Assay Validation Services

PierianDx

Overview

PierianDx's assay validation services bring clinical genomic tests to market more rapidly through experimental design, sample requirements, analytical pipeline optimization, and criteria tuning. Our assay validation services include:

- Design and validation of customized pipelines and panels
- Navigate the College of American Pathologists accreditation and inspection processes
- Strategy and setup for Clinical Laboratory Improvement Amendments (CLIA)
- Creation of assay validation processes compliant to CLIA
- Standard gene panels provided by Illumina sequencers or your customized assay
- Clinical and computational validation of clinical genomic panels and processes
- Validation planning for each panel, including details on sample types, number of samples, and other details to assess analytical specificity/sensitivity, diagnostic specificity/sensitivity, reproducibility and reliability, reference range, and reportable range
- Bioinformatics support services to process, analyze, and agglomerate data from validation runs to create reports that will be used in your official validation report

Our Approach

PierianDx has developed a standardized validation approach for use with our NGS bioinformatics pipelines. The two core components are the analytical sensitivity and specificity analysis, used to set variant filter rules and define appropriate cutoffs, and the diagnostic sensitivity and specificity analysis, in which old clinical samples are re-analyzed with the new pipeline.

The full list of validation experiments includes the following:

1) Analytical specificity and sensitivity
2) Repeatability and Reproducibility
3) Diagnostic specificity and sensitivity
4) Reportable range

Analytical Specificity and Sensitivity

Analytical specificity and sensitivity (along with the positive predictive value) for variant detection are determined using a reference standard. This analysis is typically done separately for each category of variant. Pooled hapmap cell lines derived from the 1,000 genomes project can be used for substitution detection. Pooled COSMIC lines are suitable for indels for somatic cancer assays. There are also some commercial products now that simulate a cell line enriched with somatic cancer variants that work well with some assay designs. Samples that require complete and reliable variant information are available for a significant and well-defined portion of the assay's target region.

Detection of these variants can be used to tune variant filter rules, maximizing sensitivity and specificity of the assay. Common filter criteria include: frequency, depth, and various quality metrics. These filters are then applied to the pipeline before the remaining experiments are analyzed. The samples often run multiple times to satisfy both the analytical sensitivity and specificity analysis and the repeatability analysis (described below). DNA quantity and quality for each run should be matched as closely to expected quantity and quality of clinical samples as possible.

Assay Validation Services

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Repeatability and Reproducibility

Repeatability and reproducibility are also assessed using the reference standards. Repeatability experiments demonstrate the consistency of results across potential sources of variation. Common sources of potential variation include different sequencers, different technicians performing the wet lab work, different indexes used in pooling, and different instances of the library preparation and sequencing. A reference standard (covering each variant type) run with each combination of factors enables concordance comparisons across each source of variation. Comparing all factors listed above (2 sequencers x 2 techs x 2 index sets x 2 days) would involve 16 iterations.

Repeatability is then assessed as a function of concordance of variants detected across relevant categories:

1) Between technicians
2) Between machines for the same technician
3) Between library preps/days for the same technician
4) Between indices
5) Overall concordance across all variables

Reproducibility experiments demonstrate the consistency of results over time. Reproducibility is assessed on an ongoing basis using the reference standard covering relevant variant types. Ideally, this sample is processed approximately once a week as part of a run of other validation samples (by any technician run on any machine) for about 8 weeks. Concordance of variant calls between replicates over time–as well as their frequency for somatic cancer assays—is assessed to demonstrate the consistency of results.

Diagnostic Specificity and Sensitivity

Previously characterized patient samples with known true positive variants of clinical significance are collected, sequenced, and blinded. Acquiring these samples through a sample exchange can be a slow process, so arrangements should be made at the very beginning of the validation process. To assess sensitivity, about 25-30 samples with previously identified pathogenic variants in a variety of genes should be assessed.

The blinded samples go through the informatics pipeline with the filter rules established during the analytical specificity and sensitivity phase of this validation. The variant calls in the draft reports are reviewed and classified by the clinical genomicists/sign-out team. Sensitivity is scored as the number of previously reported variants of clinical significance that are also reported during the blinded review.

To assess specificity, clinical samples or reference standards with known true negatives can be reviewed and classified by the clinical genomicists/sign-out team as part of the same blinded exercise. The results of the blinded review of these samples are assessed for any false positive calls—clinically relevant variants identified in the blinded exercise that were not part of the original report. However, new calls in regions not covered by the original analysis, in variant types not detected, or those reclassified based on more recent research should be excluded from this analysis. Additionally, this specificity analysis can utilize the same clinical samples as the above sensitivity analysis by assessing known true negative positions within the sample that carries a separate known true positive.

Reportable Range

The reportable range of the assay is determined by assessing the overall coverage of each targeted region across the diagnostic specificity and sensitivity samples. Each target region is typically defined as an exon or that portion of an exon covered by the assay. Coverage of these regions is compared to the depth-of-coverage requirement. Regions where the average coverage is low, or part of the target region is routinely below the depth cutoff, may be disclaimed from the assay's targeted regions. The exact cutoffs set vary with a panel's filter rules and assay design.

Assay Validation Services

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Definitions and Validation Formulas

Definitions

Analytical specificity and sensitivity are a measure of the panel's technical capability to report the same set of variants as those that were identified by the relevant gold standard approach for each sample. Hapmap cell lines, COSMIC cell lines, and reference standards such as those produced be Horizon Dx or the Acrometrix Oncology Hotspot control are common choices.

Diagnostic sensitivity and specificity measure the degree to which clinical genomicists using this panel on prior human clinical samples produce the same diagnosis as was generated by the original sign-out.

Sensitivity is the proportion of actual positive results identified as positive; it addresses the ability of a test or procedure to identify positive results.

Specificity is the proportion of actual negative results identified as negative; it addresses the ability of a test or procedure to identify negative results.

Positive Predictive Value is the proportion of results identified as positive that are actual positive results.

Negative Predictive Value is the proportion of results identified as negative that are actual negative results.

Accuracy is the proportion of results that are correctly classified as positive or negative.

No Calls are positions where insufficient reads are present to accurately call a variant at that position. No calls do NOT contribute to the false negative rate (i.e. each false negative is assessed for depth and if depth requirement met, then the variant is deemed a no call and is removed from the false negative list).

True Positive (TP) is a variant was identified by the assay/pipeline and is called a variant by the gold standard.

False Positive (FP) is a variant was identified by the assay/pipeline but was not called a variant by the gold standard.

True Negative (TN) is when no variant is identified by either the assay/pipeline or the gold standard.

False Negative (FN) is when a variant is not identified by the assay/pipeline but was called a variant by the gold standard.

Formulas

Sensitivity = True Positive / [True Positive + False Negative]

Specificity = True Negative / [True Negative + False Positive]

Positive Predictive Value = True Positive / [True Positive + False Positive]

Negative Predictive Value = True Negative / [True Negative + False Negative]

Pipeline

The informatics tools, versions, and parameters used are listed and discussed.

Assay Validation Services

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Validation Outline

- **Introduction**
  - **Assay overview**
  - **Summary of validation term and experiments**
  - **Bioinformatics pipeline description**
- **QC metrics**
  - **Overview**
  - **Samples used**
  - **Reference average & Reference Ranges**
    - Methodology
    - Results summary (reference averages and ranges)
      - Total, mapped, on-target, & unique read count and percentage ranges
      - Average coverage range
      - Target space coverage as a function of depth
      - Recommendation on maximum samples per run
      - Full QC dataset
    - Exon Coverage
      - Methodology
      - Summary of failing exon frequencies
      - Exon failure data
      - Boxplot of exon average coverages
    - Hotspot coverage
      - Methodology
      - Summary of failing hotspot frequencies
      - Hotspot failure data
- **Accuracy – Analytical Performance**
  - **Overview**
  - **Substitutions**
    - Methodology
    - Samples used
    - Variant filter optimization
    - Limit of Detection
    - Analytical sensitivity, specificity, positive predictive value with recommended filters
  - **Insertions, Deletions, Indels**
    - Methodology
    - Samples used
    - Variant filter optimization
    - Limit of Detection
    - Analytical sensitivity, specificity, positive predictive value with recommended filters
  - **Fusions**
    - Methodology
    - Samples used
    - Variant filter optimization
    - Limit of Detection
    - Analytical sensitivity, specificity, positive predictive value with recommended filters
  - **CNVs**
    - Methodology
    - Samples used
    - Variant filter optimization
    - Limit of Detection
    - Analytical sensitivity, specificity, positive predictive value with recommended filters
- **Precision – Repeatability and Reproducibility**
  - **Overview**
  - **Samples used**
  - **Intra-run concordance**
    - Methodology
    - Results Summary (Substitutions, InDels, Fusions, CNV)
    - Concordance Data
  - **Inter-run concordance**
    - Methodology
    - Results Summary (Substitutions, InDels, Fusions, CNV)
    - Concordance Data
  - **Concordance between Technicians**
    - Methodology
    - Results Summary (Substitutions, InDels, Fusions, CNV)
    - Concordance Data
  - **Concordance between Sequencers**

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Validation Outline

- Methodology
- Results Summary (Substitutions, InDels, Fusions, CNV)
- Concordance Data

- **Reproducibility (extended time series)**
  - Methodology
  - Results summary (Variant allele frequency, call concordance)
  - Reproducibility data

- **Diagnostic Performance**
  - **Methodology**
  - **Samples used**
  - **Results summary**
  - **Diagnostic Sensitivity Data**
    - Diagnostic Specificity Data

- **Appendices**
  - **Individual QC metrics reports for all samples**
  - **Draft reports for all samples**