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Multigene Panel NGS: Concepts for Considerations

For Discussion Purposes Only

Personalized Medicine and Molecular Diagnostics Working Group

Overview

- This is a hypothetical illustrative example designed to stimulate discussion
- Study designs are based on possible tests that might be offered as a panel

Questions of Interest

- What level of analytical validation is needed to provide confidence in system?
  - Can we test representative subsets? Use contrived samples?
- What level of clinical validation is needed? Can we treat panel as a whole to provide confidence in system performance and in support of a respective intended use?
- How do we standardize? How do we prove accuracy?
- How do we update with new panel members?
- How do we update database with new information?

General Device Description

- Device description: Multi-gene panel (“GeneX”)
  - Technology: System includes instrument software and reagents (Next-generation sequencing)
  - Sample type: Peripheral blood
  - Control concept: Coriell lines will be used as standard

General Device Description

- System Software: The System Software performs base calling with accuracy score, sequence alignment and variant detection, and reports the genotype at each locus.

- The Data Analysis Software summarizes the combinations of genotypes at the various loci and groups them by disease states. The following will be reported
  - “No variant detected” or
  - “Variant detected”, in which case, the specific genotype/variant will be identified

Interpretation Instructions

- The annotated variant information is reported, but interpretation is left up to the ordering physician/ pathologist as part of “practice of medicine”
  - They can consult with up-to-date, relevant public databases to categorize each variant as pathogenic, benign, or variant of unknown significance (VUS).

Analytical Validation

Categories of Mutations

- Representative subsets will be created that cover the following categories:
  - Single nucleotide variants (SNV),
  - Small insertion-deletion polymorphisms, large insertion-deletion polymorphisms, variants in homopolymer repeats
  - Polynucleotide indels, gross insertions
  - Mutations within high GC content regions, simple sequence repeats, and repetitive elements
  - Translocations

Analytical Validation Description

- Obtain Coriell or NIST samples that have been characterized
  Cut-offs for read quality will be set high

  For each example in representative subset we will show:
  - Analytical accuracy via side by side comparisons with Sanger sequencing.
  - Use high cut-off values for read quality (signal to noise ratio of individual reads, e.g., >Q25) as well as for the number of reads for a given genomic region (e.g., >30x coverage). If these quality and coverage cut-offs are not met, the region should not be reported (i.e., a no-call).

Analytical Validation Description

- Also include fresh native samples from individuals, hoping to get someone from each category (even if not same mutation)
  - Same accuracy, analytical performance

- For analytical accuracy studies, all findings (Pathogenic, Benign and VUS) reported from the Next Gen Sequencing test will have comparator results, regardless of their clinical significance.

Clinical Validation Description

- NGS-based GeneX panel and software used to generate variant information at each loci; followed by pathologist-performed interpretation and disease association according to DEFINED process based on ACMG guidelines, literature, and mutation databases per ACMG and other guidelines

- Calls determined as pathogenic, benign, or VUS

Clinical Validation Description

- Results Final Output
  - Clinical interpretation (by Board Certified…)
  - Variants are classified according to ACMG and other guidelines, using literature knowledge as well as *SW tools* for comparison with a variety of mutation and human variation databases to determine the pathogenicity of each variant
  - Pathogenic variants are assembled into genotypes and reported