

## **G060. Phenotypic and Genotypic Study of Patients with Hermansky-Pudlak Syndrome**

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**Introduction:** Hermansky-Pudlak syndrome (HPS) represents a group of autosomal recessive disorders due to mutations in genes involved in intracellular vesicular trafficking. Clinical presentations include oculocutaneous albinism and bleeding diathesis. There are 10 genetic subtypes of HPS: type 1 (due to mutations in *HPS1*), type 2 (*AP3B1*), type 3 (*HPS3*), type 4 (*HPS4*), type 5 (*HPS5*), type 6 (*HPS6*), type 7 (*DTNBP1*), type 8 (*BLOC1S3*), type 9 (*BLOC1S6*), and type 10 (*AP3D1*). Eventually all patients with HPS1, 2 and 4 develop pulmonary fibrosis and may require lung transplant. Since severe platelet dense granule deficiency is considered a characteristic feature of HPS, platelet whole mount transmission electron microscopy (PTEM) has been considered a good initial screening test. Potential HPS positive cases detected by PTEM still need to be confirmed and further classified by genetic testing. The goal of this study is to assess the HPS gene mutation status of the PTEM identified HPS cases in our institution.

**Methods:** Nine patients with PTEM documented severe dense granule deficiencies and one patient with ocular albinism but normal dense granules by PTEM were included in this study. Next Generation Sequencing (NGS) was performed using a targeted panel (Agilent Technologies) encompassing 9 of the HPS genes (*HPS1*, *AP3B1*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, *DTNBP1*, *BLOC1S3*, and *BLOC1S6*). DNA library preparation was performed using the SureSelect<sup>XT</sup> Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library (Agilent Technologies). The enriched indexed DNA sample was then sequenced on an Illumina MiSeq or HiSeq 2500 platform. The CLC Bio Genomics Server was used for sequence alignment and variant calling, which was then uploaded into NGS Workbench (Mayo Clinic) for review. All sequence variants were classified following the current American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines. **Results:** All 10 patients had oculocutaneous albinism and bleeding diathesis (age range 8-80 years, 3 male). The average mean dense granules/plt of the 9 PTEM positive patients was less than 0.1 (normal range >1.2). Pathogenic mutations were found in all 9 PTEM positive patients, *HPS1* ( $n=5$ ), *HPS3* ( $n=2$ ), *HPS5* ( $n=1$ ) and *HPS6* ( $n=1$ ). These included both reported ( $n=5$ ) and novel mutations ( $n=4$ ). No pathogenic mutations were found in the patient with ocular albinism but negative PTEM findings. **Conclusions:** An algorithmic approach of employing both PTEM and genetic testing can accurately identify and classify Hermansky-Pudlak syndrome to assist clinical management in such patients. The late diagnosis of many HPS patients in this cohort underscores the importance of availability of both PTEM and molecular genetic testing in clinical laboratories.

## **ID014. Evaluation of Panther Fusion System for Respiratory Viral Detection in a Pediatric Hospital**

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**Introduction:** The Panther Fusion (Hologic, Inc., San Diego, CA) respiratory assays are modular, multiplex real-time PCR (RT-PCR) diagnostic tests used in conjunction with the Panther Fusion System for the differentiation of influenza A (FluA), influenza B (FluB), respiratory syncytial virus (RSV), parainfluenza 1-4 viruses (P1-4), human Metapneumovirus (hMPV), Adenovirus (ADV), and human Rhinovirus (hRV) in less than three hours. Within the fully automated system, samples are lysed, nucleic acid is captured and transferred to the reaction tube containing the assay reagents, and RT-PCR is performed using TaqMan RT-PCR. We evaluated the performance of the Panther Fusion to detect respiratory viruses in 5 different specimen types. **Methods:** A total of 284 respiratory samples including 105 nasal washes (NW), 80 nasal/nasopharyngeal swabs (NP), 55 bronchoalveolar lavages (BAL), 42 tracheal aspirates (TA), and 2 sputums (SP) were evaluated. Results were compared to those obtained with the ProFlu+, ProAdeno+, ProParaflu+, ProhMPV+ assays (Hologic, Inc.) and a lab-developed RT-PCR test (LDT) for hRV. A subset of 135 specimens (29 NW, 28 NP, 40 BAL, and 38 TA) were also tested using the ePlex Respiratory Pathogen (RP) Panel (GenMark Diagnostics, Inc., Carlsbad, CA). Discordants were tested on the FilmArray Respiratory Panel v1.7 (Biofire Diagnostics LLC, Salt Lake City, UT). **Results:** A total of 292 respiratory viruses were identified. The Panther Fusion showed an overall agreement of 99.7%

(291/292). Percent positive agreement for each target was 100% for FluA (41/41), FluB (19/19), RSV (32/32), P1 (23/23), P2 (17/17), hMPV (54/54), ADV (39/39), and hRV (29/29), and was 96.9% (31/32) for P3. Four additional positives (3 hRV and 1 P3) were identified by the Panther Fusion but negative by ProParaflu+ or hRV LDT; all were confirmed by FilmArray. Compared to the ePlex RP, the Panther Fusion detected 2 additional P3, 1 ADV and 4 hMPV; all confirmed by in-house testing and/or FilmArray. Panther Fusion and ePlex assays correctly identified P4 in 6 specimens. **Conclusion:** The Panther Fusion System may be a viable alternative to the current batch-testing, real-time PCR assays for respiratory viruses offered at Texas Children's Hospital. The Panther Fusion is a relatively easy to perform, sample-to-answer platform with minimal hands-on time of ~10-15 minutes for approximately 30 samples. With a run time of <3 hours, this random and continuous access platform could enable same-day results not routinely available with our current high-complexity, batch-testing workflows. The flexibility of the 3 modular assays also allows for an algorithmic, reflexive approach to respiratory virus testing.

#### **TT074. Multi-patient Longitudinal Monitoring of Cancer Mutations from Circulating DNA of using Personalized Single Color Digital PCR Assays**

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**Introduction:** With the emergence of precision cancer medicine, there is an increasing need for new longitudinal diagnostic tests to evaluate patients at the initial diagnosis, during treatment, and for routine monitoring. As a high performance and flexible solution, we developed a single-color digital PCR (sc-dPCR) assay that detects and quantifies circulating DNA somatic cancer mutations collected from the plasma of cancer patients. The assay has a sensitivity of 0.1% mutation allelic fraction and can be designed for nearly any cancer mutation. Given its design flexibility, low cost, and robust performance, sc-dPCR offers many advantages for longitudinal monitoring of cancer patients during treatment.

**Methods:** We selected seven patients diagnosed with metastatic cancer of various types, whose tumors had diagnostic genotyping information available. We then developed personalized sc-dPCR assays for one or two clinically relevant mutations identified for each patient in essential cancer drivers such as *BRAF*, *KRAS* and *PIK3CA*. We collected longitudinal blood samples at each cycle of treatment and extracted circulating cell free DNA (ccfDNA) from 1mL of plasma. We then tested the ccfDNA at each time-point for the presence of somatic mutation bearing circulating tumor DNA (ctDNA) molecules.

**Results:** We successfully detected ccfDNA from plasma in all seven patients tested. In four patients we were able to identify ctDNA molecules bearing the specific mutation targeted. In addition to the ctDNA mutation molecule count, we evaluated the clinical information as well as outcome and survival. These include circulating tumor marker levels, CEA, CA-19-9, and CA-15-3, as well as PET/CT scan images at various time points. Using our sc-dPCR ctDNA molecule count, we validated trends among these patients who were receiving active treatment with chemotherapy or targeted agents. For example, one patient demonstrated low or undetectable *BRAF* V600E molecule count across all time points, thus potentially indicating the patient's disease burden was under adequate control on BRAF-targeted treatment. In another patient under active treatment, we detected increasing quantities of ctDNA molecules over time suggestive of recurrence. This hypothesis was corroborated by increases in tumor marker levels and subsequent imaging of the tumor. **Conclusions:** With our sc-dPCR technology, we identified personalized mutation bearing ctDNA molecules from individual cancer patients and identified trends in ccfDNA matching the clinical responses that were observed. Overall, our study provided longitudinal information specific to an individual patient's response to treatment, suggesting that our assay technology is a valuable tool for precision medicine and monitoring of cancer patients.

