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%).

Disclosures. **Danny Antaki, PhD**, **Twist Bioscience** (Employee, Shareholder) **Mara Couto-Rodriguez, MS**, **Biotia** (Employee) **Kristin Butcher, MS**, **Twist Bioscience** (Employee, Shareholder) **Esteban Toro, PhD**, **Twist Bioscience** (Employee) **Bryan Höglund, BS**, **Twist Bioscience** (Employee, Shareholder) **Xavier O. Jirau Serrano, B.S.**, **Biotia** (Employee) **Joseph Barrows, MS**, **Biotia** (Employee) **Christopher Mason, PhD**, **Biotia** (Board Member, Advisor or Review Panel member, Shareholder) **Niamh B. O'Hara, PhD**, **Biotia** (Board Member, Employee, Shareholder) **Dorottya Nagy-Szakal, MD PhD**, **Biotia Inc** (Employee, Shareholder)

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.

Disclosures. **Meghna Yadav, Ph.D. Molecular Biology**, **ChromaCode Inc.** (Employee, Shareholder) **Tiffany Martinez, n/a**, **ChromaCode** (Employee, Shareholder) **Isabel Regoli, MS, Bioinformatics**, **ChromaCode** (Employee, Shareholder) **Osvaldo Hernandez, B.S., Molecular Biology**, **ChromaCode** (Employee, Shareholder) **Phuong Le, B.S., Biochemistry**, **ChromaCode** (Employee, Shareholder) **Heather Carolan, Masters, Computational Molecular Biology**, **ChromaCode** (Employee, Shareholder) **Brad Brown, Ph.D Biomedical Sciences**, **ChromaCode** (Employee, Shareholder) **Karen Menge, Ph.D. Biochemistry**, **ChromaCode** (Employee, Shareholder)**ChromaCode** (Employee, Shareholder)

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

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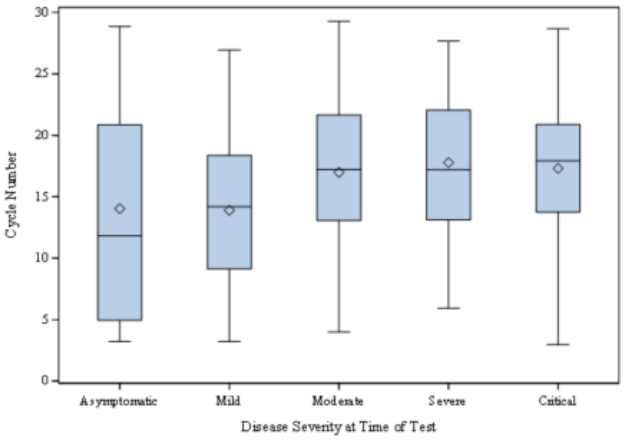
Session: P-16. COVID-19 Epidemiology and Screening

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.

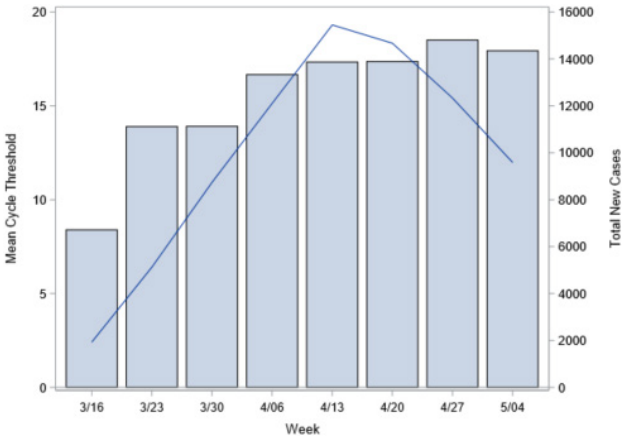
Results. The majority of the patients had mild to moderate disease. Average time since symptom onset was 5.9 days, and 92% were symptomatic. Figure 1 demonstrates the distribution of Ct by disease severity at time of testing. There was no significant difference in cycle threshold by sex, age, race or 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.

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

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.