test, one-tailed) over-represented in NYC A2a samples (Figure 4a). All five of these variants were significantly enriched (Bonferroni adjusted P value <0.05) when comparing NYC and non-NYC GISAID cases (Figure 4a). Visualizing these variants across the NextStrain derived phylogenetic tree (Figure 4b), demonstrated that the highest frequency of these variants (1059C>T, 25563G>T), define a novel subclade of A2a, which we call A2a-25563. This subclade comprised 61% (260 /423) of (NYPH and GISAID) NYC samples.

Investigating the geographical and temporal distribution of non-NYC samples in A2a-25563, we found additional samples in Western Europe and West Africa. The first noted case of A2a-25563 in GISAID was in Belgium on 2/21/2021. Since then, this putative subclade has represented a minority (<XX %) of Western European cases, though rising to prominence in West Africa (XX %) in mid-March. The SNV's defining this subclade are non-synonymous variants targeting genes encoding the non-structural protein 2 (NSP2), the viral replicase, and ORF3a, a poorly characterized SARS-CoV-2 protein with putative roles in inflammation (Siu 2019 FASEB) . This subclade defining ORF3a site (p.Gln57His) was distinct from that which defines the previously characterized L strain (XXXX) (Tang *et al*., 2020).

Probing additional rare variants in A2a-25563, we identified a 9-bp in-frame deletion (p.141_143KSF) in non-structural protein 1 (NSP1), a putative SARS-CoV-2 virulence factor (REF). This deletion was found in two of 93 samples in our cohort (**Figure 4c**). An identical deletion was observed across 14 other samples in the GISAID database, including 12 that were annotated as A2a in GISAID (**Figure 4c**), spanning samples from England, Iceland, and Canada. This deletion removes three amino acids, including a tyrosine that is not present in the SARS-CoV-1 genome (**Figure 4d**). These residues occur in the C-terminal region of NSP1, a domain that is absent from milder (common-cold associated) alphacoronavirus and may drive the pathogenicity of SARS-Cov-2 and other betacoronaviruses (SARS-Cov, MERS). The apparent polyphyletic distribution of this deletion variant in the NextStrain phylogeny suggests that it may have arisen more than once or, alternatively, may reflect errors or misclassification of a subset of these assemblies.

Defining the SARS-CoV-2 Host Transcriptome

We leveraged the comprehensive nature of the total RNA-seq profiles to define the host transcriptome under SARS-Cov-2 infection. We first integrated the LAMP, rtPCR, and total RNA-Seq viral load estimates to identify a final set of XX SARS-Cov-2 positive (COVID-POS) and XX negative (COV-NEG) cases. We then nominated differentially expressed genes (DEGs) associated with SARS-CoV-2 infection using DESeq2. (see Methods). Overall, 5,982 significant DEGs (q<0.01, >1.5-fold change) were found for the COVID-POS samples (**Supp. Table 3)** , spanning 2,942 up-regulated DEGs and 3,040 down-regulated DEGs, which could be separated into high, medium, and low viral response categories based on the three detection methodologies (RT-PCR, NGS, and LAMP) (**Figure 6b**); the host transcriptome that exhibited the greatest amount of DEGs were those with the highest viral titer.

Differentially expressed host genes indicated a wide range of antiviral responses, including some a common interferon response across all ranges of viral levels (**Figure 6b**). Notably, host epithelial cells showed an increase in angiotensin converting enzyme 2 (*ACE2)* expression (q-value=0.006), which is the SARS-CoV-2 cellular receptor. This critical gene for viral entry exhibited a dose-dependent expression concomitant with the higher levels of SARS-CoV-2 virus, along with IFI27 and IFI6 (Interferon Alpha Inducible Protein 6) (**Figure 6c**). Other interferon-related genes included IFIT1, an interferon-induced antiviral RNA-binding protein that specifically binds single-stranded RNA bearing a 5'-triphosphate group (PPP-RNA) and SHFL (Shiftless Antiviral Inhibitor of Ribosomal Frameshifting), which is associated with Dengue virus and Chikungunya infection. The DEGs also included HERC6 (HECT Containing E3 Ubiquitin Protein Ligase Family Member 6), which aids Class I MHC-mediated antigen processing and innate immune response (**Figure 6c, 6d**), underscoring the impact of the virus on these cells' immune response. Also, a subset of cytokines (CXCL10, CXCL11, CCL8) showed the highest spike of expression in the higher viral titer sub-group (**Supp. Figure 8**), matching previous results from animal models and infected cells (Blanco-Melo *et al*., 2020).

Down-regulated genes and those with a negative enrichment score (NES) were functionally distinct (**Figure 6d**). These included *ALAS2*, a gene which makes erythroid ALA-synthase that is found in developing erythroblasts (red blood cells). ALA-synthase plays an important role in the production of heme TRIM2 E3 ubiquitin ligase induced during late erythropoiesis, which indicated a connection to hematological and iron (heme) regulation during infection (**Figure 6d**). Accordingly, genes in a related biological network were significantly enriched based on Gene Ontology (GO) pathways for iron regulation (q-value =0.002, **Supp. Table 4**). Both the up-regulated and down-regulated gene expression differences in were distinct from those of house-keeping genes (**Supp. Figure 9**), which stayed mostly stable during infection.

ACEI usage increases the risk of severe COVID-19 disease

Given our observation of increased *ACE2* gene expression in patients with high SARS-Cov-2 viral load, we broadly investigated the interplay of receiving pharmacologic angiotensin converting enzyme inhibition (ACEI) for hypertension and clinical features of COVID-19 disease. Since *ACE2* expression can be increased in patients taking ACEIs, the observed correlation of viral titer with *ACE2* expression may be attributed to the pre-infection use of such

inhibitors, which is common in older patients and those with comorbidities (Fang *et al*., 2020). To address this, we analyzed an observational cohort of 8,278 patients with suspected SARS-CoV-2 infection from NYP were analyzed for their usage of ACEIs (4,574 who tested positive). We found that use of ACEIs was strongly associated with testing positive in patients suspected of SARS-CoV-2 infection (OR=3.06 95% CI: 2.38-3.94, p=2.44E^{-18}). This result was also consistent when corrected for age, sex, and IL-6, where exposure to ACEIs has an OR=1.54, and when corrected for other reported clinical covariates (1.18-2.01, p=1.41E^{-03}); patients exposed to ACEIs were 1.85 (1.40-2.44,p=1.71E^{-05}) times more likely to test positive. In the univariate analysis, ACEI usage conferred an increased risk of intubation and mortality for SARS-CoV-2 positive patients (**Table 1**) (intubation: HR=2.63 95%CI: 2.01-3.43, p=1.22E^{-12})(**Figure 6e**) and mortality: HR=1.68 95%CI: 1.22-2.31, p=1.42E^{-03} (**Figure 6f**).

In a multivariate analysis that included age, sex, and IL-6 levels, a significant association between exposure to ACEIs and those requiring mechanical respiration was also found (HR=1.83 95%CI: 1.39-2.40, p=1.27E^{-05}). Additionally, this association held when correcting for previously reported risk factors for SARS-CoV-2 morbidity and mortality with an HR=1.56 95%CI: 1.18-2.07, p=1.83E^{-03} (**Supp. Figure 10a**). Moreover, we confirmed previously reported risk factors for both mechanical respiration and mortality. For requirement of mechanical respiration, we found significant effects from male sex (p=6.18E^{-03}), diabetes (p=1.00E^{-03}), and IL-6 (p=5.85E^{-113}). In addition, for mortality we found significant effects from age (p=8.05E^{-72}), male sex (p=6.13E^{-04}), diabetes (p=6.66E^{-05}), and IL-6 (p=1.26E^{-12}). Finally, in a post-hoc comparative analysis between specific ACEIs we found that benazepril was associated with significantly increased risk of mortality (N=32, HR=2.37 95%CI: 1.05-5.35, p=3.70E^{-02}) and that trandolapril is associated with requiring mechanical respiration (N=2, HR=15.85 95%CI: 2.11-119.08, p=7.25E^{-03}; **Supp. Figure 10b**).

Discussion

These data demonstrate unique host transcriptome profiles during viral infection with SARS-CoV-2, as well as clade-specific mutational landscapes of the virus, both representing the early phase of the US pandemic (March 2020). Total RNA-seq (ribo-depleted RNA) used here enabled a comprehensive molecular map for the virus and host, including complete genotypes (including insertions and deletions) across the length of the viral genome and coding and non-coding RNA gene expression quantification. Moreover, these data show that LAMP is a quick and effective assay that could be readily deployed to aid current and future viral testing and surveillance, since it is comparable to RNA-seq and RT-PCR and can be used with saliva whole cell lysate. Widespread testing is critical to help inform individual quarantine efforts and overall management of highly infectious and long-shedding viruses such as SARS-CoV-2, and these data, methods, and results can help guide future clinical and research efforts.

Total RNA-sequencing data enabled a complete genetic map of the viruses in a significant subset of our samples. Our phylogenetic analysis nominates an A2a subclade (A2a-25563, defined by 25563G>T) which comprises the majority of NYC samples (including those sequenced outside of this study) (Gonzalez-Reiche *et al.*, 2020). Though remaining NYC cases show a wide distribution across all identified clades, the predominance of such a narrowly defined set of sequences within NYC from a rare Western European (UK, Belgium) subclade is striking. These results suggest very early introduction by a single patient harboring A2a-25563, multiple A2a-

25563 founder events, or disproportionate community transmission of strains within this subclade. The latter possibility could be consistent with A2a-25563 harboring differential fitness with respect to transmissibility or virulence relative to other A2a viruses. Future studies correlating viral genotypes with patient outcomes in larger cohorts will be necessary to determine whether any of these A2a-25563 associated variants, including the 9-bp in-frame deletion in NSP1, functionally influence viral transmission or disease severity.

Because genomes provided here were recovered from hospitalized patients with relatively severe disease, these data may only partly reflect the strains present in asymptomatic patients. Indeed, the continuous production of viral genomes in sick and healthy carriers can be used to track the pathogenicity and biology of SARS-CoV-2 strains as they move around the world, especially when paired with clinical and outcome data. Moreover, further transcriptomic and epitranscriptomic analysis and mapping through these sequencing efforts will provide new insights to the life cycle and pathogenicity of SARS-CoV-2 (Kim et al., 2020).

During a large-scale pandemic, with exponentially infectious spread as seen with COVID-19, scalable methods for diagnosis and screening of pathogens that can build the capacity to test millions of patients are desperately needed (Lan *et al*., 2020, Liu *et al*., 2020). As such, LAMP and CRISPR-based methods provide an opening for more rapid screening options, and more importantly a point-of-care test (POCT) that can translate into clinical workflow. Moreover, the data presented here represent the first full clinical validation of such rapid-response, nucleic acid-based diagnostic approaches, matched with a standard diagnostic method (RT-PCR) and total RNA-seq. The limitations of the sampling collection (e.g. swab type, swabbing method) as well as the detection technology will help inform how the results should be interpreted, and how some of the false negative results on LAMP can occur for simple technical reasons. For example, the available primers used for the clinical specimens on RT-PCR positive results can indicate if an area is infectious and a negative result (with appropriate confirmation) will possibly represent a lower risk. Indeed, these tools and methods can help create a viral "weather report" if broadly used and if partnered with continual validation.

Although much of the pathophysiology of the novel coronavirus, SARS-CoV-2, remains unknown, studies have reported the virus using the receptor ACE2 for entry into target cells (Hoffman *et al*., 2020; Lu *et al*., 2020). Further validating this, we found expression of the *ACE2* gene was significantly upregulated in the samples with higher viral load, as well as some cytokine genes, which matches observations found in other betacoronaviruses (Sajuthi *et al*., 2020). ACE inhibitors are commonly used in COVID-19 patients with comorbidities of hypertension, diabetes mellitus, and coronary heart diseases (Fang *et al*., 2020, Ferrario et al., 2005), concerning physicians that it may make patients on these medications more susceptible to SARS-CoV-2 infection. Indeed, since epidemiological studies have reported increased mortality and morbidity in COVID-19 patients with hypertension, more research needs to be done to address other confounding variables including other comorbidities and treatment with ACE inhibitors or angiotensin receptor blockers (Patel and Verma, 2020).

Although these data alone cannot establish causality between infection and ACE2 expression regulation, they provide some testable hypotheses. For example, if some patients are more susceptible because they are already expressing high levels of ACE2, this could help with

targeting ACE2 in these patients as a prophylactic method. However, if the cells respond to infection with ACE2 expression, and this leads to the cytokine storm seen in patients, then this could be used as a downstream treatment (post-infection), for when ACE2 interacts with TMPRSS2, such as the ongoing trials with camostat mesylate (Hoffman *et al*., 2020). Moreover, SARS-CoV-2 has been found to induce phosphorylation of STAT1 and increases in interferon stimulated gene proteins, a mechanism not previously seen in SARS-CoV, suggesting a potential molecular mechanism behind the upregulation of interferon response (Lokugamage et al., 2020).

Finally, it is notable that the majority of the testing for SARS-CoV-2 so far has relied on nasopharyngeal specimen collection, yet preliminary results here and elsewhere demonstrate that COVID-19 prediction from oral collection could be a more optimal path forward (Woelfel *et al*., 2020). However, further studies comparing nasopharyngeal, oropharyngeal, and buccal collection approaches, as well as a comparison of different swab types, are needed. Depending on the availability of reagents and resources, as well as automation, a LAMP-based approach on such sample types could allow facilities to increase testing capabilities by orders of magnitude. Since viral pandemics can have significant, long-lasting detrimental impacts for affected countries, it is crucial to deploy methods that can track and profile cases (e.g. RNA-seq, LAMP, RT-PCR) and provide a comprehensive view of host and viral biology. These methods can help mitigate the medical and socioeconomic harm from viral outbreaks, as well as establish protective surveillance networks that can help defend against future outbreaks.

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Author Contributions

CEM led the study design and coordination and HR led the clinical collection and validation work in the NYP CLIA laboratory, as well as with MI. Overall supervision and protocol development and implementation for the Zymo RNAClean and NEB assays (SL). DB and ChM performed the LAMP experiments to validate the assay. CeM, DD, JF, AS, JR, MM, EA, IH, DM, MI, BWL, MZ, UG, NiT, NAI, CEM performed analyses. DD, NAI, MS, BY, KR, CB coordinated and collected environmental samples. EA submitted the IRB application and helped with clinical coordination. Help and insights for analysis from EA, SL, MI, MS, LFW, ML, MC, HR all led to the figures and analyses. All authors reviewed, edited, and approved the manuscript.

Conflicts of Interest

Nathan Tanner and Bradley W. Langhorst are employees at New England Biolabs.

IRB

Samples were collected and processed through the Weill Cornell Medicine Institutional Review Board (IRB) Protocol 19-11021069. Observational cohort analysis (ACEI) was done through the Columbia University IRB Protocol AAAL0601.

Data Accessibility

Viral sequences were uploaded into GISAID (Global Initiative on Sharing All Influenza Data) site (https://www.gisaid.org), and patient data is deposited into dbGAP.

Materials and Methods

Sample Collection and Processing

Patient specimens were collected with patients' consent at New York Presbyterian Hospital (NYPH) and then processed for RT-PCR. Nasopharyngeal (NP) swab specimens were collected using the BD Universal Viral Transport Media system (Becton, Dickinson and Company, Franklin Lakes, NJ) from symptomatic patients.

Extraction of Viral RNA and RT-PCR detection

Total viral RNA was extracted from deactivated samples using automated nucleic acid extraction on the QIAsymphony and the DSP Virus/Pathogen Mini Kit (QIAGEN). One step reverse transcription to cDNA and real-time PCR (RT-PCR) amplification of viral targets, E (envelope) and S (spike) genes and internal control, was performed using the Rotor-Gene Q thermocyler (QIAGEN).

Twist Synthetic RNAs

We used two fully synthetic RNAs made by in vitro transcription (IVT) from Twist Biosciences, which were synthesized in 5kb pieces with full viral genome coverage of SARS-CoV-2. They were sequence verified to ensure >99.9% viral genome coverage, and come as 1,000,000 copies per µL, 100µL per tube. The two controls are from Wuhan, China (MN908947.3) and Melbourne, Australia (MT007544.1). Reference sequence designs came from NCBI: https://www.ncbi.nlm.nih.gov/nuccore/MT007544 and https://www.ncbi.nlm.nih.gov/nuccore/MN908947.3.

Reverse Transcriptase, quantitative real-time PCR (RT-PCR)

Clinical samples were extracted as described above and then tested with RT-PCR using primers for the E (envelope) gene, which detects all members of the lineage B of the beta-CoVs, including all SARS, SARS-like, and SARS-related viruses, and a second primer set for the S (spike) gene, which specifically detects the SARS-CoV-2 virus. The reaction also contains an internal control that served as an extraction control and a control for PCR inhibition.

Samples were annotated using RT-PCR cycle threshold (Ct) value for SARS-CoV-2 primers. Subjects with Ct less than or equal to 18 were assigned "high viral load" label, Ct between 18 and 24 were assigned "medium viral load" and Ct between 24 and 40 were assigned "low viral load" classes, with anything above Ct of 40 was classified as negative. We also predicted a combined viral load score using Ct, GloMax QuantiFluor readout from LAMP experiments and fraction of SARS-CoV-2 matching NGS reads in a sample. For this score (40-Ct), (LAMP readout) and (log10(SARS-CoV-2 fraction + 1e-6)) were all normalized between zero and one individually, and summed together using a combination weight of 5 for Ct, 3 for LAMP and 2 for NGS.

LAMP Primer Sequences

Primers were designed using PrimerExplorer (v4.0), as per guidelines in Zhang *et al*., 2020. This specifically utilized the LAMP-compatible primers for the on the COVID-19 reference genome (NCBI). LAMP's inherent specificity (using 4-6 primers vs. 2 for PCR amplification) in combination with this *in-silico* analysis revealed there is limited opportunity for cross-reactivity to allow for false-positive reporting or affect performance of the N-gene primers for SARS-CoV-2 detection (**Supp Table 5**). Overall, the primers had less than 80% homology with the vast majority of tested pathogen sequences. For any organisms where a primer hit >80% homology, only one of the primers (forward or reverse) had significant homology making an amplified product extremely unlikely. Overall, the results of this analysis predict no significant cross-reactivity or microbial interference. We also assessed the potential impact of sequence variation in circulating strains that might lead to poor amplification. In the thousands of sequences deposited in GISAID (Shu and McCauley, 2017), only one site in the priming region was observed to be polymorphic (see Supplemental Figure X+1). The polymorphism (T30359C) was only observed in 106 of 6753 (<2%) sequences with coverage of this region. This variant overlaps the priming site of the LB primer but is not near a 3-prime end and is not anticipated to cause amplification failure.

Nucleocapsid Gene: (N, 5'-3'):

GeneN-F3 TGGCTACTACCGAAGAGCT
GeneN-B3 TGCAGCATTGTTAGCAGGAT
GeneN-FIP TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG
GeneN-BIP AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
GeneN-LF GGACTGAGATCTTTCATTTTACCGT
GeneN-LB ACTGAGGGAGCCTTGAATACA

Primer Sequences: rActin (5′-3′)

ACTB-F3 AGTACCCCATCGAGCACG
ACTB-B3 AGCCTGGATAGCAACGTACA
ACTB-FIP GAGCCACACGCAGCTCATTGTATCACCAACTGGGACGACA

ACTB-BIP CTGAACCCCAAGGCCAACCGGCTGGGGTGTTGAAGGTC
ACTB-LoopF TGTGGTGCCAGATTTTCTCCA
ACTB-LoopB CGAGAAGATGACCCAGATCATGT

The LAMP Reaction Setup
For each well or Eppendorf tube, we utilized a set of six primers (above) for Gene N, the M1800 LAMP Master Mix (NEB), water, and 11.5µL of the sample. The protocol is as follows:

1. Reagents added:
    a. 12.5 µL M1800 LAMP mix (NEB)
    b. 1 µL LAMP primers (Gene N)
    c. 1-11.5 µL of sample
    d. Remaining volume (to 25 µl) H_2O
2. Vortex, spin down;
3. Place on Thermocycler at 65ºC for 30 minutes with lid at 105 ºC;
4. Remove tubes, place on ice for 5 seconds;
5. Visualize over lab bench/ice/paper.

Light Intensity and Data Processing
Completed reactions were analyzed with the Promega GloMax Explorer (Promega GM3500) fluorometer using the QuantiFluor ONE dsDNA system (Promega E4871). This system recorded light intensity from each well using an emission filter of 500-550nm, an excitation filter set at blue 475 nm, and a high sensitivity setting on the Glomax software. Values were then tabulated and compared with controls (positive and negative). The intensity threshold of 2.5x negative control was used as the threshold for positive detection.

DNAse treatment, rRNA depletion, and RNAseq library construction
All samples' total nucleic acid (TNA) were treated with DNAse 1(Zymo Research, Catalog # E1010), which cuts both double-stranded and single-stranded DNA. Post-DNAse digested samples were then put into the NEBNext rRNA depletion v2 (Human/Mouse/Rat), Ultra II Directional RNA (10ng), and Unique Dual Index Primer Pairs were used following the vendor protocols from New England Biolabs (except for the first flowcell, see supplelmental figures). Kits were supplied from a single manufacturer lot. Completed libraries were quantified by Qubit or equivalent and run on a Bioanalyzer or equivalent for size determination. Libraries were pooled and sent to the WCM Genomics Core, HudsonAlpha, or New York Genome Center for final quantification by Qubit fluorometer (ThermoFisher Scientific), TapeStation 2200 (Agilent), and RT-qPCR using the Kapa Biosystems Illumina library quantification kit.

Taxonomic Classification of Sequence Data
All complete genome or chromosome level assemblies from RefSeq database for archaea, bacteria, protozoa, fungi, human and viruses including SARS-CoV and SARS-CoV-2 genomes were downloaded and used for building a classification database for Kraken2 (k=35, ℓ=31) (O'Leary *et al.,* 2016; Wood *et al.,* 2019).

To get an approximation for the positive and negative classification rate, the BBMap random-reads script was used to simulate 10 million 150bp paired-end Illumina reads from the database

sequences (Segata *et al.,* 2016). For the negative test all sequences in the database excluding SARS-CoV and SARS-CoV-2 genome were removed from the sequences and the simulated reads were mapped with the Kraken2 database (Supp Table 1).

For the positive test, the same process was repeated using only SARS-CoV-2 genome (**Supp Table 1**). Positive results show >99% of SARS-CoV-2 reads uniquely map to either SARS-CoV or SARS-CoV-2, with the remaining 1% are ambiguous, potentially matching multiple taxa (**Supp Table 2**). All sequences were classified using the Kraken2 database. To remove the potential contamination of reads that are homologous across multiple species we used Kraken2 outputs to filter sequences to either human (uniquely matching Homo sapiens and no other taxon in our database), SARS-CoV-2 (either matching SARS-CoV or SARS-CoV-2 due to homology between these two viruses), and remaining reads that may be coming from unclassified, archaeal, bacterial, viral, fungal, protozoan or ambiguously mapping reads to human or SARS-CoV (*Li*, 2015).

Reference- and Fragment-based Viral Mapping and Assembly
Reads unambiguously mapping to SARS-CoV or SARS-CoV-2 were aligned to the Wuhan-Hu-1 (Genbank accession MN908947.3) reference using bwa mem (Li, 2013). Variants were called using iVar, and pileups and consensus sequences were generated using samtools (Li *et al.,* 2009; Grubaugh *et al.,* 2019; Greenfield *et al.,* 2020). Any sample with more than 30,000 SARS-CoV-2 mapping reads and >99% coverage above 10x depth were taken as reliable samples, which resulted in 95 samples (90 positive, 2 negative, 2 positive controls and 1 negative control). 92 clinical samples were compared to 4872 SARS-CoV-2 sequences from GISAID (as of April 10, 2020) (9, 10). All sequence filtering, alignments, phylogenetic inference, temporal ordering of sequences and geographic reconstruction of likely transmission events were done using nextstrain (Katoh and Standley, 2013; Sagulenko *et al.,* 2018; Hadfield *et al.,* 2018).

Fragment assembly was also performed using IRMA (Sehpard *et al.,* 2016). The Wuhan-Hu-1 genome (Genbank accession MN908047.3) was used as a reference with the poly-A tail trimmed to reduce the likelihood of false low-complexity matching. An HMM model of this reference sequence was created using the native modelfromalign script. IRMA was run with the COV module with the following parameters adjusted from the default: minimum read length = 25bp; minimum read pattern count = 5; minimum read count = 2; minimum count for alternative finished assembly = 20. Consensus assemblies were aligned to the Wuhan-Hu-1 reference using MAFFT (Katoh and Standley, 2013) with default settings, and sequence identity and coverage metrics were calculated using Mview (Brown *et al.,* 1998). Phylogenetic trees were created using nextstrain's augur as described above, and visualized using the ggtree package in R (Yu, 2020).

Viral SNV Calling
Subsequent to alignment, SNV were identified by xAtlas (Farek *et al.,* 2018) (parameters: -g -m 10 -n 10) producing a gVCF per sample. The individual call sets were merged using bcftools (Li, 2011) (merge -g) and allele counts were added with bcftools view (-q 0.01) . Population-level variants across all samples were converted in a presence/absence matrix using a customized script where every SNV was counted as present if it was supported by at least 5% of the reads. This matrix was loaded in R and underwent hierarchical clustering using the function dist with the euclidean method and hclust using the average method. All samples were further analyzed

using Manta (Chen *et al.,* 2016), and subsequently summarized and filtered using the SURVIVOR pipeline (Jeffares *et al.,* 2017). Subsequently, SV calls were re-genotyped using SVTyper (doi:10.1038/nmeth.3505) and a population-level VCF was generated using SURIVOR merge (allowing for 100bp maximum distance). Events were filtered for only <1000bp insertions and deletions that were homozygous in a sample.

Human Transcriptome Analysis

The reads that mapped unambiguously to the human reference genome via Kraken2 were used to detect the host transcriptional response to the virus. Reads matching *Homo sapiens* were trimmed with TrimGalore, aligned with STAR (v2.6.1d) to the human reference build GRCh38 and the GENCODE v33 transcriptome reference, gene expression was quantified using featureCounts, stringTie and salmon using the nf-core RNAseq pipeline (Pertea *et al.,* 2015; Malinen *et al.,* 2005; Johnson *et al.,* 2007; Robinson *et al.,* 2010; Naccache *et al.,* 2014; Zamani *et al.,* 2017; Ewels *et al.,* 2019). Sample QC was reported using fastqc, RSeQC, qualimap, dupradar, Preseq and MultiQC (Okonechnikov *et al.,* 2016; Andrews, 2015; Ewesl *et al.,* 2016; Sayols *et al.,* 2016; Wang *et al.,* 2012). Samples that had more than 10 million human mapped reads were used for differential expression analysis. Reads, as reported by featureCounts, were normalized using variance-stabilizing transform (vst) in DESeq2 package in R (Love *et al.,* 2014) for visualization purposes in log-scale. DESeq2 was used to call differential expression with either Positive cases vs Negative, or viral load (High/Medium/Low/None) as reported by either RT-PCR cycle threshold (Ct) values or the combination viral load method as explained before. Genes with BH-adjusted p-value < 0.01 and absolute log2 fold-change greater than 0.58 (at least 50% change in either direction) were taken as significantly differentially regulated (Benjamini and Hochberg, 1995). The complete gene list for all comparisons are given in Supp Table 3. Resulting gene sets were ranked using log2 fold-change values within each comparison and put into GSEA to calculate gene set enrichment for molecular signatures database (MSigDB), MGI Mammalian Phenotypes database and ENCODE transcription factor binding sets (Liberzon *et al.,* 2011; Subramanian *et al.,* 2005; Sergushichev, 2016; Smith *et al.,* 2018). Any signature with adjusted p-value < 0.01 and absolute normalized enrichment score (NES) >= 1.5 were reported (Supp Table 3).

Statistics and Visualization

Statistics and visualization of single nucleotide variants and indels were completed in R. Visualization of phylogenies was completed using Auspice and the 'ape' library for R.

Cross-reactivity Analysis

Primers were compared with a list of sequences from organism from the same genetic family as SARS-CoV-2 and other high-priority organisms listed in the United States Food and Drug Administration's Emergency Use Authorization Template (https://www.fda.gov/media/135900/download). Using the sequence names in the EUA template, the NCBI taxonomy database was queried to find the highest quality representative sequences for more detailed analysis. Primers were compared to this database using Blast 2.8.1 and the following parameters (word size: 7, match score: 2, mismatch score: -3, gap open cost: 5, gap extend cost: 2). Up to 1000 hits with e-value > 10 were reported.

Inclusivity Analysis

Unique, full-length, human-sample sequences were downloaded from the GISAID web interface. These sequences were aligned to NC_045512.2 (Wuhan SARS-CoV-2) using minimap2 -x asm5 and visually inspected using IGV 2.8.0 with allele frequency threshold set to 0.01.

ACE Inhibitor Cohort Analysis

We compared usage of ACE inhibitors in an observational cohort analysis of 8,278 patients with suspected SARS-CoV-2 infection (4,574 of which tested positive). ACE inhibitors are commonly taken continuously for several years (Bonarjee et al., 2001). We defined a cohort of ACE inhibitor-exposed patients as those that have an ACE inhibitor prescription order sometime after January 1st, 2019. We compared the frequency of ACE inhibitor exposure in three cohort comparisons:

i. SARS-CoV-2 tested positive patients versus SARS-CoV-2 tested negative patients,
ii. SARS-CoV-2 positive patients who require mechanical ventilation versus those who did not, and
iii. SARS-CoV-2 positive patient survival versus death.

In addition, we perform one post-hoc comparison to evaluate the individual effects of particular ACE inhibitors among SARS-CoV-2 positive patients who are exposed to ACE inhibitors.

Cohort and Data Source

Our cohort data for SARS-CoV-2 suspected patients is extracted from the electronic health records at New York-Presbyterian/Columbia University Irving Medical Center (NYP/CUIMC). We used data collected starting on March 10th, 2020 through April 16th, 2020. In addition, we used data from 279,487 patients, who were not tested for SARS-CoV-2 infection, with available electronic health records from January 1st, 2019 through September 24th, 2019 to represent a comparison population of patients. In both cases, data extracted included disease diagnoses, laboratory measurements, medication and pharmacy orders, and patient demographics. We derived mortality from a death note filed by a resident or primary provider that records the date and time of death. Intubation was used as an intermediary endpoint and is a proxy for a patient requiring mechanical respiration. We used note types that were developed for patients with SARS-CoV-2 infection to record that this procedure was completed. We validated outcome data derived from notes against the patient's medical record using manual review.

Experimental and Statistical Methods

We conducted univariate analysis of the frequency differences of ACE inhibitor exposure and multivariate regression analysis to account for established risk factors for SARS-CoV-2 outcomes (i.e. age, sex, and baseline IL-6 upon admission (Zhou et al., 2020). We use logistic regression for analysis for comparing cohort (i) as this cohort is retrospective analysis on patients with definitive outcomes. We perform survival analysis using a Cox proportional hazards model for cohort comparisons (ii) and (iii) as some patients from these cohorts are currently being treated and their outcomes are unknown (i.e., censored). We cannot determine from our data the date of infection. For the study start date for the patient, we use the date of testing positive minus seven days. Quantitative variables (i.e., age and the first IL-6 measurement) are scaled to [0,1] to facilitate comparison of model coefficients to those of dichotomous variables. Prior to conducting our multivariate analysis, we evaluated and removed correlated covariates. We include a model built with all covariates as well in the supplemental tables.

Comorbidity Definitions
Risk factors were assigned using OMOP CDM concept IDs 317576 for "Coronary Arteriosclerosis", 201820 for "Diabetes mellitus", and 437525 for "Overweight". In each case, the concept ID and all descendant concepts were used to define the risk factor phenotype, and individuals were assigned the phenotype if they were assigned any of the codes.

Statistical Software
All analyses were done in using Python 3.7 and all models were fit using R 3.6.3. Survival analyses (Cox regressions and survival curves) were performed with the survival package for R, version 3.1-12.

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Figures and Legends

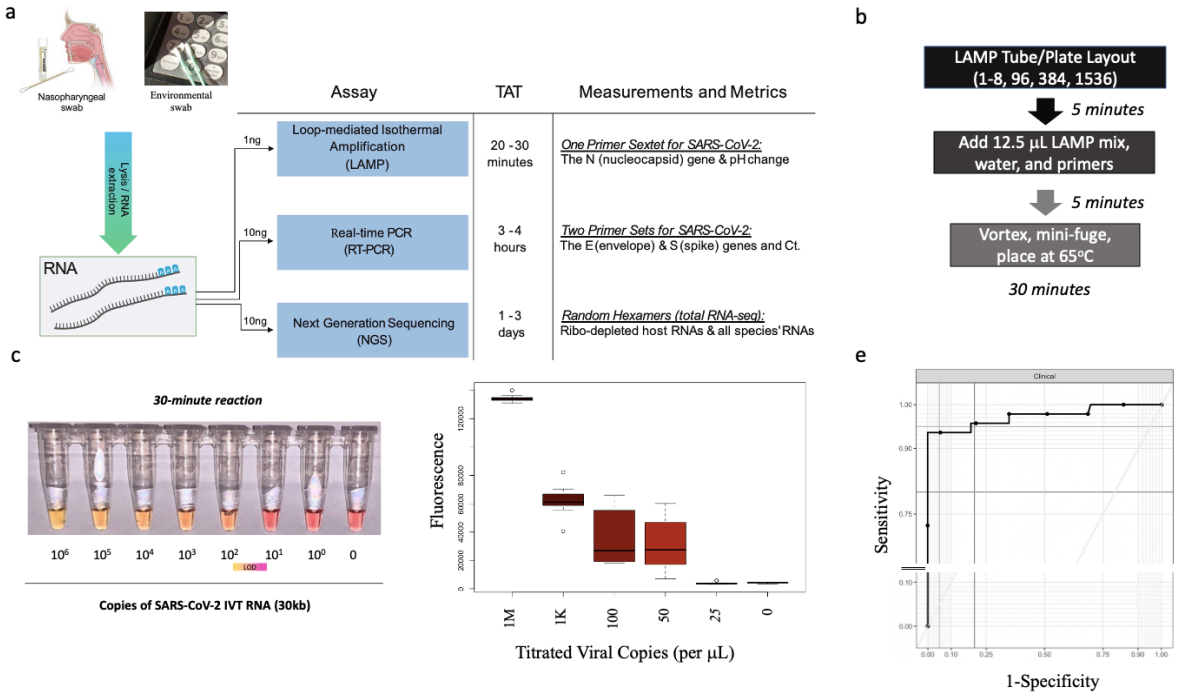


Figure 1. Sample Processing, the Loop-Mediated Isothermal (LAMP) Reaction and Synthetic RNA Validation. (A) Clinical and environmental samples collected with Nasopharyngeal (NP) and isohelix swabs respectively, were tested with RNA-sequencing, RT-PCR, and LAMP. (B) The test samples were prepared using an optimized LAMP protocol from NEB, with a reaction time of 30 minutes. (C) Reaction progress was measured for the Twist COVID-19 synthetic RNA (*MT007544.1*) from 1 million molecules of virus (10^6), then titrated down by $\log_{10}$ dilutions. The colorimetric findings of the LAMP assay are based on a yellow to pink gradient with higher copies of SARS-CoV-2 RNA corresponding to a yellow color. The limit of detection (LoD) range is shown with a gradient after 30 minutes between 10 and 100 viral copies (lower right). (D) Replicates of the titrated viral copes using LAMP, as measured by Quantifluor fluorescence. (E) The sensitivity and specificity of the LAMP assay from 182 patients (118 negative and 62 positive for SARS-CoV-2, as measured by RT-PCR).

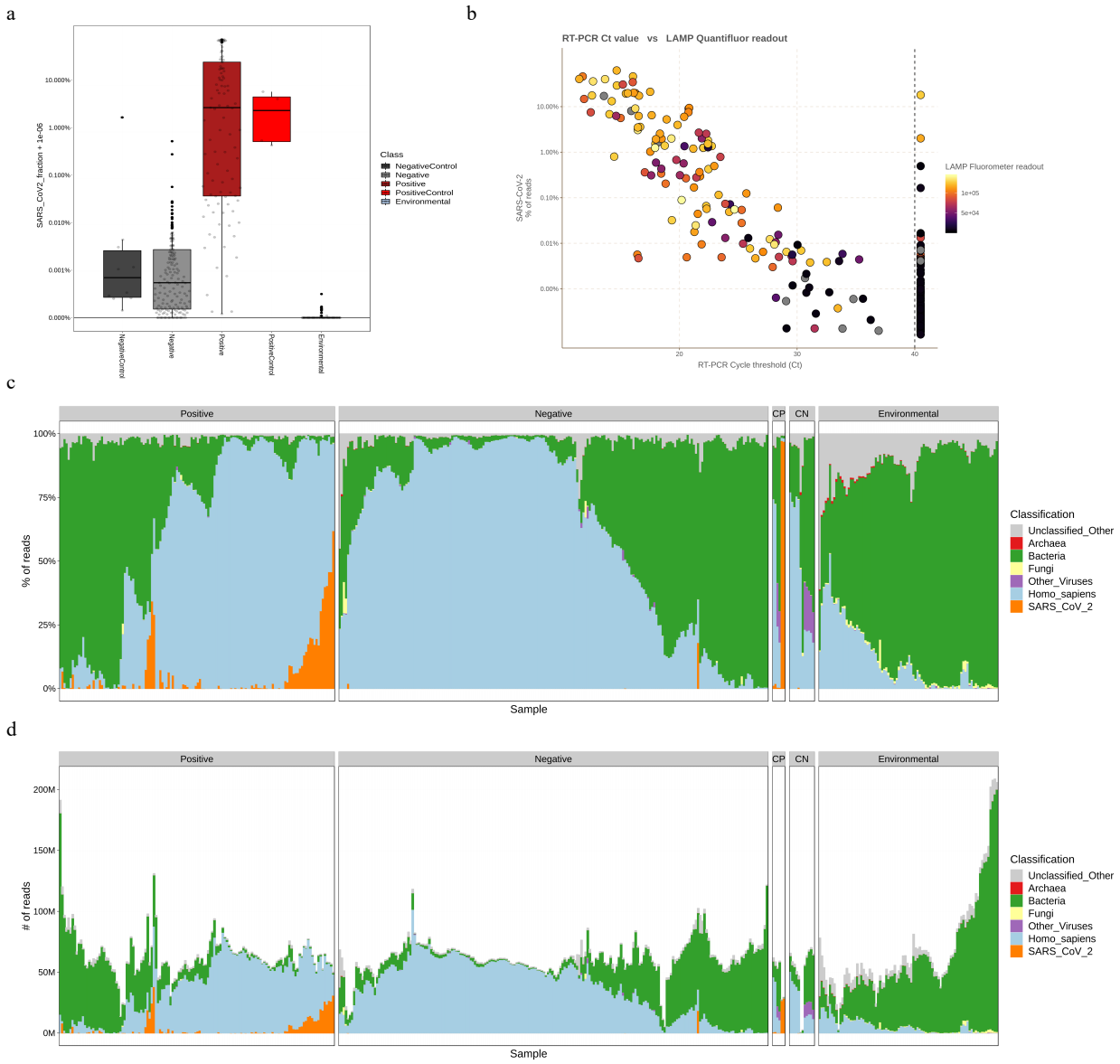


Figure 2. Full transcriptome profiles of SARS-CoV-2 Patients with NGS, RT-PCR, and LAMP. (a) Clinical samples tested by RT-PCR (Positive, dark red or Negative, light grey) were sequenced and run through the LAMP assay. These results were compared to the buffer blanks (Negative Control), dark grey, Synthetic RNAs or Vero 6 cell extracts with SARS-CoV-2 infection (Positive Controls, light red), and Subway Samples (Environmental, blue). Read proportions are shown on the y-axis. (b) SARS-CoV-2 abundance, as measured with NGS and percentage of reads (y-axis) is compared to the Ct Threshold for RT-PCR (x-axis), with lower Ct values representing higher viral abundance, and the LAMP reaction output (Fluorimeter values, black to yellow scale). (c,d) Read mapping to archaea (red), bacteria (blue), fungi (turquiose), chordates (brown), viruses (purple), human (green), and SARS-CoV-2 (tan) across the clinical controls (CN, CP), environmental samples (E), RT-PCR negative (N), and RT-PCR positive (P) samples.

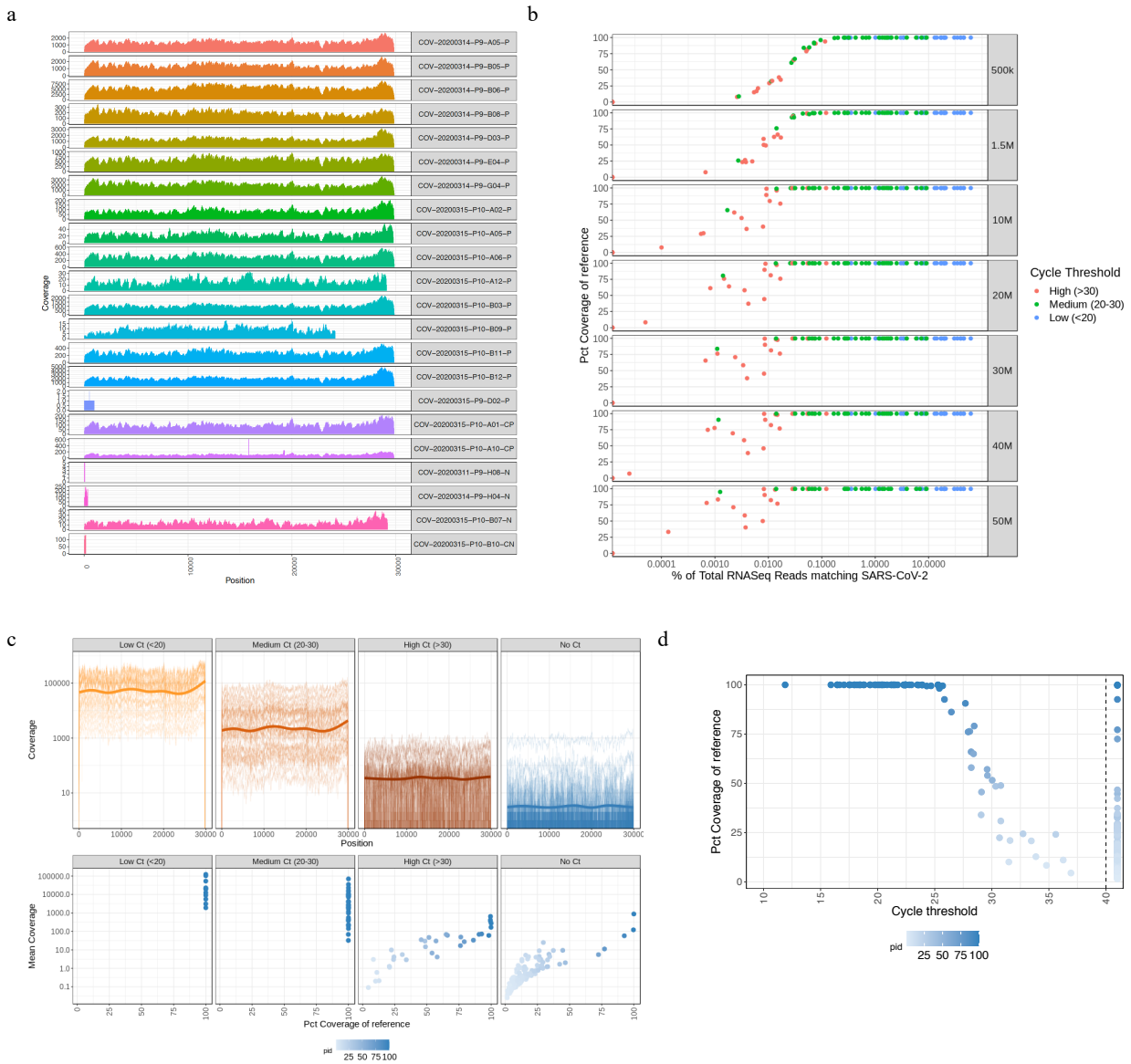


Figure 3: Viral genomes from RNA-seq data and titration of coverage (a) The coverage plot across the SARS-CoV-2 genomes (viral coordinate on bottom, colored by sample) from a representative set of clinical positive samples. CN samples and N samples are clinical negative (buffer), P samples are RT-PCR positive, and CP samples are Vero 6 cells with virus (CP). (b) Downsampling (right annotation) of the samples and mapping to the SARS-CoV-2 genome to gauge the percent coverage (y-axis) as a function of the viral quantification by RT-PCR (Ct thresholds, low <20, medium 20-30, and high>30). (c) Average coverage statistics for the low, medium, and high Ct samples, as well as the mean coverage for each of these samples. (d) The cycle threshold (x-axis) vs. the coverage of the genome (y-axis and color depth) for the total RNA-seq.

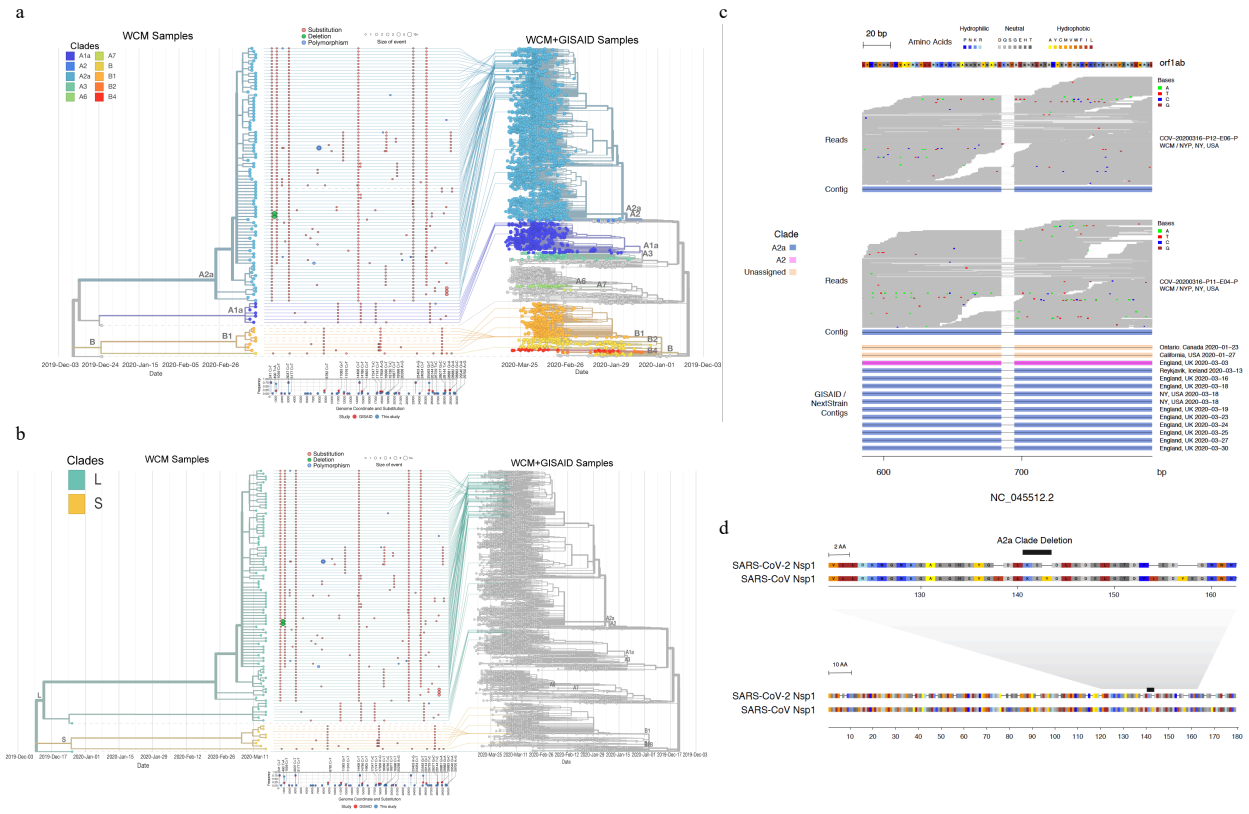


Figure 4: Variation and phylogenetics of NYC COVID-19 Cases. (a) The phylogenetic placement of these COVID samples is shown on the tree (left) and the global map of known COVID genomes (right). Genetic variants called from the RNA-seq data a range of variants that are distinct from the Wuhan reference strain, and the samples from this study, highlighted in blue, show enrichment for European and Asian alleles. (b) Proportion of the L (green) and S strain (yellow) are shown for the NYC viruses. Phylogeny of samples from this study on the left and total GISAID samples on the right, with a map of variants in this study's samples in the middle, colored by event type and sized by number of nucleotides impacted. Annotation track on the bottom shows frequency of alternate alleles in this study and in the GISAID database. (c) Two patients that showed the 9-bp deletion in ORRF1b (NSP1) were confirmed in the GISAID database (below). (d) The ORF1b deletion represents a clade-specific (A2a) deletion that is in a region of the CoV-2 genome unique relative to CoV-1.

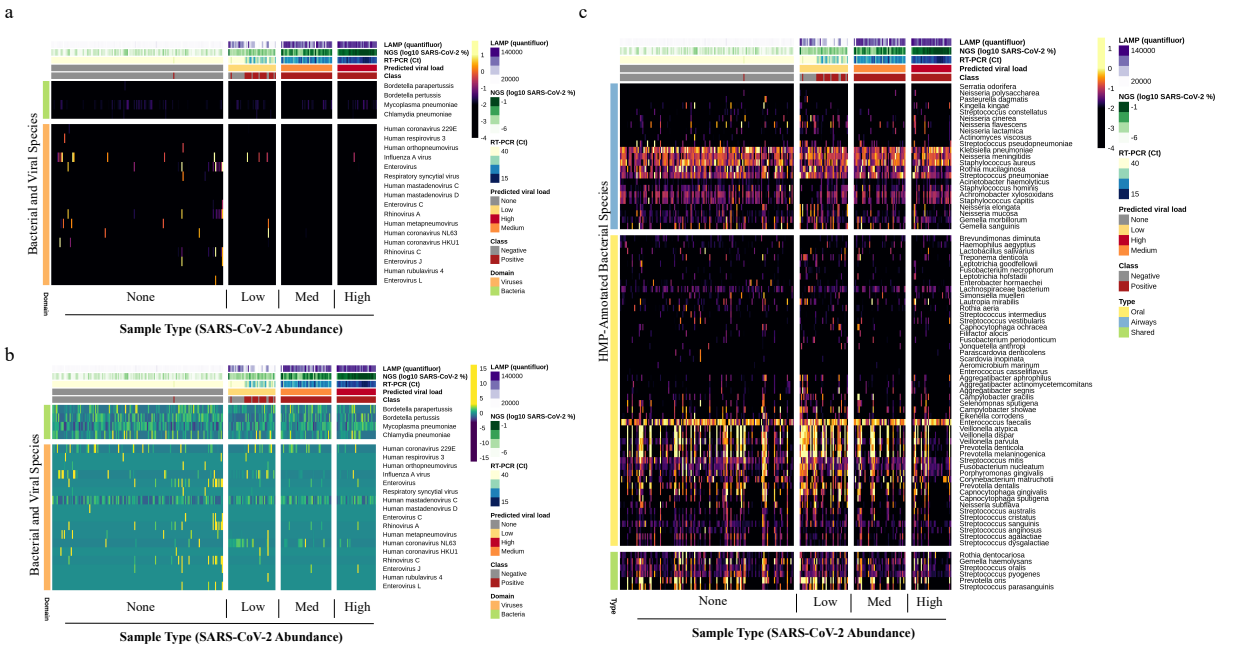


Figure 5: Metatranscriptome profiles of the patient cohorts. (top row, all panels) Samples were quantified by a range of viral detection methods, including LAMP (Quantifluor), RNA-seq (log10 SARS-CoV-2 % of reads), and RT-PCR (Ct values) to create a three-tier range of viral load for the positive samples (right) compared to the clinically-annotated negative samples (class, red or grey). (bottom) The detected microbial species (horizontal lines) are plotted as a z-score of their divergence from the patient cohort (vertical lines). (a) Common respiratory pathogens plotted as a $\log_{10}$ abundance of mapped reads, with each organism as a line and each vertical column as a patient. (b) The same plot as (a), but with z-score for statistical divergence. (c) The same layout as samples, but with bacteria from annotated from the Human Microbiome Project (HMP) as normal airway (blue, top portion), oral (yellow, middle set), or both oral and airway flora (green, bottom).

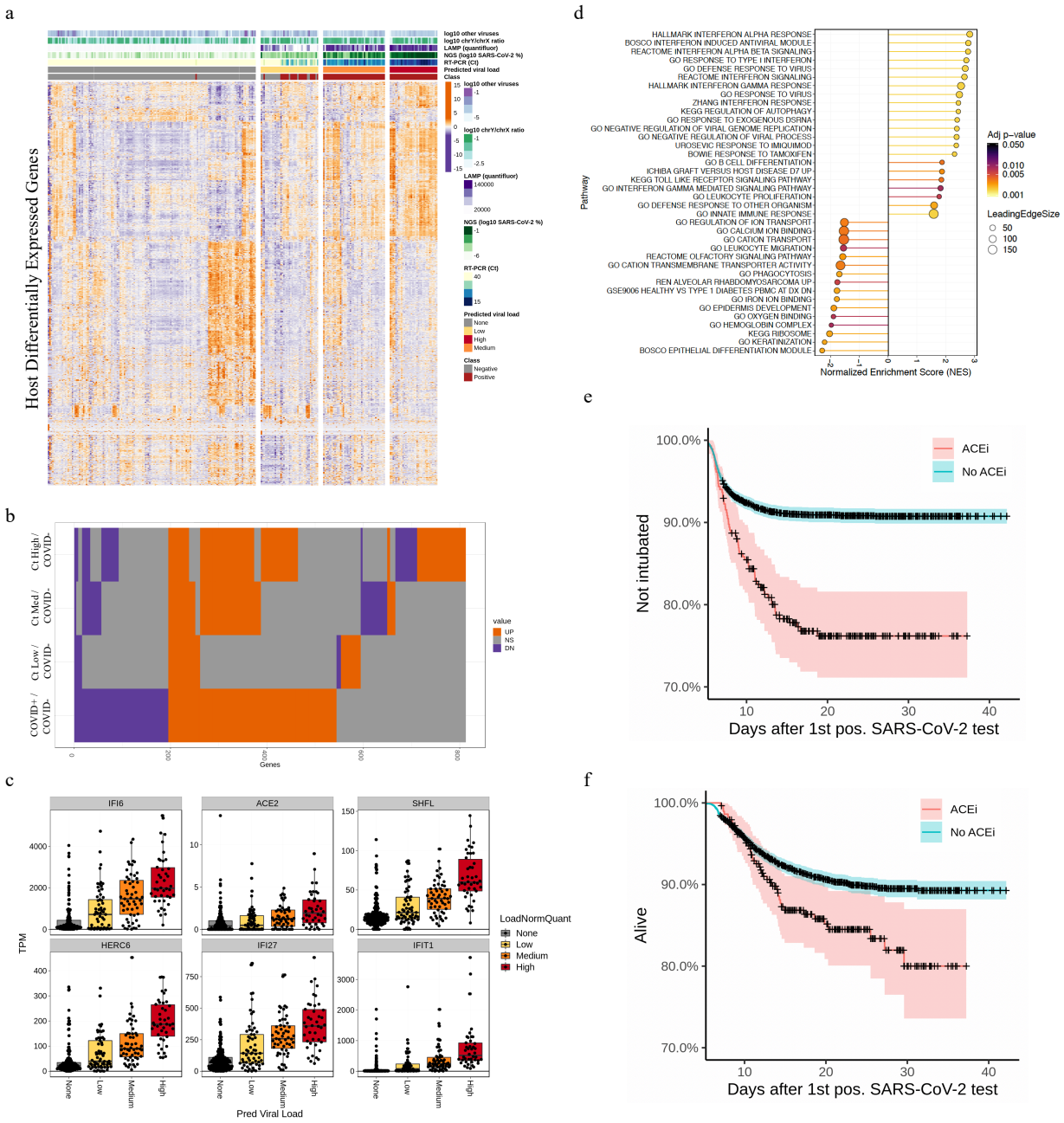


Figure 6: Host transcriptome responses and risk to SARS-CoV-2. (top row) Samples were quantified by a range of viral detection methods, including LAMP (Quantifluor), RNA-seq (log10 SARS-CoV-2 % of reads), and RT-PCR (Ct values) to create a three-tier range of viral load for the positive samples (right) compared to the clinically-annotated negative samples (class, red or grey). (bottom) The differentially expressed genes of COVID+ patients compared to COVID- patients showed up-regulated (orange) genes as well as down-regulated (purple) genes. (b) Up-regulated genes, with boxplots across all samples, include IFI6, ACE2, SHFL, HERC6, IFI27, and IFIT1, based on data from (c), which is the total set of DEGs. The full set is shown in an intersecting heat map, with a core set of up-regulated genes (orange) distinct form the set of down-regulated genes (purple), compared to genes that are not significantly differently

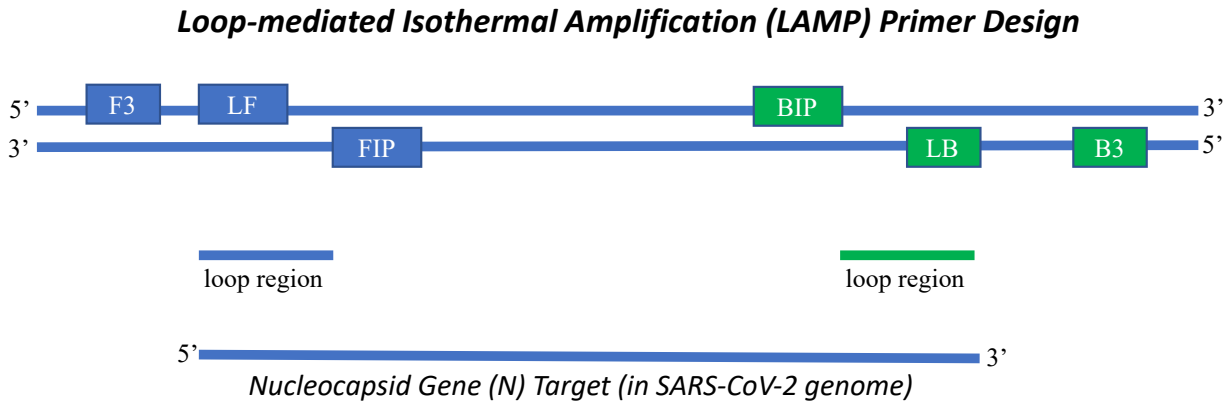
expressed (grey) in any comparison (DESeq2, q-value <0.01, |logFC| >0.58). (d) Significantly different gene ontology (GO) pathways between COVID-POS and COVID-NEG patients include interferon response and host response to infection, as well as some ion and heme transport mechanisms. (e)Survival curves for requiring mechanical respiration (identified by intubation procedure notes) (e) and mortality (f). Patients with a history of ACE inhibitor exposure were more likely to require intubation (HR=2.63 95%CI 2.01-3.43, p=1.22E-12; (e) and less likely to survive (HR=1.68 95%CI: 1.22-2.31, p=1.42E-03; (f). Because several individuals were intubated shortly before they first tested positive for SARS-CoV-2 infection, each first positive test was set back by seven days to account for testing delays.

Table 1. Baseline characteristics of SARS-CoV-2 suspected cohort. General population refers to a comparison cohort of individuals administered drugs at NYP/CUIMC in 2019 who were not later tested for SARS-COV-2 infection.

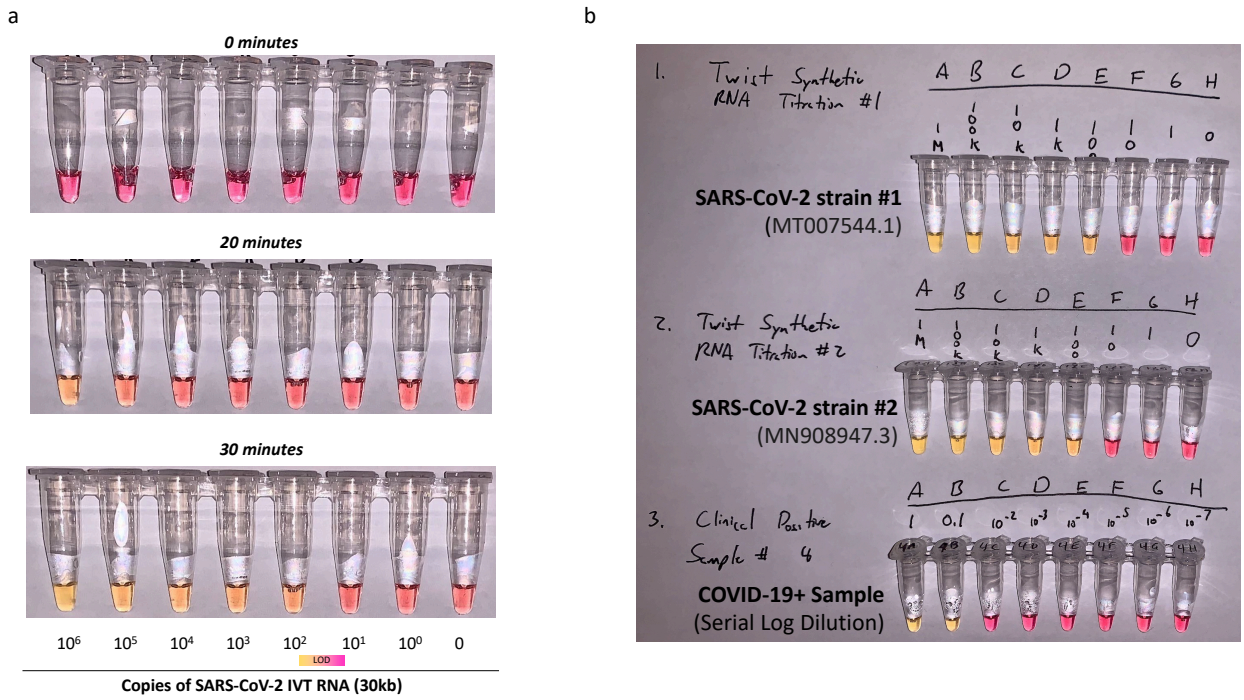
	General Population	**Tested**	**COV+**	**COV+/Intubated**	**COV+/Died**
N (% of tested)	279487	8278 (100%)	4574 (55.3%)	493 (6%)	423 (5.1%)
ACEi	20017 (7.2%)	370 (4.5%)	290 (6.3%)	69 (14%)	42 (9.9%)
Median age (95%)	28.8 (1.4-49.3)	55.7 (0.5-91.3)	62.1 (23.3-92.5)	65.1 (19.3-89.3)	79.2 (49-96.1)
Male	118555 (42.4%)	3806 (46%)	2413 (52.8%)	308 (62.5%)	251 (59.3%)
Black/ African-American	80449 (28.8%)	917 (11.1%)	531 (11.6%)	71 (14.4%)	59 (13.9%)
Caucasian	24267 (8.7%)	1392 (16.8%)	682 (14.9%)	89 (18.1%)	89 (21%)
Asian or Pacific Islander	9 (0.003%)	88 (1.1%)	30 (0.7%)	3 (0.6%)	2 (0.5%)
Other race	167 (0.06%)	1934 (23.4%)	1170 (25.6%)	165 (33.5%)	164 (38.8%)
Missing race	174595 (62.5%)	3947 (47.7%)	2161 (47.2%)	165 (33.5%)	109 (25.8%)
Hispanic/Latino	36905 (13.2%)	1750 (21.1%)	1088 (23.8%)	171 (34.7%)	159 (37.6%)
Non-Hispanic/Latino	87562 (31.3%)	1547 (18.7%)	746 (16.3%)	85 (17.2%)	87 (20.6%)
Other listed ethnicity	155020 (55.5%)	1034 (12.5%)	579 (12.7%)	72 (14.6%)	68 (16.1%)
Hypertension	104925 (38.4%)	2168 (26.2%)	1368 (29.9%)	221 (44.8%)	260 (61.5%)
CAD/CHD	155561 (56.9%)	816 (9.9%)	503 (11%)	84 (17%)	115 (27.2%)

Diabetes	46908 (17.2%)	1274 (15.4%)	834 (18.2%)	151 (30.6%)	174 (41.1%)
Overweight	39192 (14.3%)	452 (5.5%)	259 (5.7%)	43 (8.7%)	38 (9%)
No risk factors	110460 (40.4%)	5830 (70.4%)	3052 (66.7%)	248 (50.3%)	150 (35.5%)
Median IL-6 (50%)	-	30.6 (8-94.1)	31.1 (8-94.9)	68.2 (16-178)	68 (18-153.8)

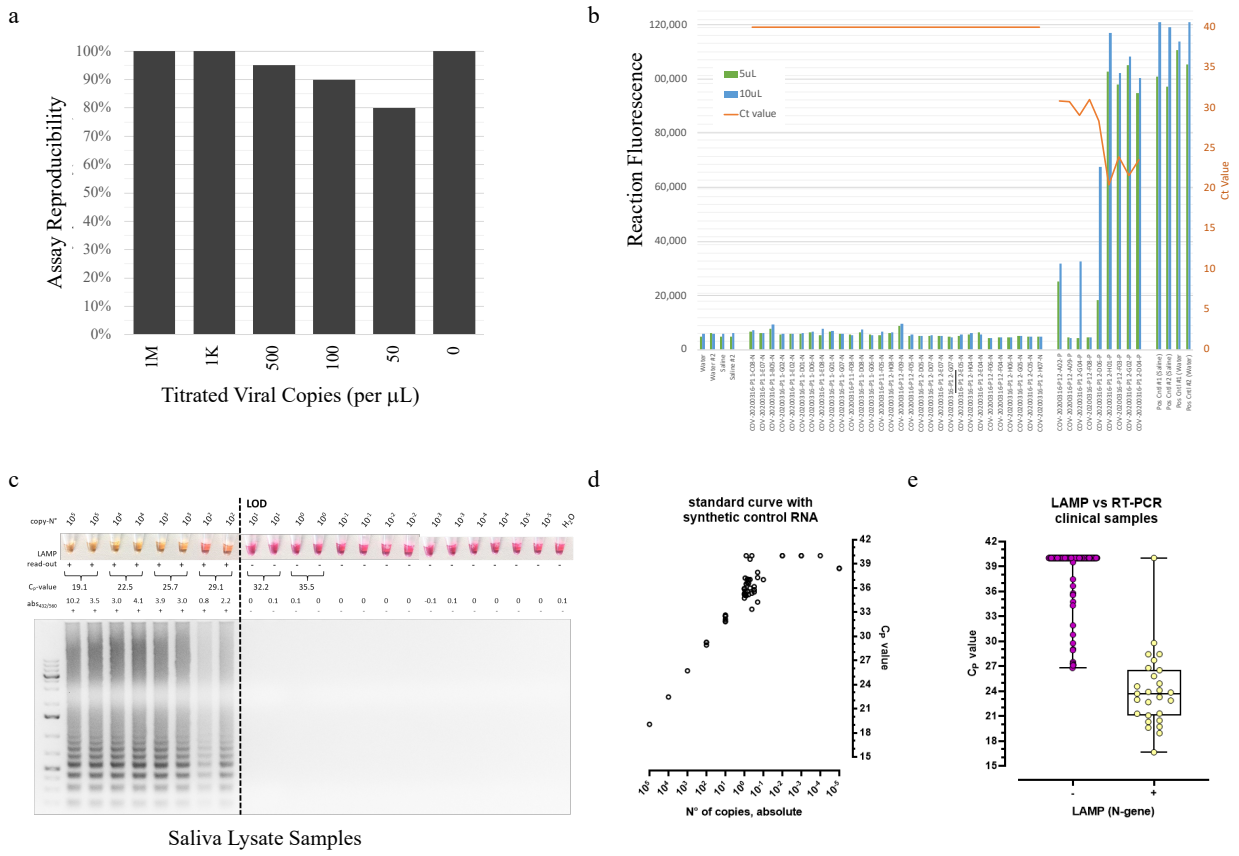
Supplemental Figures



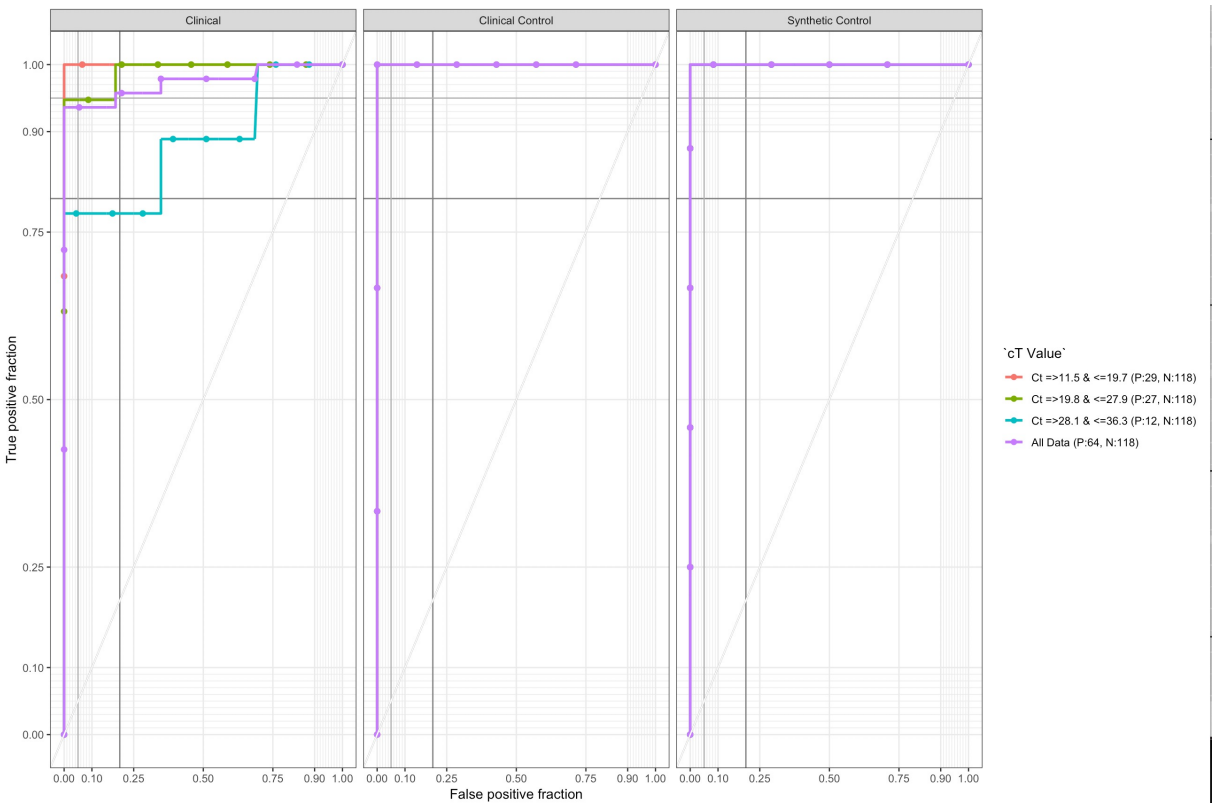
Supplemental Figure 1: LAMP Primer Design. PrimerDesigner (v4) was used to create a set of six primers that would specifically target the nucleocapsid gene (N) in the COVID-19 genome. Primer sequences are listed in the methods section.



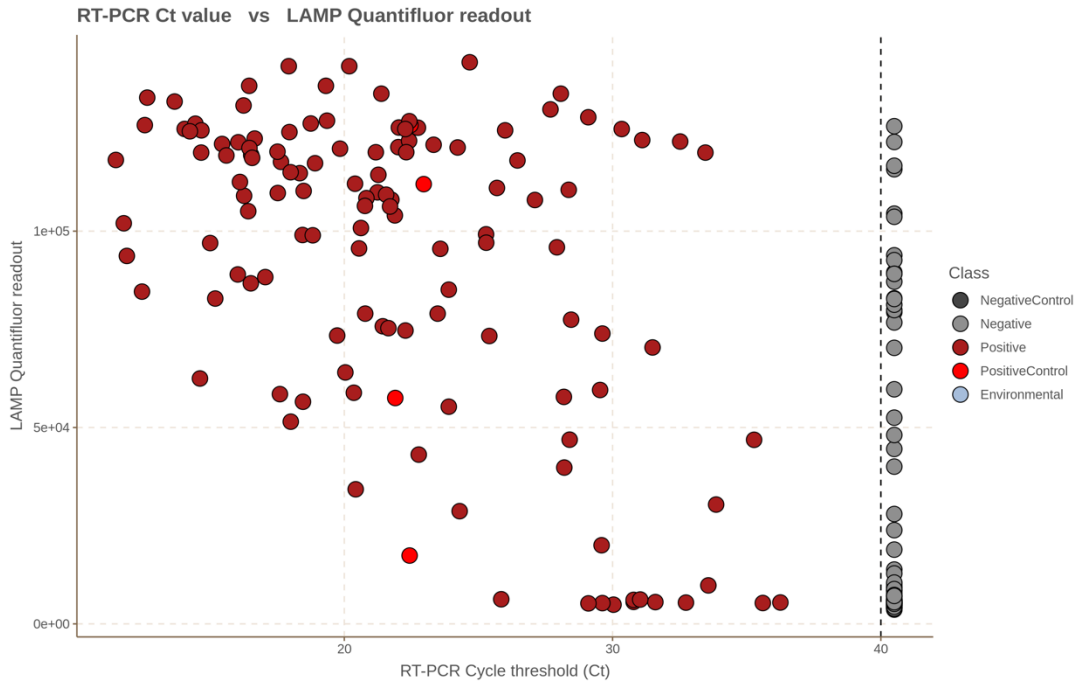
Supplemental Figure 2. Additional Testing and Titration of the LAMP Assay with Synthetic and Clinical Samples. Samples were prepared using the LAMP protocol with a reaction time of 30 minutes. Reaction progress was measured (a) from 0, 20, and 30 minutes. (b) This was repeated for both Twist COVID-19 synthetic RNAs (*MT007544.1*, top and MN908947.3, middle*)* from 1 million molecules of virus (10^6), then titrated down by log10 dilutions. Limit of Detection (LOD) range is shown with a gradient after 30 minutes between 10 and 100 viral copies. (bottom) A clinically positive sample that was not detectible by Qubit (<0.05 ng/mL) was nonetheless detected by LAMP, in accordance with detection of low viral titer samples.



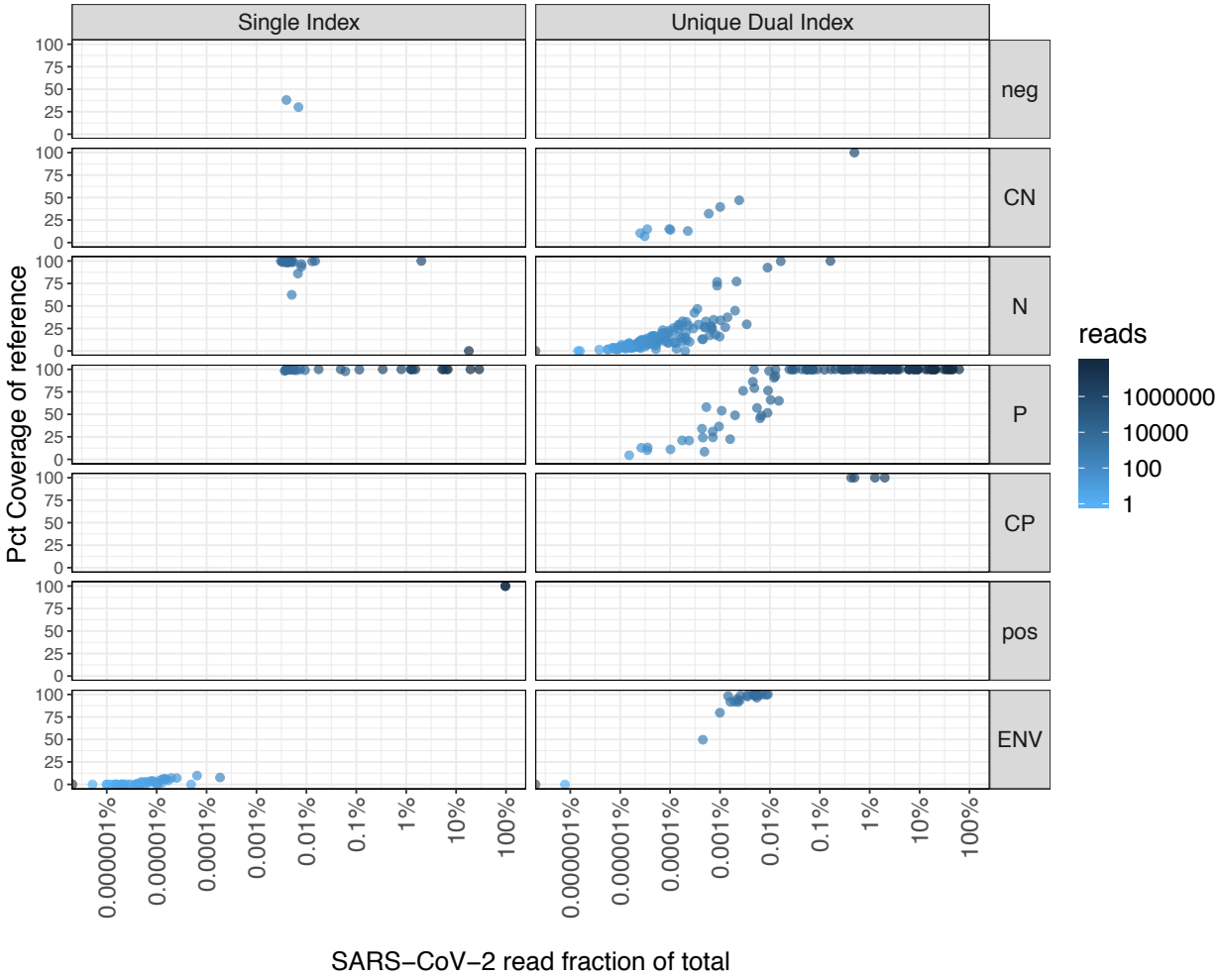
Supplemental Figure 3. Reproducibility, sensitivity, and specificity for the LAMP Assay. (a) Testing with a synthetic SARS-CoV-2 RNA that was serially titrated and measured in replicates (n=10) showed 100% and 95% reproducibility at 1,000 and 500 copies, respectively, with lower rates at lower viral titers. (b) Replicates of a clinically positive (by RT-PCR) sample at 10 uL (blue) compared to 5uL (green) showed high concordance, with greater sensitivity with increased reaction volume. (c) Whole oropharyngeal swab lysates from clinical positive (Cp-value >0) and negative samples (Cp = NA) were used to test the LAMP reaction. (d) The standard curve with synthetic RNA was also tested relative to absolute number of copies (x-axis) and the Cp value (y-axis). (e) The Cp value for the LAMP positive (+, right) and negative (-, left) were compared to the Cp value from RT-PCR (y-axis).



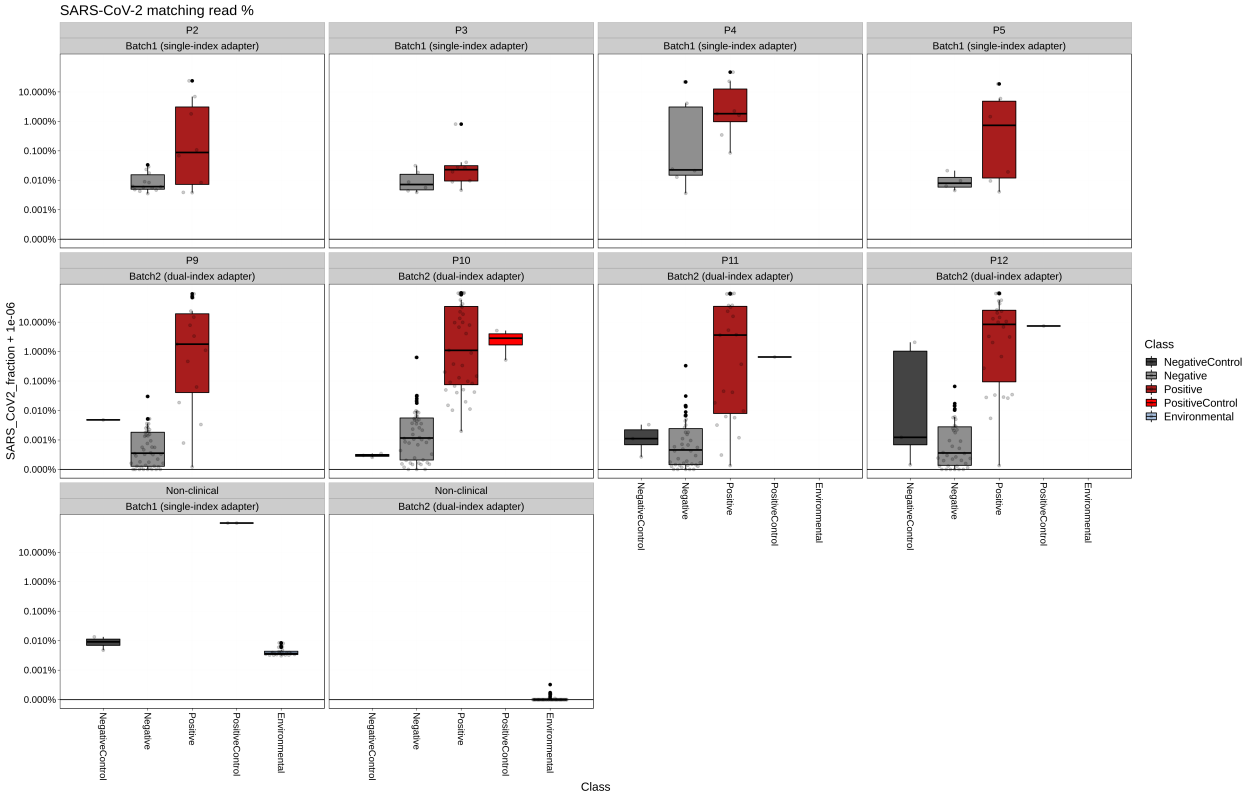
Supplemental Figure 4. Ct Thresholds for the LAMP Assay. With increasing viral load, as measured by RT-PCR, the LAMP assay shows an increased sensitivity (y-axis) and specificity (y-axis). For high and medium viral count samples (Ct < 28, red and green line), we see 100% sensitivity and specificity, and for low viral count, we see nearly 80% sensitivity and 100% specificity (blue). Across all data (purple), we see 94% sensitivity and 100% specificity.



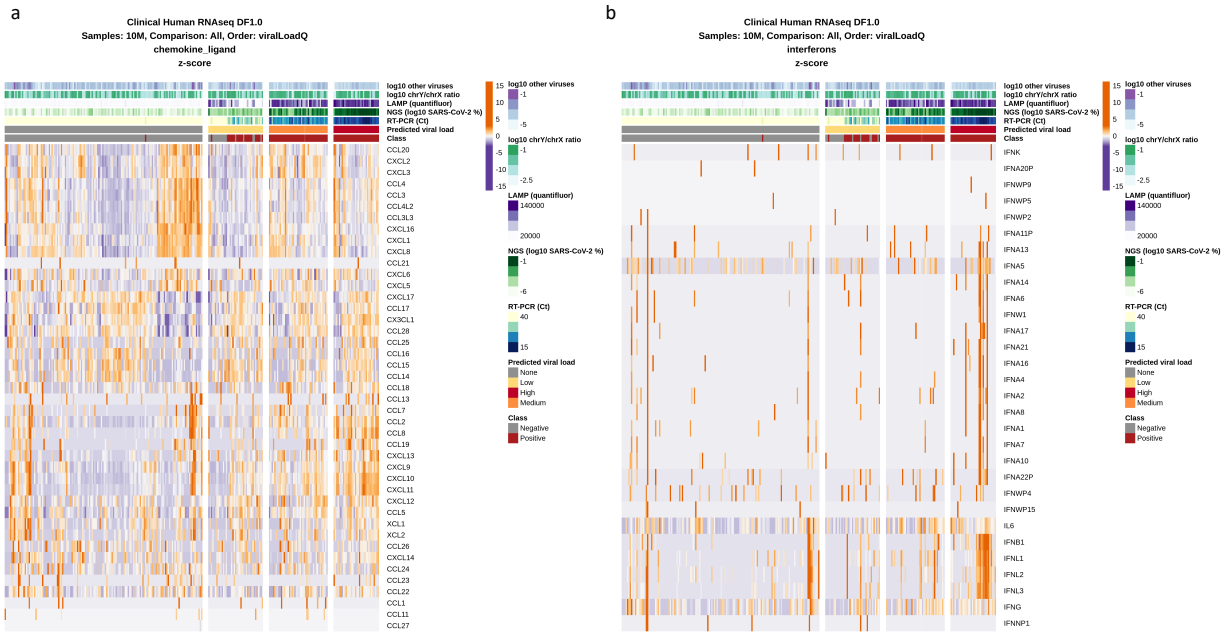
Supplementary Figure 5. Correlation between LAMP Reaction Output and RT-PCR. Clinical samples tested by RT-PCR (Positive, dark red or Negative, light grey) were run with the LAMP assay and compared to the buffer blanks (Negative Control), dark grey, Synthetic RNAs or Vero 6 cell extracts with SARS-CoV-2 infection (Positive Controls, light red), and Subway Samples (Environmental, blue, lower right). The DNA abundance, as measured with the GloMax Quantifluor (y-axis) is compared to the Ct Threshold for RT-PCR (x-axis), with lower Ct values representing higher viral abundance.



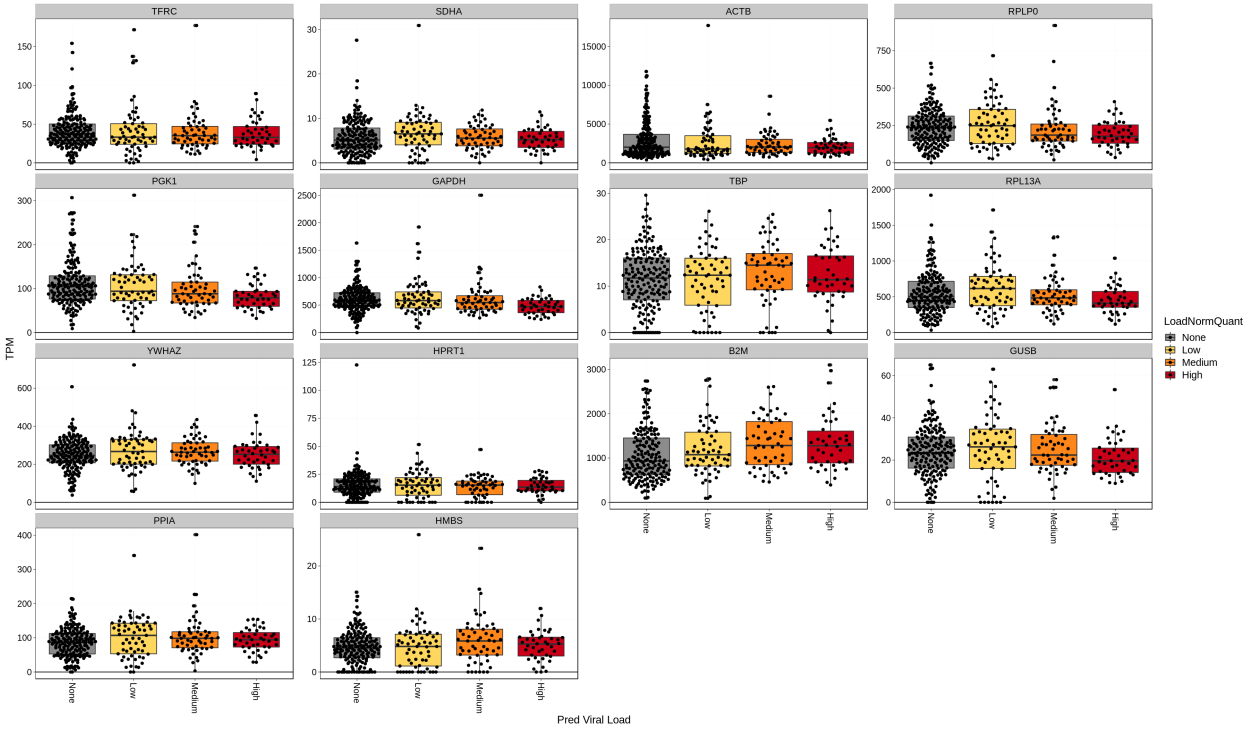
Supplementary Figure 6. Coverage of COVID samples by Index and Sample Type . Clinical samples tested by RT-PCR (Positive, P, Negative, N) were compared to the buffer blanks (Negative Control, N and neg), Synthetic RNAs or Vero 6 cell extracts with SARS-CoV-2 infection (CP and pos) and Subway Samples (Environmental, ENV). The %identity to the SAS-CoV-2 reference (y-axis) is shown relative to the proportion of reads that mapped from NGS (x-axis). The unique, dual-index runs (left panels) are shown relative to the single-index runs (right panels).



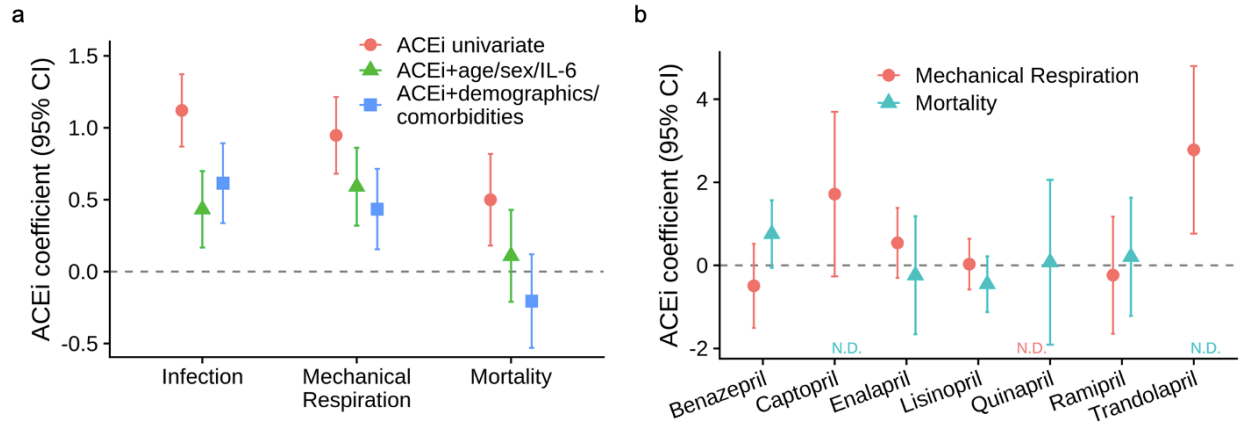
Supplemental Figure 7. Proportion of RNA-seq reads mapping SARS-CoV-2. Clinical samples tested by RT-PCR (Positive, dark red or Negative, light grey) were sequenced and compared to the buffer blanks (Negative Control), dark grey, Synthetic RNAs or Vero 6 cell extracts with SARS-CoV-2 infection (Positive Controls, light red), and Subway Samples (Environmental, blue). Read totals are shown on the y-axis. Differences between single index barcodes are plotted across each of the plates of samples that were processed.



Supplemental Figure 8. Cytokine and interferon profiles of the host transcriptome. Top rows: samples were quantified by a range of viral detection methods, including LAMP (Quantifluor), RNA-seq (log10 SARS-CoV-2 % of reads), and RT-PCR (Ct values) to create a three-tier range of viral load for the positive samples (right) compared to the clinically-annotated negative samples (class, red or grey). (bottom) The differentially expressed genes of COVID+ patients compared to COVID- patients showed up-regulated (orange) genes as well as down-regulated (purple) genes. (a) Chemokine profiles for the samples (x-axis) is plotted for each related gene (y-axis), and the same plotting function shows in (b) the interform and interleukin gene profiles of IL6.



Supplemental Figure 9. Housekeeping host genes relative to SARS-CoV-2 infection. Clinical samples were sequenced with RNA-seq and quantified to a set of genes for their expression levels. Samples with no virus (grey) were compared to those with low (yellow), medium (orange), and high (red) expression levels, based on RT-PCR.



Supplemental Figure 10. ACEI Multivariate and Comparative Analyses. (a) Regression coefficients for variables indicating exposure/history of exposure to ACE inhibitors for each of the three cohort comparisons: (left) test outcome in a cohort of patients suspected of SARS-CoV-2 infection, (middle) requirement of mechanical respiration in patients who tested positive, (right) mortality in patients who tested positive. Univariate analyses are shown as red circles. The green triangles coefficients are when correcting for age, sex, and baseline IL-6 levels take upon admission. The blue squares are from a model that includes age, sex, and IL-6 as well as comorbidities including CAD/CHD, diabetes, obesity, and self-reported race and ethnicity. (b) Comparison of the effects of different ACE inhibitors. We directly compared the effects of specific ACE inhibitors among those patients with evidence of exposure to ACE inhibitors. We found two significant associations: benazepril was significantly associated with

higher mortality (HR=2.37 95%CI 1.05-5.35, p=3.70E-02) and trandolapril was associated with requirement of mechanical ventilation (HR=15.85 95% CI: 2.11-119.08, p=7.25E-03). N.D. indicates that a result was not displayed due to low sample size and, therefore, very large errors.

Supplementary Table 1: Metatranscriptome Profiles of All Samples. Appended.

Supplementary Table 2: Taxonomic Mis-assignment Filter. The entire SARS-CoV-2 genome was fragmented (wgsim) and then re-mapping to the entire Kraken2 database. These species were then used to flag putative false positives from SARS-CoV-2 genome segments that are similar to other organisms.

Supplementary Table 2 : Other Taxonomic Alignments for SARS-CoV-2 (High-risk false positives)

Taxonomic group (domain, phylum, class, order, family, genus, species, strain)	TaxonName	% match
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Betacoronavirus|s__Severe acute respiratory syndrome-related coronavirus	Severe acute respiratory syndrome-related coronavirus (SARS)	95.0813%
d__Viruses|o__Nidovirales|f__Coronaviridae|s__Bat coronavirus BM48-31/BGR/2008	Bat coronavirus BM48-31/BGR/2008	0.3937%
d__Eukaryota|k__Metazoa|p__Chordata|c__Mammalia|o__Primates|f__Hominidae|g__Homo|s__Homo sapiens	Homo sapiens	0.0170%
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Alphacoronavirus|s__Rhinolophus ferrumequinum alphacoronavirus HuB-2013	Rhinolophus ferrumequinum alphacoronavirus HuB-2013	0.0164%
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Betacoronavirus|s__Betacoronavirus 1	Betacoronavirus 1	0.0099%
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Betacoronavirus|s__Murine coronavirus	Murine coronavirus	0.0089%
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Betacoronavirus|s__Rousettus bat coronavirus GCCDC1	Rousettus bat coronavirus GCCDC1	0.0059%
d__Viruses|o__Nidovirales|f__Coronaviridae|s__Bat coronavirus	Bat coronavirus	0.0049%
d__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae|g__Clostridium|s__Clostridium botulinum	Clostridium botulinum	0.0045%
d__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae|g__Clostridium|s__Clostridium perfringens	Clostridium perfringens	0.0037%
d__Bacteria|p__Fusobacteria|c__Fusobacteriia|o__Fusobacteriales|f__Fusobacteriaceae|g__Fusobacterium|s__Fusobacterium varium	Fusobacterium varium	0.0027%
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Alphacoronavirus|s__Myotis ricketti alphacoronavirus Sax-2011	Myotis ricketti alphacoronavirus Sax-2011	0.0025%
d__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Erwiniaceae|g__Buchnera|s__Buchnera aphidicola	Buchnera aphidicola	0.0024%
d__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Thermoactinomycetaceae|g__Thermoactinomyces|s__Thermoactinomyces vulgaris	Thermoactinomyces vulgaris	0.0023%
d__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__Streptococcus|s__Streptococcus pyogenes	Streptococcus pyogenes	0.0023%
d__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rickettsiales|f__Anaplasmataceae|g__Ehrlichia|s__Ehrlichia sp. HF	Ehrlichia sp. HF	0.0018%
d__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillaceae|g__Bacillus|s__Bacillus cereus	Bacillus cereus	0.0017%
d__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|g__Candidatus Azobacteroides|s__Candidatus Azobacteroides pseudotrichonymphae	Candidatus Azobacteroides pseudotrichonymphae	0.0017%
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Betacoronavirus|s__Rousettus bat coronavirus HKU9	Rousettus bat coronavirus HKU9	0.0015%
d__Viruses|s__Beihai picorna-like virus 116	Beihai picorna-like virus 116	0.0015%
d__Bacteria|p__Proteobacteria|c__Epsilonproteobacteria|o__Campylobacterales|f__Helicobacteraceae|g__Helicobacter|s__Helicobacter pylori	Helicobacter pylori	0.0014%
d__Bacteria|p__Proteobacteria|c__Betaproteobacteria|o__Burkholderiales|f__Oxalobacteraceae|g__Janthinobacterium|s__Janthinobacterium sp. B9-8	Janthinobacterium sp. B9-8	0.0014%
d__Eukaryota|p__Apicomplexa|c__Aconoidasida|o__Haemosporida|f__Plasmodiidae|g__Plasmodium|s__Plasmodium coatneyi	Plasmodium coatneyi	0.0014%
d__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Yersiniaceae|g__Serratia|s__Serratia symbiotica	Serratia symbiotica	0.0013%
d__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Pseudomonadales|f__Moraxellaceae|g__Acinetobacter|s__Acinetobacter baumannii	Acinetobacter baumannii	0.0013%
d__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae|g__Clostridium|s__Clostridium septicum	Clostridium septicum	0.0013%
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Betacoronavirus|s__Tylonycteris bat coronavirus HKU4	Tylonycteris bat coronavirus HKU4	0.0012%
d__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Staphylococcaceae|g__Staphylococcus|s__Staphylococcus cohnii	Staphylococcus cohnii	0.0012%
d__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Clostridiaceae|g__Caloranaerobacter|s__Caloranaerobacter azorensis	Caloranaerobacter azorensis	0.0012%
d__Bacteria|p__Cyanobacteria|o__Nostocales|f__Calotrichaceae|g__Calothrix|s__Calothrix sp. NIES-2098	Calothrix sp. NIES-2098	0.0012%
d__Viruses|o__Nidovirales|f__Coronaviridae|g__Betacoronavirus|s__Bat Hp-betacoronavirus Zhejiang2013	Bat Hp-betacoronavirus Zhejiang2013	0.0011%
d__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Erwiniaceae|g__Erwinia|s__Candidatus Erwinia haradaeae	Candidatus Erwinia haradaeae	0.0011%
d__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Thiotrichales|f__Thiotrichaceae|g__Thioploca|s__Thioploca ingrica	Thioploca ingrica	0.0011%
d__Bacteria|p__Proteobacteria|c__Alphaproteobacteria|o__Rhodospirillales|f__Acetobacteraceae|g__Bombella|s__Bombella sp. KACC 21507	Bombella sp. KACC 21507	0.0011%
d__Bacteria|p__Cyanobacteria|o__Chroococcales|f__Aphanothecaceae|g__Candidatus Atelocyanobacterium|s__Candidatus Atelocyanobacterium thalassa	Candidatus Atelocyanobacterium thalassa	0.0011%
d__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Erwiniaceae|g__Wigglesworthia|s__Wigglesworthia glossinidia	Wigglesworthia glossinidia	0.0010%
d__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Enterobacterales|f__Enterobacteriaceae|g__Enterobacter|s__Enterobacter hormaechei	Enterobacter hormaechei	0.0010%
d__Bacteria|p__Proteobacteria|c__Gammaproteobacteria|o__Alteromonadales|f__Colwelliaceae|g__Colwellia|s__Colwellia sp. PAMC 20917	Colwellia sp. PAMC 20917	0.0010%
d__Bacteria|p__Proteobacteria|c__Betaproteobacteria|o__Neisseriales|f__Neisseriaceae|g__Neisseria|s__Neisseria flavescens	Neisseria flavescens	0.0010%
d__Bacteria|p__Bacteroidetes|c__Flavobacteriia|o__Flavobacteriales|f__Flavobacteriaceae|g__Lutibacter|s__Lutibacter profundi	Lutibacter profundi	0.0010%

Supplementary Table 3: Differentially Expressed Genes in COVID-19+/- patients. Appended.

Supplemental Table 4: Gene Ontology Pathways.
Appended

Supplementary Table 5: COVID-19 Rapid Colorimetric LAMP Detection Test: N Primers Specificity

Supplemental Table 5: COVID-19 Rapid Colorimetric LAMP Detection Test: N Primers						
Organism	Range % Homology with F3 Primer	Range % Homology with B3 Primer	Range % Homology with FIP Primer	Range % Homology with BIP Primer	Range % Homology with LF Primer	Range % Homology with LB Primer
Human coronavirus 229E (https://www.ncbi.nlm.nih.gov/nuccore/NC_002645.1)	0%	0%	0%	50%	0%	0%
Human coronavirus OC43 (https://www.ncbi.nlm.nih.gov/assembly/GCF_003972325	0%	0%	0%	0%	0%	0%
Human coronavirus HKU1 (https://www.ncbi.nlm.nih.gov/nuccore/NC_006577.2)	0%	0%	0%	0%	0%	0%
Human coronavirus NL63 (https://www.ncbi.nlm.nih.gov/nuccore/NC_005831.2)	0%	0%	0%	0%	0%	0%
SARS-coronavirus (https://www.ncbi.nlm.nih.gov/nuccore/NC_004718.3)	100%	85%	46%	43-50%	88%	100%
MERS-coronavirus (https://www.ncbi.nlm.nih.gov/nuccore/NC_038294.1)	0%	0%	0%	0%	0%	0%
SARS-coronavirus 2 (https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2)	100%	100%	46%-54%	45%-55%	100%	100%
Bordetella pertussis 18323, complete sequence	0%	0%	37%-41%	65%-73%	72%	0%
Candida albicans SC5314 chromosome 1 sequence	0%	65%-75%	0%	35%	0%	0%
Candida albicans SC5314 chromosome 2 sequence	0%	0%	0%	38%	64%	86%
Candida albicans SC5314 chromosome 3 sequence	0%	0%	0%	0%	64%	76%
Candida albicans SC5314 chromosome 4 sequence	0%	0%	0%	0%	68%	0%
Candida albicans SC5314 chromosome 5 sequence	0%	65%	0%	0%	0%	0%
Candida albicans SC5314 chromosome 7 sequence	0%	65%	0%	35%	0%	0%
Candida albicans SC5314 chromosome R sequence	0%	0%	0%	0%	68%	0%
Chlamydia pneumoniae TW-183, complete sequence	79%	0%	0%	0%	0%	0%
Haemophilus influenzae genome assembly NCTC8143, chromosome : 1	0%	0%	0%	0%	64%-72%	0%
Legionella pneumophila subsp. pascullei strain NCTC12273, chromosome: 1	0%	65%-75%	0%	0%	72%	0%
Mycobacterium tuberculosis H37Rv, complete genome	0%	0%	37%-59%	0%	0%	0%
Mycoplasma pneumoniae FH chromosome, complete genome	0%	0%	0%	0%	72%	0%
Pneumocystis jirovecii RU7 chromosome Unknown supercont1.14	0%	0%	0%	0%	76%	0%
Pneumocystis jirovecii RU7 chromosome Unknown supercont1.15	0%	0%	0%	0%	64%-72%	0%
Pneumocystis jirovecii RU7 chromosome Unknown supercont1.8	0%	85%	0%	0%	64%	0%
Pneumocystis jirovecii RU7 chromosome Unknown supercont1.9	0%	0%	0%	0%	64%	0%
Rothia mucilaginosa DY-18 DNA, complete genome	0%	80%	0%	0%	0%	62%
Staphylococcus epidermidis ATCC 12228, complete sequence	0%	75%	0%	35%	76%	0%
Streptococcus pneumoniae genome assembly NCTC7465, chromosome : 1	0%	85%	41%	0%	64%	62%
Streptococcus pyogenes genome assembly NCTC8198, chromosome : 1	0%	0%	0%	35%	64%-68%	62%