G004. **Diagnosis of Germline and Somatic Mismatch Repair Deficient Cancers by Low-Pass Whole-Genome Sequencing**

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Introduction: There is increasing recognition of the role of mutations in the mismatch repair of DNA polymerase genes that leads to replication repair deficiency (RRD) as one of the major cancer mechanisms. RRD is prevalent in common malignancies, such as brain and hematological in children, and colon, endometrial, and ovarian in adults. RRD poses 2 major issues to clinicians. First, these cancers are lethal if unrecognized, as they are inherently resistant to standard chemotherapies. However, RRD hypermutant cancers are exquisitely sensitive to targeted and immunotherapies. Second, a significant proportion of RRD cancers are hereditary, creating an opportunity for early detection and intervention for the patient and family members. Unfortunately, current methods and commercial tests to screen for RRD are inaccurate and/or expensive, and often require multiple sequential tests that significantly increase the turnaround time and cost. Robust tools for identifying these cancers are critical for patient care and represent a significant opportunity. **Methods:** Using low-pass whole-genome sequencing and bioinformatic approaches, we have recently developed **LOGIC**, a robust tool to detect RRD in cancer, which we call: **LO**w-pass **G**enomic **I**nstability **C**haracterization. LOGIC can characterize microsatellite instability and copy number variations in one simple test. We validated this tool for clinical use on a cohort of 357 cases consisting of colorectal, endometrial, small bowel, stomach, and ovarian tissues. These included 68 RRD tumor samples, and 289 non-RRD samples, consisting of 27 replication repair proficient tumors, 86 ovarian *BRCA* tumors, and 176 normal match samples. **Results:** The algorithm generated MMRDness scores ranging from -1.121 to -0.685 (n = 357), with non-RRD samples falling between -1.121 and -0.997 (n = 289), whereas RRD samples ranged from -1.070 to -0.685 (n = 68). An MMRDness threshold was then calculated and set at -1.027 (SD = 0.0002), which allowed accurate diagnosis of RRD samples (being above the threshold), resulting in 96.9% sensitivity (SD = 4.5) and 99.7% specificity (SD = 0.7) in our cohort. **Conclusions:** LOGIC successfully diagnosed RRD in a variety of cancers. The streamlined, accurate, and cost-saving nature that such a tool presents can become invaluable in the clinical setting for recognizing RRD cancers.

G005. **A Two-Year Experience of Bench-to-Bedside Clinical Germline Whole-Exome Sequencing and Reporting of More Than 600 Samples in a Rural Academic Medical Center**

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Introduction: Nearly 2 years ago, the section for Clinical Genomics and Advanced Technologies (CGAT) at Dartmouth-Hitchcock Medical Center began offering in-house clinical germline whole-exome sequencing to its patients. A complete front-to-end in-house service from DNA extraction through next-generation sequencing (NGS) library preparation, to curated variant interpretation and reporting is provided by a single laboratory team. We present an overview of the complete workflow as well as data on diagnostic rates, case load, and test request breakdown. We also highlight the important benefit of the front-to-end service for providing expedited reports for our most critical patients. **Methods:** Blood/buccal-extracted DNA (Qiagen) was procured for library preparation using the Magnis NGS prep system (Agilent). Libraries were quality control (QC) assessed using the TapeStation (Agilent), and sequenced on the NextSeq 500 or NovaSeq 6000 (Illumina). Alissa Reporter was used for FASTQ analysis and alignment, and Alissa Interpret performed analysis for variant calling (Agilent). Variant curation and reporting were performed in-house by a small, experienced team of variant curators and laboratory directors, with weekly exome sign-out board meetings. Test catalogue included germline whole-exome, a neurodevelopmental disease (NDD) panel, and a connective tissue disorder (CTD) panel. **Results:** From November 2022 to date of writing, CGAT has received 677 clinical samples to process for NGS library preparation. Of these samples, 62% were ordered for singleton/duo/trio exomes, and 38% for panels (NDD or CTD) and familial variant segregation analyses. QC for NGS libraries show >90% of fragments consistently between 200bp and 700bp. Depth of coverage per sample averaged >100x with 95% to 99% of analyzable target bases with at least 20 reads. Diagnostic yield was approximately 33%, dependent on test type. Sample extraction through variant annotation can be completed in 4 to 8 business days. Remarkably, for expedited requests for our most critical patients, preliminary reports have been issued in as few as 6 days after blood collection. **Conclusions:** We demonstrate a framework in which laboratories expanding to genomic-scale sequencing and reporting can internalize the entire bench-to-bedside workflow for exome sequencing, providing personalized results for patients directly under our care. In particular, such a framework can result in expeditious reporting of answers for our most in-need critical patients such as newborn infants and children admitted to the intensive care units.

G006. **Genetic Testing in Atypical Hemolytic Uremic Syndrome: The Mayo Clinic Experience**

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Introduction: Atypical hemolytic uremic syndrome (aHUS)/complement-mediated thrombotic microangiopathy (CM-TMA) is a rare disorder caused by alternative complement pathway dysregulation that presents with thrombocytopenia, microangiopathic hemolytic anemia, and renal injury. aHUS/CM-TMA is multifactorial, with inherited and environmental contributors. Genetic testing is complex due to the highly homologous *CFH/CFHR* gene family. Mayo Clinic has performed aHUS genetic testing since 2018, with a major update in 2022 to include detection of copy number variation (CNV) across 14 genes, including the *CFH/CFHR* gene family, 3 risk haplotypes, and *C5* eculizumab resistance variants. Due to gene overlap, the test is also useful for *C3* glomerulopathy. Here we describe the Mayo Clinic experience of aHUS/TMA/*C3G* gene panel testing. **Methods:** Fifteen genes (*ADAMTS13, C3, CD46, CFB, CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, CFI, DGKE, MMACHC,* and *THBD;* and *C5* eculizumab resistance variants) were interrogated using the IDT xGEN Exome Panel v1 capture supplemented with amplicon-based next-generation sequencing (NGS). For *CFH/CFHR1-5,* the results of the exome capture were combined with results from 6 PCR amplicons (3 short-range and 3 long-range) using specific primers that uniquely amplified the region of interest. The amplicons were processed with the Illumina TruSeq Nano NGS chemistry and aligned to a modified reference genome file with select regions of homology masked. CNV was bioinformatically detected for non-homologous regions by a custom pipeline. CNV detection for *CFHR1* and *CFHR3* utilized a training set of samples known to have 2 copies, enabling detection of the common polymorphic deletions. **Results:** Among the first 695 patients tested, 48% were female and 52% were male, with ages ranging from 4 months to 94 years old (mean 45 years). At least 1 variant of uncertain significance, likely pathogenic (LP), or pathogenic variant was detected in 33% of cases. Five percent (35/695) had a pathogenic or LP variant. Homozygous *CFHR1* gene deletion, which is associated with antibodies to FH, was present in 9.3% (65/695), in alignment with literature, while 3.6% (25/695) had other CNV(s) in the *CFHR* gene cluster. Other findings included rare hybrid alleles and 1 patient with a *C5* eculizumab resistance variant. **Conclusions:** The test offered at Mayo Clinic has aided in diagnosis and treatment decisions for patients with features of aHUS. Due to the low disease penetrance, environmental contributions to the disorder, and the technical complexity of the genes involved, variant classification is challenging. Therefore, it is imperative to look at the combined impact of genetic results, serology, and clinical findings to aid in diagnosis and patient management.

ID009. **Adenovirus Genotypes Detected in a Community-Based Longitudinal Cohort of US Children from Birth to Two Years of Age**

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Introduction: Adenoviruses (AdVs) are a common cause of acute respiratory illness in children. There is an incomplete understanding of the community prevalence of AdV infections due to lack of studies outside the hospital setting. The aim of this study was to characterize AdV infections in early childhood within the community setting. **Methods:** Children were followed from birth to 2 years of age from 2017 to 2020 for acute respiratory illness. Weekly nasal swabs were collected and tested for respiratory pathogens with the Luminex Respiratory Pathogen Panel. For AdV-positive specimens, the hexon gene was genotyped via Sanger sequencing. Pattern of AdV-positive detections was defined as either persistent (from subjects with ≥5 AdV detections) or acute (from subjects with <5 AdV detections) over the study period. **Results:** Overall, 245 children were recruited and followed; 13,781 nasal swabs were tested, and 364 samples were positive for AdV in 119 children (median of 2 samples positive for AdV per child; range 1 to 18). Of the 364 samples, 71% (n = 257) were successfully sequenced; samples that failed sequencing had high median cycle threshold value of 35, suggesting low viral load. Resulting genotypes included 42% C1 (n = 109); 32% C2 (n = 81); 16% B3 (n = 40); 8% C5 (n = 21); 1% E4 (n = 3); and <1% for each of B7, C6, and F41 (each n = 1). In the 24 children with persistent detections, 139/180 (77%) samples were successfully sequenced. In contrast, in the 95 subjects with acute detections, 118/184 (64%) samples were successfully sequenced. AdV C1 accounted for 55% (n = 77) of persistent detections as compared to 27% (n = 32) of acute detections (p <0.0001). The duration between AdV-positive detections significantly varied among persistent subjects (median = 365 days, interquartile range (IQR) = 272 days, Q1 = 197.5 days, Q3 = 469.5 days) and those with acute detections (median = 6 days, IQR = 76 days, Q1 = 0 days, Q3 = 76 days) (p <0.001). Genotype switches were significantly more prevalent in persistent (13/24 = 54%) versus acute detections (5/52 = 10%) (p <0.0001). In 13 children with persistent detections and genotype switching, AdV C1 was detected in 11 with a switch to AdV B3 in 8 children. **Conclusions:** AdV species C was the most prevalent among early childhood detections in our community cohort. AdV C1 and B3 predominated in persistent detections, whereas AdV C2 was more common in acute detections. Multiple genotypes were detected in persistent detections, suggesting lack of cross-reactive immunity against other AdV genotypes.

ID010. **Rapid Identification of Infectious Pathogens in Sterile Body Fluids**

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Introduction: Prompt identification of pathogens causing infections in sterile body sites is critical to optimize antibacterial therapy and improve clinical outcomes. Collecting cultures often requires invasive procedures, leading to delays. Rapid molecular methods for direct pathogen detection in fluids from commonly sterile body sites are needed but have not been commercially available. This study assesses the ability of the BIOFIRE BCID2 Panel, consisting of 34 targets, to detect pathogens directly in body fluid specimens. **Methods:** Body fluid specimens were tested using the BIOFIRE TORCH analyzer with the Blood Culture Identification 2 (BCID2) Panel (bioMérieux, Marcy-l'Étoile, France) and standard microbiology laboratory methods. A total of 304 specimens were tested: synovial fluid (36), pericardial fluid (62), peritoneal fluid (61), pleural fluid (61), cerebrospinal fluid (CSF) (8), and abscesses from liver (61), pelvis (6), abdomen (4), breast (1), brain (1), and psoas muscle (3). BCID2 testing followed the manufacturer's instructions, except for sample type adjustments. Viscous or swab-collected samples were diluted 1:1 with molecular-grade water before analysis. BCID2 reproducibility was assessed by testing 7 pooled samples in duplicate over 2 days by 2 operators. Stability for ambient and refrigerated conditions was assessed using 7 pooled samples, tested at baseline, 72 hours, 5 days, and 7 days. Potential interference by whole blood was evaluated using 12 contrived synovial fluid specimens spiked with various microorganisms and whole blood. **Results:** The initial study of 36 contrived synovial fluid samples showed 100% agreement for 4 positives and 140 negatives per target. The second study of 245 contrived samples, including ascites, liver abscess, pericardial, and pleural fluids, demonstrated 100% agreement for 16 positives and 560 negatives per target. The final study of 23 patient samples, including CSF and abscesses, showed 90.6% accuracy. BCID2 results were concordant with 11 of 11 negative cultures (100%) and 9 of 12 positive cultures (75%). Discordant samples included rare *Staphylococcus* colonies by culture, but not by BCID2, and *Streptococcus pyogenes* and *E. coli* detected by BCID2 but not by culture. Further evaluation suspected *Staphylococcus* organisms to be contaminants and organisms detected by BCID2 to be nonviable bacteria detected by PCR. Reproducibility studies demonstrated 100% agreement. Stability studies showed 100% recovery at all time points. Whole blood addition did not interfere with the results. **Conclusions:** Direct testing of fluid specimens from normally sterile body sites using the BCID2 panel is a potentially useful method for rapidly detecting and identifying pathogens in deep-seated infections.

ID011. **Adenovirus Quantification in Transplant Patients: The Need for Assay Standardization**

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Introduction: Accurate quantification of human adenovirus (HAdV) in plasma plays an important role in the diagnosis and management of post-transplant recipients but suffers from interlaboratory variability mainly due to calibration material from a variety of sources and types. The 1st WHO International Standard for HAdV DNA for nucleic acid amplification techniques provides the opportunity for assay harmonization that can reduce that variability, but there is no commercial WHO-calibrated standard material. Here, we describe the analytical performance of a laboratory-developed test (LDT) calibrated with a commercial panel quantified by digital droplet PCR (ddPCR) and the agreement of quantitative results of clinical samples with another test that was calibrated using quantitation standards. **Methods:** Real-time PCR of the nucleic acid extracted material was performed using the RealStar Adenovirus PCR kit v1.0 reagents (Altona Diagnostics) on the Rotor-Gene Q (Qiagen). The ADVP100 Verification Panel (Exact Diagnostics) quantified by ddPCR was used for system calibration. Results were analyzed with optimized settings. The lower limit of detection and quantification, precision, and linearity of the LDT were evaluated using serial dilutions of inactivated virus in ethylenediaminetetraacetic acid (EDTA) plasma. Thirty clinical EDTA plasma specimens previously tested with a test calibrated using quantitation standards were used for agreement between the tests. **Results:** The 95% lower limit of detection and quantification was 200 (±1.3) copies/mL. The LDT was linear from 2.3 to 8.0 log10 copies/mL (R2 >0.99). Precision of results across 4 runs performed on different days by different operators and instruments showed high reproducibility with standard deviations ranging from 0.03 to 0.25 log copies/mL. For the clinical specimens, positive and negative percent agreements were 100%. A correlation coefficient of r = 0.99 of specimens quantifiable by both methods revealed that results were highly correlated; there was negative proportional bias with the comparative test with a mean difference of -0.619 ± 0.142 but no systematic bias. Results agreed when the LDT was calibrated with the same quantitation standards (mean difference = 0.187). **Conclusions:** The LDT system showed excellent analytical performance but poor agreement with the comparator lab for clinical samples due to differences in calibration. Assay harmonization using commutable reference and control materials traceable to WHO international standard (IU) for calibration can reduce interlaboratory variability and improve clinical utility in transplant patients. In the absence of that, calibrators quantified with ddPCR provide absolute quantification and closer estimation to the true value that could facilitate comparability among different laboratories.

for both saliva and urine specimens, that labs may implement for clinical use in neonatal cCMV screening within their health system.

ID006. **Human Papillomavirus Subtyping Using Whole-Transcriptome Sequencing**

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Introduction: Human papillomavirus (HPV) is a most common sexually transmitted disease that is linked to the development of several types of cancers. HPV is classified into 2 groups: high-risk and low-risk subtypes. A persistent high-risk HPV subtype infection can develop into a cancer, whereas low-risk subtypes rarely do. There are more than 200 HPV subtypes that have been classified. Common HPV tests include PCR- and fluorescence *in-situ* hybridization (FISH)-based tests, which are limited in the number of subtypes they can identify because a probe or primer is needed to be designed for each subtype. Whole-transcriptome sequencing (WTS) is a technique that allows for comprehensive profiling of all RNA transcripts from a given organism. It is commonly used to detect gene fusions, splice variants, as well as assess gene expression. It utilizes an unbiased approach, not requiring probes for a particular sequence, to detect both expected and novel transcripts from a sample. To leverage this advantage, we developed a bioinformatics pipeline that allows us to identify and subtype HPV from libraries using an in-house-developed WTS assay. **Methods:** RNA was extracted from formalin-fixed, paraffin-embedded sections from patients (n = 39) that were previously tested for HPV. The RNA was used to prepare a library of the whole transcriptome and then sequenced on an Illumina NovaSeq 6000. The sequencing output data were analyzed by a custom bioinformatics pipeline to identify and subtype HPV using the *L1* gene. HPV detected from the pipeline was compared to in-house HPV assays (*e.g.,* Sanger sequencing and/or RNA FISH). **Results:** The analysis of HPV subtyping was separated between high-risk and low-risk HPV to assess the ability of the bioinformatics pipeline to identify subtypes from each group. For the low-risk HPV subtypes, the sensitivity, specificity, and accuracy of HPV subtyping by WTS compared to traditional HPV tests were 100%, 100%, and 100%, respectively. When compared to high-risk HPV subtypes that were detected, the sensitivity, specificity, and accuracy were 86.4%, 100%, and 94.1%, respectively. **Conclusions:** We demonstrate that whole-transcriptome sequencing can be used to classify HPV into subtypes with similar performance to traditional methods to identify and subtype HPV. This application represents added value, potential cost savings, and a reduction in sample requirements for patients requiring HPV screening, in addition to the more common results provided by WTS, such as detecting gene fusions.

ID007. **Adding Infectious Disease Samples to a Cancer Next-Generation Sequencing Workflow Decreases Costs and Increases Efficiency of Whole-Genome Sequencing for Pathogen Identification and Surveillance**

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Introduction: Whole-genome sequencing can be used for organism identification, epidemiologic surveillance, and outbreak analysis, but the cost of next-generation sequencing can be prohibitive for routine and timely use by many hospitals. Here we explored if we could eliminate sequencing costs and enable more frequent testing of epidemiologic samples by replacing PhiX control with libraries created from culture isolates (CI) in a routine clinical solid tumor sequencing run. **Methods:** *Staphylococcal aureus* isolates were cultured from stock provided by the Centers for Disease Control Antimicrobial Resistance Isolate Bank. *Acinetobacter baumanni* isolates were cultured from 2 patient samples in a suspected transmission cluster at our institution. CI libraries were constructed using the DNA PCR-Free Prep (Illumina, San Diego, CA) with minimum insert size of 350 bp, and spiked in place of PhiX on the TruSight Oncology 500 Assay (TSO500) clinical validation run on the NovaSeq 6000 with sequencing cycle 101x2. For comparison, the same TSO500 samples were run at the same time but with PhiX in a dual flow cell setup on the NovaSeq 6000. Analysis of FASTQ files was performed using our in-house, modified TSO500 pipeline and the BugSeq (Vancouver, British Columbia) analysis platform. **Results:** Cancer sample sequencing quality-control (QC) metrics such as total reads, median depth, and coverage and pipeline output such as variant calls, tumor mutation burden, microsatellite instability, and copy number variation were not affected by the replacement of PhiX by CI libraries. There were minimal bacterial DNA sequences present in unmapped reads in the cancer samples (<0.0001% of unmapped reads). CI samples had variable reads depending on genome size and number of libraries spiked in per run, with target total reads 1 million per library. This minimum cut-off allowed for accurate genome assembly, multi-locus sequence typing, plasmid detection, and outbreak analysis. Repeat testing of the same CI and cancer sample libraries showed excellent reproducibility of results. **Conclusions:** Sequencing QC metrics and variant output of cancer samples were not affected by inclusion of libraries from CI, and the sequencing settings for the cancer panel run did not affect coverage of CI genomes. By using sequencing space freed by replacing PhiX, sequencing costs are eliminated, and CIs can be more frequently run, though the number of CIs that can be sequenced per run is restricted by organism genome size. Overall, this approach provides a cost-effective method for sequencing of CIs for organism identification, epidemiologic surveillance, and outbreak analysis.

ID008. **Multiplex Fragment Analysis Quickly Detects and Differentiates Non-tuberculous Mycobacterial Subspecies**

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Introduction: The incidence of tuberculous and non-tuberculous mycobacterial infections continues to increase worldwide, posing significant diagnostic challenges for clinical laboratories. Culture-based identification is hampered by relatively slow growth rates (2 to 8 weeks for some species) and the discontinuation of widely utilized commercial molecular probe-based systems for rapid identification of mycobacterial species. The current lack of FDA-approved alternatives for mycobacterial identification underscores the need for novel diagnostic approaches in this area. Beyond cursory identification, highly discriminatory techniques such as gene sequencing to guide empiric therapy of *Mycobacterium abscessus* complex infections based on subspeciation are generally unavailable outside the reference laboratory setting. To address these diagnostic gaps, we developed and validated a molecular fragment analysis assay with capillary electrophoresis to identify the most clinically relevant mycobacterial species grown in solid and liquid media in a single, 3-hour reaction. **Methods:** A novel multiplex fragment analysis PCR (MYCOPLEX) assay was developed and validated using the amplification of mycobacterial gene targets and analysis by an electropherogram. DNA was extracted from heat-inactivated clinical isolates grown either on Lowenstein-Jensen media or mycobacterial growth indicator tube broth, PCR amplified, and separated using capillary electrophoresis (ABI 3730XL). Six conserved regions were selected to identify *M. tuberculosis* complex (MTB), *M. avium* complex (including *M. avium*, *M. intracellulare/chimera*), *M. abscessus* complex (*abscessus/massiliense* subspeciation), *M. kansasii*, and *M. gordonae/paragordonae*. *M. abscessus* subspeciation based on an intact (*M. abscessus* subsp. *abscessus*) or deleted (*M. abscessus* subsp. *massiliense*) *erm*41 gene was also developed. **Results:** Sixty-eight clinical isolates recovered from pulmonary and extrapulmonary specimens in solid and liquid media were analyzed by MYCOPLEX. Overall, sensitivity was 96.1% (n = 2/51 false negatives) and specificity was 100%. The diagnostic accuracy was 97.1% with no misclassifications. The limit of detection (LOD) varied by species, with the MTB achieving an LOD of 1 copy/reaction. **Conclusions:** MYCOPLEX is a multiplex fragment analysis approach that rapidly and robustly identifies clinically relevant mycobacterial species, including *M. abscessus* subsp. *abscessus* from *M. abscessus* subsp. *massiliense* and *M. intracellulare/chimera* from *M. avium*. This approach is flexible and scalable, allowing for expanded throughput to accommodate laboratory workflows of various sizes. Future work aimed at adaptation of this method with direct specimens is ongoing.