ID46. **A Systematic Review of the Genomic Diversity of SARS-CoV-2 Virus Detected in Dartmouth-Hitchcock Hospital**

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Introduction: As the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became a pandemic, sequencing initiatives such as Nextstrain demonstrated the global genomic diversity of the virus. There has also been documentation of intra-host viral mutations. The ability for a hospital laboratory to genotype SARS-CoV-2 and understand the diversity in its patient population has implications for tracking and controlling new outbreaks. **Methods:** Archival total nucleic acid extracted from viral transport media or formalin-fixed, paraffin-embedded (FFPE) tissue from a pilot group of 45 cases and control viral RNA was subjected to real-time reverse transcriptase-polymerase chain reaction. Ion AmpliSeq SARS-CoV-2 Research Panel libraries were prepared on the Ion Chef Instrument using the Ion AmpliSeq Kit for Chef DL8. Base-calling and alignment were performed on Torrent Suite v5.12.0. Variant calling was performed on the Torrent Suite variantCaller plugin v5.12.0.2 using the Germ line – low stringency configuration. Variant calls were annotated by the COVID19AnnotateSnpEff v1.0.0.1 plugin, and IRMAreport plugin v1.0.0 was used to generate assembled consensus FASTA for each sample. A multiple sequence alignment was produced, using MUSCLE, for samples that contained coverage across all targeted regions and several reference sequences. Sequence variants were called based on the reference sequence NC_045512.2. **Results:** Of the first 45 samples sequenced, 31 had sufficient coverage. Of these, 20 contained the same six variations g.3037C >T, g.241C >T, g.23403A >G, g.14408C >T, g.25563G >T, and g.1059C >T, which corresponds to Nextstrain clade 20C. Additionally, nine more samples shared five of these variants but lacked g.1059C >T, which corresponds to Nextstrain clade 20A. Both of these clades are associated with a European origin. Most samples contained unique secondary variations not seen in the rest of the populations. The most frequent types of variants seen were missense followed by synonymous. Most variants were detected at ~100% variant allele fraction, but lower VAFs in some samples suggest the possibility of intra-host viral evolution. Despite the high degree of viral genome similarity in most samples, two contained none of the previously mentioned variants. These two viral genomes belong to a subset of clade 19A, which also localizes to Europe. **Conclusions:** Sequencing residual SARS-CoV-2 samples has provided insight into the genomic diversity of infections in New Hampshire. This understanding may prove useful in tracking the origin of new nosocomial or community-acquired infections. Sequencing additional samples will inform on the clustering of viral strains in our cohort and enhance our understanding of the SARS-CoV-2 landscape in our patient population.

ST24. **Benefits of Rapid Genotyping of *KRAS* Mutations versus NGS in Pancreatic Cyst Fluids**

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Introduction: Pancreatic cancer necessitates early intervention to improve outcomes; this requires genotyping to identify targetable mutations rapidly and with high sensitivity. Next-generation sequencing (NGS) based genotyping of pancreatic cyst fluids is sensitive and comprehensive but is time- and cost-intensive, with a 10 to 14 day turnaround time (TAT) and a moderate failure rate. A rapid test to identify common, actionable mutations in cyst fluid samples could allow faster diagnosis and initiation of treatment. Further, an orthogonal test could rescue samples that fail NGS. Here we assess the utility of rapid *KRAS* genotyping on cyst fluids to complement NGS. **Methods:** Cyst fluids received between 10/12/19 to 12/18/19 were subjected to both rapid *KRAS* genotyping and comprehensive NGS. Rapid genotyping relied on Idylla ctKRAS Mutation Assay, which enables qualitative detection of 21 mutations in codons 12, 13, 59, 61, 117, and 146. Comprehensive genotyping was performed using target enrichment via anchored multiplex PCR in combination with NGS on an Illumina NextSeq. Our NGS panel detects mutations in *KRAS* in addition to 90 other hotspot-containing genes. Concordance was assessed by comparing *KRAS* mutations uncovered by rapid testing with those identified by NGS. **Results:** *KRAS* genotyping was performed in 32 pancreatic cyst fluid samples. NGS detected *KRAS* mutations in 21 samples and rapid testing in 20 samples. A total of 22/32 samples (68.8%) were concordant between rapid testing and NGS. Of the discordant results, 3 samples (9.4%) had differing *KRAS* SNVs detected by rapid genotyping versus NGS, and 1 sample (3.1%) had a *KRAS* SNV detected by rapid genotyping undetected by NGS. Further, although 4 samples failed rapid testing but passed NGS, 2 samples passed rapid testing after NGS failed, thereby demonstrating sample rescue post-NGS failure. Our historical data

revealed that 20% cyst fluids failed NGS versus 14.4% of formalin-fixed, paraffin-embedded (FFPE) samples, indicating the need for rescue of cyst fluid samples (Fisher's Exact Test, P <0.05). **Conclusions:** Rapid *KRAS* genotyping of pancreatic cysts is complementary to NGS-based profiling. Although not as comprehensive as NGS, rapid testing offers faster results, ease of use, and the ability to provide actionable results when NGS fails. Our data demonstrate that rapid testing of pancreatic cyst fluid samples enables same-day resulting, achieving sample to result in 2.5 hours versus 10 to 14 days for NGS.

ST40. **Rapid qPCR Testing in the NGS Era Enables Same-Day Resulting of *EGFR* Mutant NSCLC**

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Introduction: Effective treatment of advanced *EGFR*-mutant non-small-cell lung cancers (NSCLC) depends on delineating the actionable mutations. Next-generation sequencing (NGS) is the standard for delineating the EGFR mutation status but can take several weeks to provide these results. Our targeted, PCR-based molecular assays specific to common *EGFR* mutations can reduce turn-around time (TAT), but in many cases cannot provide same-day turnaround for rapid initiation of treatment. Here, we report our ultra-rapid workflow which aims to provide same-day, comprehensive *EGFR* genotyping for expedient genomic characterization and initiation of treatment. **Methods:** Our PCR-based rapid panel assesses L858R and exon 19 deletion after nucleic acid extraction from frozen, ethanol fixed samples via single base pair extension and sizing via capillary electrophoresis (CE), respectively. Our ultra-rapid workflow relies on the Biocartis Idylla *EGFR* assay and detects *EGFR* G719A, G719S, G719C in exon 18; del 9, del 12, del 15, del 18, del 21, and del 24 in exon 19; T790M, S768I, insG, insASV9, insASV11, insSVD, and insH in exon 20, and L858R and L861Q in exon 21. Frozen, ethanol fixed tissue is microdissected and placed directly into the assay cartridge for testing. **Results:** With PCR-based rapid testing, optimal turnaround time (TAT) was 2 days from biopsy to result, which is 80% faster than the 10 ± 4-day TAT for NGS. With the ultra-rapid workflow, optimal TAT is reduced to 2.5 ± 1 hours from receipt of tissue. Using frozen, ethanol-fixed tissue instead of formalin-fixed, paraffin-embedded (FFPE) enables same-day resulting and bypassing manual nucleic acid extraction further shortens in-lab TAT over PCR-based methods. The ultra-rapid workflow is a 90% improvement over optimal TAT for NGS and direct technical manipulation of samples in our lab was reduced by >99%. The ultra-rapid method is also more comprehensive than the PCR/CE-based workflow; it allows for detection of 51 individual *EGFR* mutations, compared to only L858R point mutations and exon-19 deletions covered by PCR/CE. **Conclusions:** Overall TAT for the ultra-rapid workflow was reduced by ~50% compared to PCR/CE, and in most cases, results are delivered on the same day as tumor sampling. The lack of nucleic acid extraction and single-slide sample input enables targeted profiling of even scant tumor samples. Although both PCR/CE and the ultra-rapid workflow provide drastic improvement of TAT compared to NGS-based profiling, more comprehensive coverage of actionable *EGFR* mutations in the ultra-rapid workflow allows for faster and more definitive treatment of NSCLC.