Conclusion. Of the markers studied, both d-dimer and CRP were considered useful by most respondents. LDH and ferritin were used less frequently and were not considered as useful in guiding medical decision making. Discontinuation of standing daily LDH and ferritin orders is believed to have potential to result in cost savings to the health care system with no adverse patient outcomes.

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366. Abbott BinaxNOW Rapid Antigen Test Performance in Detecting SARS-CoV-2 Infections in a COVID-19 Outbreak Among Horse Racetrack Workers

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Session: P-15. COVID-19 Diagnostics

Background. Rapid antigen tests (e.g., Abbott's BinaxNOW) are cheaper and faster than nucleic acid amplification tests (e.g., real-time reverse transcription polymerase chain reaction [RT-PCR]) for SARS-CoV-2 infection, with variable reported sensitivity. A horse racetrack in California experienced a COVID-19 outbreak among staff and used BinaxNOW to supplement RT-PCR. Utility of BinaxNOW in detecting SARS-CoV-2 infection in a workplace outbreak was assessed.

Methods. Between November 25–December 22, 2020, anterior nasal swabs were collected from racetrack staff for six rounds of paired BinaxNOW and RT-PCR tests. BinaxNOW tests were interpreted according to manufacturer instructions. RT-PCR was performed at the state public health lab using the ThermoFisher TaqPath COVID-19 Combo Kit. Staff with positive results on either test were isolated and removed from subsequent testing. Viral cultures were attempted on specimens with cycle threshold $(C_t) < 30$.

Results. Overall, 769 paired results from 342 staff were analyzed. Most were of Hispanic ethnicity (62.0%) and ages ranged from 18 to 92 years (median 52). BinaxNOW performance compared to RT-PCR (95% CI) was as follows: positive percent agreement (PPA) 43.3% (34.6%–52.4%); negative percent agreement (NPA) 100% (99.4%–100%); positive predictive value (PPV) 100% (93.5%–100%); negative predictive value 89.9% (87.5%–92.0%). Among 127 RT-PCR-positive specimens, those with paired BinaxNOW-positive results (n = 55) had a lower mean C_t value than those with paired BinaxNOW-negative results (n = 72) (17.8 vs. 28.5) (p < 0.001). In dual positive pairs, median time from specimen collected to RT-PCR result reported was 4 days (range 1-6), compared to the 15-minute BinaxNOW reporting time. Of 100 $C_t < 30$ specimens, 51 resulted in positive virus isolation, 45 (88.2%) of which were BinaxNOW-positive.

Conclusion. High NPA and PPV support immediate isolation of BinaxNOW-positive individuals, while low PPA supports confirmatory testing following BinaxNOW-negative results. BinaxNOW performed better in paired specimens with lower C_t value and positive viral cultures, which could suggest that among RT-PCR-positive specimens, those that are BinaxNOW-negative may be less likely to contain infectious virus than those that are BinaxNOW-positive.

Disclosures. **David Seftel, M.D., M.D., M.B.A.**, **Enable Biosciences, Inc** (Board Member, Employee, Scientific Research Study Investigator, Shareholder)

367. Role of Conventional Biomarker for Prediction of Chest CT-confirmed COVID-19 Pneumonia

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Session: P-15. COVID-19 Diagnostics

Background. The coronavirus disease 2019 (COVID-19) has a wide range of severity. Chest computed tomography (CT) had high sensitivity and specificity to identify COVID-19 pneumonia. However, chest CT was not available in almost all hospitals in pandemic settings, including developed countries. This study is to evaluate the potential role of conventional inflammatory biomarkers to predict COVID-19 pneumonia.

Methods. All 155 RT-PCR-confirmed COVID-19 patients were evaluated for pneumonia by chest CT from April 10, 2021 to May 3, 2021 in the outpatient unit, a Thai university hospital. The inflammatory biomarkers were evaluated the sensitivity, specificity, LR+, LR-, and ROC to predict COVID-19 pneumonia.

Results. Of all 155 patients, pneumonia was diagnosed by chest CT in 117 patients. The pneumonia patients had a median (IQR) age of 38 (30, 55) years old. The BMI was higher in pneumonia than mild illness in 25.5 (22.0, 29.5) and 22.9 (19.4, 26.9) kg/m^2, respectively (p=0.031). In univariate analysis, serum high-sensitivity C-reactive protein (hsCRP), lactate dehydrogenase (LDH), ferritin, total lymphocyte count (TLC), and albumin were associated with pneumonia, but the only hsCRP demonstrated association by multivariate analysis. The area under the ROC curves (AUC) was 0.82, 0.74, 0.68, 0.38, and 0.37 in hsCRP, LDH, ferritin, TLC, and albumin, respectively. The optimal cut-off level for CRP to diagnose COVID-19 pneumonia was 2.00 mg/L given sensitivity, specificity, LR+, LR- of 81.9%, 70.3%, 2.75, and 0.26 respectively (Figure 1 and Table 1).

Table 1. Demonstrated sensitivity, specificity, LR+, and LR- for each specific cut-off value of hsCRP

Cut-off for hsCRP ($\geq$ mg/L)	Sensitivity (%)	Specificity (%)	LR+	LR-
1.90	81.9	64.9	2.33	0.28
1.95	81.9	67.6	2.53	0.27
2.00*	81.9	70.3	2.75	0.26
2.05	80.2	70.3	2.70	0.28
2.10	80.2	70.3	2.70	0.28

*Indicated optimal cut-off value for hsCRP to predict chest CT-confirmed pneumonia.

ROC Curve of hsCRP to Diagnose of COVID-19 Pneumonia
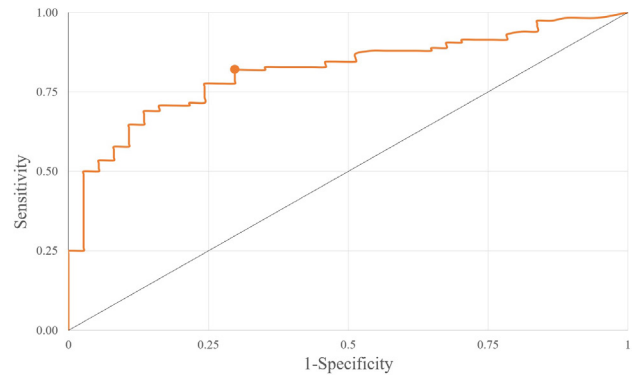
This figure shows ROC curve for hsCRP to diagnose of chest CT-confirmed COVID-19 pneumonia. The area under the ROC curve is 0.82. The optimal cut-off value for hsCRP is 2.00 given sensitivity of 81.9% and specificity of 70.3%.

Conclusion. The hsCRP was the conventional biomarker that had an excellent performance in predicting COVID-19 pneumonia lead to early anti-SARS-CoV-2 treatment. This study demonstrated the potential role of hsCRP combined with clinical assessment in negative chest X-rays to replace chest CT in a high burden COVID-19 country during pandemic situations.

Disclosures. **All Authors**: No reported disclosures

368. Performance Characteristics of Sequencing Assays for Identification of the SARS-CoV-2 Viral Genome

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Session: P-15. COVID-19 Diagnostics

Background. As the SARS-CoV-2 (SCV-2) virus evolves, diagnostics and vaccines against novel strains rely on viral genome sequencing. Researchers have gravitated towards the cost-effective and highly sensitive amplicon-based (e.g. ARTIC) and hybrid capture sequencing (e.g. SARS-CoV-2 NGS Assay) to selectively target the SCV-2 genome. We provide an *in silico* model to compare these 2 technologies and present data on the high scalability of the Research Use Only (RUO) workflow of the SARS-CoV-2 NGS Assay.

Methods. *In silico* work included alignments of 383,656 high-quality genome sequences belonging to variant of concern (VOC) or variant of interest (VOI) isolates (GISAID). We profiled mismatches and sequencing dropouts using the ARTIC V3 primers, SARS-CoV-2 NGS Assay probes (Twist Bioscience) and 11 synthesized viral sequences containing mutations and compared the performance of these assays using clinical samples. Further, the miniaturized hybrid capture workflow was optimized and evaluated to support high-throughput (384-plex). The sequencing data was processed by COVID-DX software.

Results. We detected 101,432 viruses (27%) with > = 1 mismatch in the last 6 base pairs of the 3' end of ARTIC primers; of these, 413 had > = 2 mismatches in one primer. In contrast, only 38 viruses (0.01%) had enough mutations (> = 10) in a hybrid capture probe to have a similar effect on coverage. We observed that mutations in ARTIC primers led to complete dropout of the amplicon for 4/11 isolates and diminished coverage in additional 4. Twist probes showed uniform coverage throughout with little to no dropouts. Both assays detected a wide range of variants (~99.9% coverage at 5X depth) in clinical samples (CT value < 30) collected in NY (Spring 2020-Spring 2021). The distribution of the number of reads and on target rates were more uniform among specimens within amplicon-based sequencing. However, uneven genome coverage and primer dropouts, some in the spike protein, were observed on VOC/VOI and other isolates highlighting limitations of an amplicon-based approach.

Conclusion. The RUO workflow of the SARS-CoV-2 NGS Assay is a comprehensive and scalable sequencing tool for variant profiling, yields more consistent coverage and smaller dropout rate compared to ARTIC (0.05% vs. 7.7%).

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369. Alternative Workflow for SARS-CoV-2 Testing Using a Heat Lysis Protocol for Respiratory Specimens

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Session: P-15. COVID-19 Diagnostics

Background. The SARS-CoV-2 pandemic has demonstrated the need for streamlined workflows in high-throughput testing. In extraction-based testing, limited extraction reagents and required proprietary instrumentation may pose a bottleneck for labs. As a solution, ChromaCode developed a Direct Extraction protocol for the HDPCR™ SARS-CoV-2 Assay, distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C., which allows for the processing of specimens without an extraction system. In lieu of an extraction system, the Direct Extraction protocol uses a thermal cycler to lyse and inactivate specimens which are directly added to the Polymerase Chain Reaction (PCR).

Methods. The Limit of Detection (LoD), Clinical Performance, and effect of Interfering Substances was determined for the Direct Extraction protocol. The LoD was established on 6 PCR platforms with dilutions of inactivated SARS-CoV-2 virus spiked into residual, negative nasopharyngeal swab (NPS) matrix. Clinical performance was assessed with 48 positive and 50 negative frozen retrospective samples using the Direct Extraction protocol compared to an external Emergency Use Authorized (EUA) comparator assays (cobas® Liat® SARS-CoV-2 & Influenza A/B assay and the Hologic Panther Fusion® SARS-CoV-2 Assay respectively) on three PCR platforms. The Direct Extraction protocol was evaluated for performance in the presence of 13 potentially interfering substances that can be present in a respiratory specimen.

Results. The LoD of the Direct Extraction protocol ranges from 1000 – 3000 genomic equivalents (GE)/mL. The clinical performance of the assay was 95.8% positive agreement (95% CI of 84.6% - 99.3%) and 100% negative agreement (95% CI of 90.9% - 100% or 91.1% – 100%) across all three PCR platforms tested. The viral target was detected at 3X LoD for all interferents tested.

Conclusion. The Direct Extraction protocol of ChromaCode's SARS-CoV-2 Assay is a sensitive test that eliminates the need for sample extraction and performs very well against traditional extraction-based workflows. The inclusion of this protocol can reduce costs, reliance on extraction systems, and time associated with extraction-based protocols.

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370. Examining the Relationship Between SARS-CoV-2 PCR Cycle Threshold, Disease Severity and Epidemiologic Trends

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**Session:** P-16. COVID-19 Epidemiology and Screening

Background. Real-time reverse transcriptase PCR (rRT-PCR) has become the primary method for detection of SARS-CoV-2. Specific measurements of cycle threshold (Ct) values can give an estimate of viral load. Previous studies have shown temporal trends in Ct values, which could be used to predict the phase of the pandemic. This study's goal was to examine the relationship between Ct and disease severity, as well as Ct trends.

Methods. Testing was performed using the Abbott M2000 SARS-CoV-2 assay. Data was collected for 262 SARS-CoV-2 positive patients from March-May 2020. Kruskal-Wallis testing was performed to determine differences in median Ct based on age, gender, race and ethnicity. To determine relationship between symptom onset and clinical severity with Ct, linear and logistic regression were performed.

Results. The majority of the patients had mild to moderate disease. Average time since symptom onset was 5.9 days, and 92% were symptomatic. Figure 1 demonstrates the distribution of Ct by disease severity at time of testing. There was no significant difference in cycle threshold by sex, age, race or ethnicity. Figure 2 shows weekly mean cycle threshold by total new cases in Massachusetts to reflect temporal trend of Ct and cases. In the multivariable linear regression model, Ct increased with days since symptom onset (P< 0.001). Cycle threshold was inversely associated with disease severity in multivariable logistic regression though (OR 1.06, 95%CI 1.01-1.11, p=0.03), even when controlling for time since symptom onset.

Figure 1. Distribution of Ct by disease severity at time of SARS-CoV-2 testing
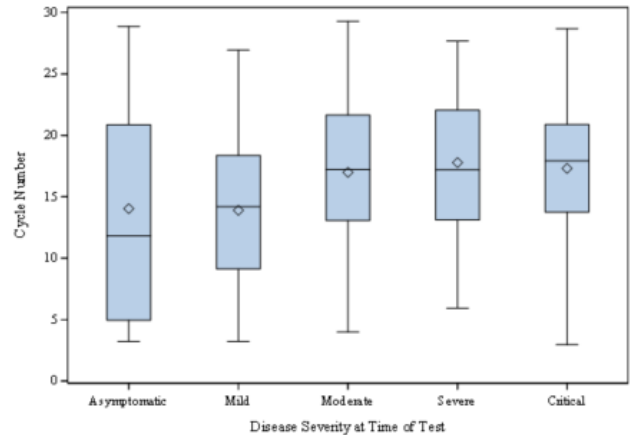
Boxplot demonstrating distribution of Ct by disease severity at time of testing. There was no significant difference between groups.

Figure 2. Weekly Mean Cycle Threshold by Total New MA Cases
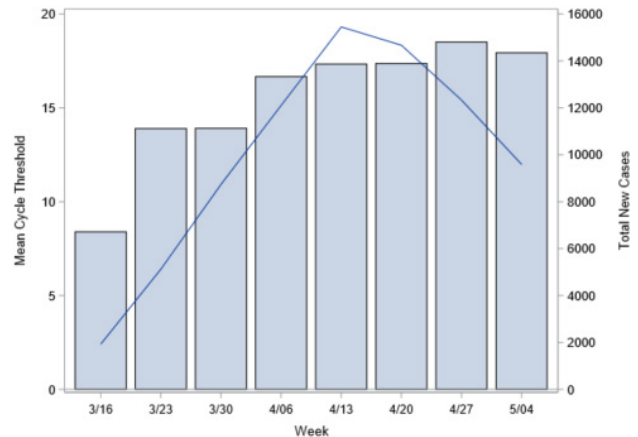
Line represents mean Ct over time period included in this study overlaid on total new cases in Massachusetts. Lower Ct were seen in the course as cases were increasing which peaked as cases stabilized.

Conclusion. Cycle threshold increased with time since symptom onset, consistent with prior data showing increasing Ct from time since infection due to decreasing viral replication. This study showed an inverse relationship between cycle threshold and disease severity, which differs from previous studies which demonstrated higher odds of progression to severe disease and mortality with lower Ct. This finding may reflect the disease severity associated with the secondary inflammatory phase of SARS-CoV-2 seen later in the disease course, although there was only moderate correlation between Ct and time since symptom onset. Further research is needed to better understand the role of Ct in predicting clinical severity of SARS-CoV-2 infections.

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371. Estimating SARS-CoV-2 Seroprevalence from Spent Blood Samples, January–March 2021

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Session: P-16. COVID-19 Epidemiology and Screening

Background. Measuring SARS-CoV-2 antibody prevalence in spent samples at serial time points can determine seropositivity in a diverse pool of individuals to inform understanding of trends as vaccinations are implemented.