Submission Code: 13369

Category: Genetics

Corresponding Author: Joseph Blommel

TITLE: Clinical Impact of Characterizing Genomic Alterations Using Whole-Genome Mate Pair Sequencing

ABSTRACT:

Abstract

Introduction: Mate pair sequencing (MPseq) is used in the characterization of rearrangements, particularly balanced rearrangements, in order to identify clinically significant genes at/near the breakpoints, which can aid in determining pathogenicity, diagnosis, prognosis, and in some cases therapeutic options. Due to the nature of its unique library preparation method, MPseq has higher sensitivity than conventional NGS for detecting structural variation in the human genome.

Methods: As previously described by Cao *et al*, we have validated the first clinical MPseq assay using the Illumina mate pair library preparation in conjunction with Illumina HiSeq Rapid (2x101bp) sequencing chemistry and BIMAv3 mapping and alignment tool for the detection of structural variants. This is a multi-laboratory effort in which DNA is isolated from either blood, bone marrow, or fresh/frozen tissue in the extraction core, prepped and sequenced using MPseq in our next-generation sequencing core laboratory, and aligned and analyzed for structural variation in our bioinformatics core. Results are interpreted in a targeted fashion depending on the rearrangement that is to be characterized for each particular patient, and a clinical report is issued to the patient's provider. All results are also confirmed by an orthogonal method such as Sanger sequencing.

Clinical case: In our first clinical sample, MPseq was offered for a 10 y/o patient with relapsed B lymphoblastic leukemia. At diagnosis, conventional chromosome studies identified a 12;15 translocation. Interphase and metaphase fluorescence *in-situ* hybridization (FISH) confirmed a rearrangement involving the *ETV6* gene (at 12p13) with an unknown partner gene located on chromosome 15. MPseq was peformed to determine the partner gene in hopes that prognosis or treatment for the patient would be better defined.

Results: MPseq confirmed the presence of a t(12;15)(p13.2:q25.3) rearrangement resulting in fusion of *ETV6* and *NTRK3*. Specifically, this rearrangement leads to fusion of exons 1-4 of *ETV6* with exons 15-20 of *NTRK3*. This fusion has been described previously in several malignancies, including a pediatric patient with Ph-like B lymphoblastic leukemia. Most importantly, this fusion is targetable using tyrosine kinase inhibitors, and this patient's therapeutic regimen now has the potential to be altered as a result of this clinical assay.

Conclusions: The validation and implementation of MPseq has already, with the first patient tested, demonstrated its clinical utility and potential impact on patient care. Because of these results, this patient will be treated with tyrosine kinase inhibitors in addition to other chemotherapies in hopes of achieving and sustaining remission long-term.

Submission Code: 13207

Category: Infectious Diseases

Corresponding Author: Scott McClellan

TITLE: Analytical Validation of an Analyte Specific Reagent (ASR) for Mycoplasma genitalium Detection and Point Prevalence Assessment

ABSTRACT:

Introduction: *Mycoplasma genitalium* is associated with non-chlamydial, non-gonococcal urethritis in men, as well as with pelvic inflammatory disease in women. Assessing *Mycoplasma genitalium* prevalence has been hampered by the lack of a suitable diagnostic method. Hologic has developed ASRs that use transcription mediated amplification (TMA) for detection of the 16s rRNA of *M. genitalium* on the automated Panther system.

Methods: A total of 985 specimens submitted for CT/GC and TV testing were analyzed for *M. genitalium*. Specimen types included urine (female and male), vaginal, cervical, endocervical, urogenital, rectal and oropharyngeal. Positive specimens were confirmed by testing with an alternate *M. genitalium* target TMA ASR. Urine specimens previously tested for *M. genitalium* with the Hologic ASRs were obtained from an outside laboratory for accuracy evaluation: 41 female urines (16 positive, 25 negative) and 41 male urines (16 positive, 25 negative). Analytical sensitivity utilized replicate testing of a serially diluted *in vitro* RNA Transcript (50,000 copies/mL) with probit analysis (SPSS 23, IBM). Precision was assessed by testing 1,000 copies/mL replicates of 5 on 4 separate days and using Analyse-it Method Validation Edition software (v.4.0, Analyse-it Software, Leeds, UK) for variance component calculations. Analytical specificity and interference used a panel of viruses, bacteria and fungi that could potentially be found in the urogenital tract were tested in neat and spiked positive *M. genitalium* Aptima Urine Transport tubes. Specimen matrix interference was assessed by selecting 10 vaginal, 10 cervical and 10 endocervical specimens that had tested negative with the Aptima Combo 2. The specimens were tested neat and again after spiking with *Mycoplasma genitalium*.

Results: Prevalence in the population tested was determined to be 5.2%, which ranked second to *Trichomonas vaginalis* (6.0% from April 2016 to March 2017). Alternate target testing confirmed 41/43 positive results. Concordance with outside laboratory specimens was 97.6%. Analytical sensitivity was calculated to be 14.8 copies/mL at 95% confidence of detection. Testing of potential urogenital organisms found no cross reactivity, nor did they interfere with detection of *M. genitalium*. Within day, between day and total coefficients of variance were 2.5%, 2.7% and 3.7%, respectively. There was no matrix interference for vaginal, cervical or endocervical specimens.

Conclusions: The performance characteristics of the Hologic ASRs for the qualitative detection of *M. genitalium* indicate it is a sensitive, specific and reproducible molecular assay. It is robust enough to be used with a variety of specimen types.

Submission Code: 13657

Category: Solid Tumors

Corresponding Author: Stefano Rosati

TITLE: Validation of a Clinical Targeted CNS Next Generation Sequencing Panel for Detection of SNPs, Indels and 1p/19q Co-deletion

ABSTRACT:

Introduction: The recent 2016 WHO classification of central nervous system (CNS) tumors describes an integrated approach for the diagnosis of gliomas based on histological features and genetic alterations. This approach has been demonstrated to improve prognostics and support treatment decision. The major diagnostic biomarkers in the 2016 WHO classification of glioma are *H3F3A* K27M, *IDH1/2* mutations, and 1p19/q codeletion. Additional molecular testing of *ATRX*, *BRAF*, *TERT*, *EGFR*, and *PTEN* are used to further classify gliomas. A next-generation sequencing (NGS) based assay was designed to target these molecular biomarkers in a single assay and analysis pipeline.

Methods: 24 cases were analyzed in this study: 20 glioma cases (formalin fixed paraffin embedded), 3 CAP glioma samples, and 1 normal genomic control. DNA was extracted using Zymo Pinpoint Slide Isolation system. Genetic targets include ATRX (CDS), BRAF (hotspots), H3F3A (hotspot), IDH1 (hotspot), IDH2 (hotspot), EGFR (exons 1-10, 15-22), PTEN (CDS), TERT promoter, TP53(CDS), 15 SNPs on 19q, and 29 SNPs on 1p. Libraries and templates were prepared with the Ion Chef and sequencing was performed on the Life Technologies PGM. Variant calling was performed by the Torrent Suite Variant caller (v5.2.0.34) and annotations were performed by ANNOVAR (v0.2). Loss of heterozygosity testing was performed using custom Python algorithms in Pandas from Torrent Suite VCF files.

Results: Successful sequencing was performed in 24 of 24 cases. Eight ATRX variants were detected in 10/24 samples. Two of the ATRX variants p.E2246X and p.1090_1091del were predicted deleterious, which were confirmed by IHC. 1p/19q co-deletions were identified in 6/24 cases; 3 positive cases were tested and 1p/19q status was confirmed by FISH using Vysis LSI Dual-Color Probe Kit. IDH1 R132H was found in 4/24 cases. 3 of 4 IDH1 R132 variants were tested and confirmed via IHC and the IonTorrent Cancer Hotspot Panel. IDH2 R172K and IDH2 R172M mutations were detected in 1 case each. BRAF V600E was present in 1 case. One or more TP53 missense mutation was found in 10 of 24 cases. PTEN variants were present in 8 of 24 cases. Copy number variation (CNV) results of the TERT promoter region and EGFR were inconclusive.

Conclusions: A custom NGS-based assay designed for the detection of clinically significant genetic variants in genes ATRX, IDH1 and TP53 and the codeletion 1p/19q for gliomas is a viable solution for classifying gliomas following the 2016 WHO recommendations. Further modification of the genetic targets, assay design and/or data analysis tools are required to conclusively identify and annotate the entire spectrum of molecular biomarkers recommended by the 2016 WHO CNS classification.