Extraction-free rapid cycle RT-qPCR and extreme RT-PCR for SARS-CoV-2 virus detection

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Introduction/Objective: Since the start of the coronavirus disease 2019 (COVID-19) pandemic, molecular diagnostic testing for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has faced substantial supply chain shortages and noteworthy delays in result reporting after sample collection. Supply chain shortages have been most evident in reagents for RNA extraction and rapid diagnostic testing. In this study, we explored the kinetic limitations of extraction-free rapid cycle RT-qPCR for SARS-CoV-2 virus detection using the commercially available capillary based LightCycler.
Methods/Case Report: We optimized reverse transcription and PCR under extraction-free and rapid thermocycling conditions utilizing hydrolysis probe-based detection methods using a Roche LightCycler.
Results (if a Case Study enter NA): This protocol improves detection speed while maintaining the sensitivity and specificity of hydrolysis probe-based detection. Percentage agreement between the developed assay and previously tested positive patient samples was 97.6% (n= 40/41) and negative patient samples was 100% (40/40). We further demonstrate that using purified RNA, SARS-CoV-2 testing using extreme RT-PCR and product verification by melting can be completed in less than 3 minutes.
Conclusion: We developed a protocol for sensitive and specific RT-qPCR of SARS-CoV-2 RNA from nasopharyngeal swabs in less than 20 minutes, with minimal hands-on time requirements. Overall, these studies provide a framework for increasing the speed of SARS-CoV-2 and other infectious disease testing.

Validation and Implementation of molecular RT-PCR COVID-19 Testing Platforms.

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Introduction/Objective: COVID-19 is caused by the SARS-CoV-2 coronavirus that has led to a worldwide pandemic with an unprecedented need for fast and accurate testing under FDA Emergency Use Authorizations (EUA). Despite the promise of the Alinity-m as an upgrade from the Abbott m2000 for optimization of high throughput testing and random access for laboratory workflow, validation studies comparing Alinity-m to already used Abbott m2000 are sparse in the literature, particularly for the veteran population.
Methods/Case Report: The validation study for the Alinity m (Chicago IL) was performed in three parts 1) Method to method sample/patient correlation study, 2) precision study (at 250 virus copies/mL, 500 virus copies/mL and 1000 virus copies/mL versus 4 negatives), and 3) verification and confirmation of the accuracy of the assay at the lower limit of detection (LOD). For the validation study. The patient specimens were used side by side for both assays. The results from the Abbott m2000 (Chicago IL), which was already established in our lab, were used as a reference for validation.
Results (if a Case Study enter NA): The validation with a method to method correlation included 157 valid specimens from which concordant results were obtained for all 157 specimens on both the Alinity-m and Abbott m2000. The precision or reproducibility of the Alinity-m was verified at all concentrations. The limit of detection verification on diluted samples determined the limit of detection to be 20 virus copies/mL (>95% of dilution samples agreed with positive results at this level), which confirmed even below the manufacturer provided LOD of 100 virus copies/mL.
Conclusion: Alinity m was validated for clinical use with study demonstrating that it is 1) equivalent in the method to method correlation, precision, and LOD determination. 2)The validation of Alinity m, holds great promise with its optimized throughput for testing of large numbers of specimens with less technician handling and random access, is a valuable addition to the literature of test validations under the FDA EUA. 3)The validation of tests under the FDA EUA is unprecedented and provides an important way to improve patient care during this extraordinary pandemic.

Molecular Profiling in Thyroid cancer- Using next generation sequencing to differentiate rare cases of papillary thyroid hyperplasia from papillary thyroid carcinoma

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Introduction/Objective: Differentiating papillary thyroid hyperplasia from papillary thyroid carcinoma is made primarily on differences in key histologic and cytomorphologic features. These include architectural

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