node metastases (p=0.0295) and to PD-L1 positivity on immune cells (p<0.0026).
Conclusion: Marked differences exist in the number of CTLA-4+ lymphocytes between tumors. Analyzing two independent antibodies by a deep learning framework can facilitate automated quantification of immunohistochemically analyzed target proteins such as CTLA-4.

Multiplex Fragment analysis detects all COVID-19 variants of concern

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Introduction/Objective: The majority of tracking methods have employed whole genome sequencing, which can be very expensive and time consuming. An alternative method has been to use genotyping of specific mutations to identify variants. However, tracking SARS-CoV-2 variants by targeted methods has been a moving target. Most methods only multiplex four targets per reaction, but we have multiplexed 8 targets in a single tube using fragment analysis.
Methods/Case Report: Fluorescently labeled primers targeted a combination of insertion/ deletion mutations and single nucleotide mutations. The PCR amplified products, amplicons, were separated by capillary electrophoresis. Primers were designed to detect changes in size indicative of insertion or deletion mutations including: ORF1A:Del3675_3677, S:Del69_70, S:Del144, S:Del157_158, S:Del242_244, ORF8:Del119_120, and ORF8:ins28269-28273. Allele-specific primers were designed to detect both the wild-type and mutated versions of S:N501Y, S:E484K, and S:L452R.
Residual nasopharyngeal and nasal specimens testing positive for SARS-CoV-2 by RT-PCR or isothermal amplification (IDnow) methods were selected from May 1- June 24, 2021. Variant analysis was performed by multiplex targeted PCR and whole genome sequencing in parallel on the same specimens to determine positive percent agreement.
Results (if a Case Study enter NA): Variant analysis was performed on 250 specimens detecting each of the major variants of concern Alpha (B.1.1.7, U.K. origin, n= 108), Beta (B.1.351, South Africa origin, n=3), Gamma (P.1, Brazil origin, n=12), Delta (B.1.617.2, Indian origin, n=17), and Iota (B.1.526, New York, n=5). Some specimens with low viral load were detected by only PCR (n=18), only WGS (n=41), or neither (n=20). Overall positive percent agreement was 95% (163/171).
Conclusion: This adjustable method robustly and accurately identifies COVID-19 VOCs utilizing a platform amenable to multiple targets (20-40 targets ranging from 100-500b.p. across four fluorescent channels) using equipment commonly found in routine molecular pathology laboratories. Future directions include adjusting targets to detect new variants.

Comparison of Abbott COVID-19 testing Platforms for timely results- CMCVAMC experience

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Introduction/Objective: COVID-19 is a new disease, caused by the SARS-CoV-2 coronavirus capable of causing severe disease and death. The Alinity-m has a high throughput and random-access features that are not on the Abbott m2000, both of which had been validated and brought into clinical use for high throughput SARS-CoV-2 testing. The additional features of Alinity-m would be expected to improve turnaround time; however, there are no published reports in the English literature comparing the turnaround time between the Abbott m2000 and Alinity-m.
Methods/Case Report: A retrospective quality assurance search for all SARS-CoV-2 tests performed on the Abbott m2000 and Alinity-m (both Chicago IL) between February 1st 2021 to March 1st, 2021, to capture the turnaround time differences for the Abbott m2000 versus the Alinity-m for the month after the Alinity-m was brought into clinical service after validation.
Results (if a Case Study enter NA): There was a total of 318 tests performed on the Abbott m2000 and 1329 tests performed on the Alinity-m during this time period. The average turnaround time on the Alinity was 6 hours, compared with 11 hours on the Abbott m2000. This difference was statistically significant by the t-test (p-value = <0.01). Both the optimized throughput and random-access features of the Alinity-m contributed significantly to this improvement.
The Alinity-m is capable of producing results within 115 minutes for the first specimen and then 3 minutes for each sequential specimen. On the other hand, the Abbott m2000 must be batched in limited 8-12 hour runs without random access capability. All the results were reported and communicated to the clinical teams, so the timely patient management can be administrated and surveillance of the same can be done in real time.
Conclusion: Alinity M has a significant advantage for a random access as well as improved TAT for detection of SARS-CoV-2, leading to prompt patient care and management.

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