Validation of clinical testing

Setting a new standard for clinical testing through fully transparent validation.

Blueprint Genetics

Analytic validation of diagnostic tests at Blueprint Genetics

Comprehensive analytic validation is a critical step in transparent genetic diagnostics. It demonstrates the quality and performance of the sequencing methods and data analysis pipelines and is the foundation for setting quality standards for the carried tests.

Blueprint Genetics performs analytic validation of all laboratory and data analysis assays per ACMG guidelines

(1). Metrics included in the analytic validation of Blueprint Genetics diagnostics assays are described in **Table 1.** Blueprint Genetics applies independent and publicly available sample materials and data sets as the reference materials in all validation studies, to ensure full traceability of the validation results. Comparisons are performed using all available data and no post-analysis corrections have been applied.

Table 1 Metrics applied in the analytic validation of Blueprint Genetics' diagnostic tests.

Validation metric	
PRECISION	*is a statistical measure of the performance of the assay to generate correct test results. For estimation of the precision, true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) are measured and sensitivity, specificity, positive predictive value, and accuracy are calculated.*
CLINICAL SENSITIVITY	*provides a statistical measure of the assay's clinical performance. It reflects the assay's ability to provide a diagnosis in specific clinical cases. Clinical sensitivity is measured by calculating the extent to which all clinical diagnosis scenarios are met.*
REPORTABLE RANGE	*is the functional range of an assay over which the analyte can be analyzed.*
REPEATABILITY	*is the technical variation in measurements taken by a single person on the same instrument, on the same experiment, under the same conditions, and in a short time.*
REPRODUCIBILITY	*is the ability of a test result to be duplicated under all variable conditions (different users, between reagent lots, using different instruments, and different testing times).*
INTENDED USE	*is the test's ability to perform under all testing conditions.*

Analytic validation of diagnostic tests

Gene panels and Whole Exome Sequencing

Blueprint Genetics utilizes Whole Exome Sequencing (WES) with boosted clinical content and high-throughput next-generation sequencing to generate high quality data. In addition to CCDS genes and 20 bps of flanking intronic sequences, over 1,479 non-coding and deep intronic variants with clinical relevance have been included in the boosted WES assay. Diagnostic gene panels are sliced from the clinically boosted WES data. Our data analysis pipeline has been validated for detection of single nucleotide variants (SNVs) and insertions and deletions (INDELs) **(Table 2)** as

well as copy number variation (CNV; ≥1 exon level deletions and duplications) **(Table 3).**

22 platinum genome samples and Genome-in-a-bottle (GIAB) reference samples with confirmed SNVs and INDELs, 80 samples with confirmed CNVs and 240 test samples with confirmed mutations were applied in the validation experiments. Results from clinical validation of challenging genomic regions are described in **Table 4.**

Table 2 Analytic validation of the SNV and INDEL detection.

Performance metric	Value	Approach
Accuracy (SNVs)	*99.9%*	*TN: 922,349,615*
Sensitivity (SNVs)	*99.7%*	*TP: 412,456*
Specificity (SNVs)	*99.9%*	*FP: 9,928*
		FN: 1,437
Sensitivity (1–10 bp INDELs)	*96.9%*	*TP/FN: 17,070/538*
Sensitivity (11–20 bp INDELs)	*98.9%*	*TP/FN: 791/9*
Sensitivity (21–30 bp INDELs)	*100.0%*	*TP/FN: 145/0*
Sensitivity (31–40 bp INDELs)	*100.0%*	*TP/FN: 19/0*
Sequencing (nucleotides with >20x sequencing depth)	*99.4%*	
Mean sequencing depth at nucleotide level (CCDS genes and boosted content)	*174x*	
Reportable range (SNVs)	*Hom, Het*	
Reportable range (insertions)	*1–221 bp*	
Reportable range (deletions)	*1–210 bp*	
Repeatability	*99.7%*	
Reproducibility	*99.7%*	
Intended use (blood samples with >98% of nucleotides with >20x sequencing depth)	*100%*	
Intended use (saliva samples with >98% of nucleotides with >20x sequencing depth)	*100%*	
Intended use (DBS samples with >98% of nucleotides with >20x sequencing depth)	*100%*	

SNVs, single nucleotide variants; INDELs, insertions and deletions; TN, true negative; TP, true positive; FP, false positive; FN, false negative; DBS, dried blood spots. Validation experiments were performed by subsampling the data to 100M sequencing reads.

* The human genome contains regions that are affected by pseudogenes, repeats and extreme GC-content that are not reproducibly analyzed using short read sequencing.

Table 3 Analytic validation for copy number variations (CNVs; 1 exon or larger deletions and duplications).

Performance metric	Value	Approach
Clinical sensitivity* (1 exon)	*92.3%*	*24/26 detected*
Clinical sensitivity* (2 exon)	*100.0%*	*11/11 detected*
Clinical sensitivity* (3–7 exon)	*93.3%*	*14/15 detected*
Clinical sensitivity** (Microdeletion syndromes, 0.10−47 Mb)	*100.0%*	*37/37 detected*
Clinical sensitivity (proportion of target exons covered***)	*97.01%*	*29,992/30,916 exons*
Simulated sensitivity** (2 exon) *Simulated specificity** (2 exon)*	*90.1%* *100.0%*	*TP/FN: 7,357/8,086*
Simulated sensitivity** (5 exon) *Simulated specificity** (5 exon)*	*98.6%* *100.0%*	*TP/FN: 7,975/8,086*
Simulated sensitivity** (10 exon) *Simulated specificity** (10 exon)*	*99.9%* *100.0%*	*TP/FN: 8,076/8,086*
Repeatability	*98.6%*	
Reproducibility	*98.6%*	
Intended use (blood samples with >98% of nucleotides with >20x sequencing depth)	*100%*	
Intended use (saliva samples with >98% of nucleotides with >20x sequencing depth)	*100%*	
Intended use (DBS samples with >98% of nucleotides with >20x sequencing depth)	*100%*	

TP, true positive; TN, true negative; FP, false positive; FN, false negative; DBS, dried blood spots.

*   High resolution CNV detection algorithm was applied to detect small CNVs (1–7 exons)

**   CNV detection algorithm was applied to detect large CNVs (≥5 exons and microdeletion/-duplication syndromes)

*** The human genome contains regions that are affected by pseudogenes, repeats and extreme GC-content that are not reproducibly analyzed using short read sequencing.

Table 4 Clinical validation of challenging genomic regions.

Challenging genomic regions	Value	Approach
RPGR ORF15 exon chrX:38,144,793-38,146,499 (nucleotides with >20x sequencing depth)	*99.9%*	*11/11 detected*
RPGR ORF15 gap, chrX:38,145,185-38,145,807 (nucleotides with >20x sequencing depth)	*99.8%*	
Clinical sensitivity* (2 exon)	*100.0%*	*11/11 detected*

Sanger sequencing

Direct Sanger sequencing of PCR amplicons is applied to confirm pathogenic and likely pathogenic SNV and INDEL variants from probands as well as for screening of disease-causing mutations in the family members.

20 samples with confirmed variants were applied in the validation of Sanger sequencing **(Table 5)**.

Table 5 Analytic validation of Sanger sequencing assay.

Performance metric	Value	Approach
Sensitivity	*100.0%*	*TP/FN: 88/0*
Repeatability	*100.0%*	
Reproducibility	*100.0%*	

TP, true positive; FN, false negative.

Clinically actionable test design

Maximizing diagnostic yield

Blueprint Genetics diagnostic panels have been designed together with clinicians to maximize the tests' impact for clinical practice. Each panel has been carefully compiled to be used in specific clinical applications where differential diagnosis requirements and complex disease phenotypes need to be taken into consideration. Sequencing analysis covers all coding exons based on the widest possible gene models, including 20 bases of flanking intronic sequences. Our panels offer high sensitivity to detect different types of genetic variants, e.g. SNVs, INDELs, and CNVs.

Wide variety of accepted sample types

Our tests have been validated for variety a of specimen types, including blood, saliva, DNA and dried blood spots (DBSs), to ensure flexible sampling procedures.

Rapid turnaround time

Test results are provided in 28 days to facilitate effective clinical workflow and to minimize patients' anxiety when waiting for test result

Our panels have been designed together with clinicians to maximize the tests' impact for clinical practice.

Test reporting that produces clinical value

Experienced interpretation team

Our result interpretation team consists of 20 internationally recognized geneticists (medical, clinical, ABMGC certified clinical molecular and other). The average number of peer reviewed publications per team member is 41. This level of experience guarantees an unmatched quality of interpretation.

Similarly, Blueprint Genetics has a team of 17 clinical consultants with expertise covering all medical specialties. The clinical consultation team consists of professors and assistant professors from top universities. All consultants are MD, PhD and have specialist degree in their medical field of expertise.

Clinical interpretation system and databases

Blueprint Genetics has developed an efficient software platform for clinical interpretation of genetic tests and management of clinical genetic information. Clinical interpretation utilizes a cloud-based big data environment to handle a colossal amount of unstructured and structured data and enables a high precision view into case-specific clinical-genetic data that augments improved speed, higher reproducibility and better accuracy for the geneticists to create comprehensive and clinically actionable reports.

Clinical interpretation utilizes a systematic and high-quality process to interpret genetic test results and records geneticists' and clinical consultants' judgments and scientific justifications when evaluating a case. Knowledge from previously analyzed cases is applied, and experimental, genetic and clinical data is presented intuitively and comprehensively at each analysis step. Genetic and clinical information is automatically collected from various databases **(Table 6)** in real-time, and access is provided to our in-house curated knowledge base. The system provides workflows for annotation and management of genetic tests, automates product information synchronization, and creates a transparent audit trail for future scrutiny of the interpretation work.

With advanced analytics, we can provide our customers with reporting that is based on up-to-date, clinically relevant, and thoroughly vetted scientific information.

Table 6 Databases applied in the clinical interpretation.

Databases applied in the clinical interpretation
HGMD
ClinVar
UMD-Be
LOVD
UniProt
ExAC
GnomAD
GeneReviews
OMIM
Multiple disease/gene specific databases

Clinical statement

The Blueprint Genetics clinical statement provided for customers, is a thorough report depicting the entire diagnostic process. It presents all relevant genetic findings and scientific justification, and provides information about the clinical follow-up. The reports fulfil all requirements provided in the **ISO15189, CAP,** and **CLIA** standards. Blueprint Genetics diagnostic tests are **CE-marked.**

The clinical statement contains detailed case-specific information about testing performance, including a test's analytic precision (sensitivity) and clinical sensitivity, that allows the clinician to accurately estimate the clinical impact of the test result. The most relevant test results are concisely presented on the first page of the statement. Additional pages include basic information of the variant's allele frequencies in the reference populations and the *in-silico* predictions. The most important part of the statement is the literature review, which carefully presents all the evidence gathered for the variant classification. This includes the biomedical role of the affected gene, number of patients with the same variant, their phenotype, available segregation data and citations to publications and mutation databases, and a list of possible additional analysis used, such as paralogue annotation.

Blueprint Genetics interpretation team follows a systematic variant classification scheme according to the guideline set by the **ACMG.**

Nucleus is the on-line portal where clinicians can place orders, follow the progress of analysis, and both retrieve and manage test results securely.

Clinical validation

Blueprint Genetics participates in scientific research of inherited disorder genetics.

Dilated cardiomyopathy

The clinical utility and the yield of clinically meaningful findings in comprehensive NGS-based genetic diagnostics in dilated cardiomyopathy (DCM) has been previously studied **(2)**. The study utilized a high-quality targeted sequencing panel to analyze 145 unrelated patients and to investigate the impact of genetic testing in DCM. The researchers found that the diagnostic yield was 35.2% (familial 47.6% and sporadic 25.6%, P = 0.004) when both pathogenic and likely pathogenic variants were considered as disease causing.

Pulmonary arterial hypertension

Targeted NGS and a custom data analysis and interpretation pipeline was applied to identify pathogenic base substitutions, insertions, and deletions in seven genes associated with pulmonary arterial hypertension (PAH) **(3)**. The study included 21 PAH patients, whose *BMPR2* and *ACVRL1* mutation status had been previously analyzed using Sanger sequencing. In 29% of the cases, pathogenic base substitutions were identified in the *BMPR2* gene. Two of the pathogenic variant-positive patients had been previously tested negative using Sanger sequencing.

Role of Titin mutations in cardiomyopathy

Clinical interpretation of the *TTN* gene variants was elucidated by systematic analysis of truncating variants in publicly available reference populations **(4)**. The findings indicated that one in 500 in general population carries a truncating TTN mutation in the A-band, suggesting that some of these variants do not manifest as autosomal dominant DCM. The researchers called for caution when interpreting truncating *TTN* variants in individuals and families with no history of DCM. In a follow-up study, truncating *TTN* variants were found to be more prevalent in DCM patients compared with a reference population, and it was estimated that the probability of pathogenicity of truncations that were affecting all *TTN* transcripts was 97.8% **(5).**

Quality management system

Accreditations and certifications

Blueprint Genetics is a **CLIA-certified** (#99D2092375) laboratory, inspected by the US Centers for Medicare & Medicaid Services (CMS), which gives us a permission to analyze samples from the US. In addition, we have licenses from five states (California, Florida, Maryland, Pennsylvania and Rhode Island) which are not covered by the federal CLIA and require separate laboratory permits. CLIA regulation focuses on protecting the analysis process of the clinical samples.

Our laboratory has been accredited by the **College of American Pathologists** (**CAP** #9257331) and by **FINAS** Finnish Accreditation Service, laboratory no. T292, accreditation requirement SFS-EN **ISO 15189**:2013, thus, our processes and documentation have been built to comply with two highly recognized quality standards. These two accreditations ensure the quality of all our functions from the management of the laboratory and testing of samples to the safety of the staff and customer satisfaction.

Blueprint Genetics diagnostic tests have been **CE-marked.**

Quality management

CAP accreditation covers all functions related to patient testing. In addition to the requirements for clinical sample handling and testing, the CAP standard provides detailed checklists e.g. for method validation, proficiency testing, testing environment, and laboratory safety and staff qualifications. The ISO 15189 standard, which is the most widely used standard worldwide, is recognized by the International Laboratory Accreditation Cooperation (ILAC), the InterAmerican Accreditation Cooperation (IAAC), the Asia Pacific Laboratory Accreditation Cooperation (APLAC), and the European Cooperation for Accreditation (EA). It takes a broader view to quality management by setting guidelines for management of the laboratory e.g., management responsibility and commitment. Furthermore, supplier management and customer satisfaction are included in the guidance.

Our laboratory is among a few that have received **ISO 15189** accreditation with a scope covering all next-generation sequencing testing functions.

External audits

FINAS (Finnish Accreditation Service) audits Blueprint Genetics once a year for **ISO 15189** accreditation. FINAS is the national accreditation body in Finland and its operations are regulated under law (920/2005). Like other EU countries, Finland has only one accreditation body. Accreditation policies are agreed upon by international organizations in the field, with members from the accreditation bodies in different countries. FINAS operates in both European and international organizations (EA, ILAC, and IAF). FINAS's operations are internationally consistent and recognized through the activities of these organizations.

CAP (College of American Pathologists) inspects us biennially and requires a self-inspection in the year between the inspections. CAP is the world's largest organization of board-certified pathologists. It inspects and accredits medical laboratories worldwide under deemed authority of the US Centers for Medicare & Medicaid Services (CMS). There are more than 7,000 CAP-accredited laboratories worldwide, including mostly hospital laboratories as well as commercial laboratories and contract research organizations.

In-process quality control

We perform 17 in-process quality control checks during processing of the clinical samples. These QC steps ensure that sample handling, examination processes, data analysis and interpretation have been executed successfully, and that the obtained results conform to validated assay performance.

We include a well-characterized golden-standard reference sample (NA12878) into each sample processing, sequencing, and data analysis batch to control for quality of the testing and to assess the sensitivity of the performed assays. For each clinical sample, we measure a quality value that ensures each analysis fulfills the criteria derived from the analytic validation studies **(Table 7)**.

Table 7 Quality metrics and acceptance criteria for clinical testing.

Quality indicator	Quality metrics	Acceptance criteria	Actual*
Sequencing coverage	*Test sample coverage (nucleotides with >20x sequencing depth)*	*>98%*	*99.6%*
	Reference sample coverage (nucleotides with >20x sequencing depth)	*N.A*	*99.5%*
Sequencing depth	*Test sample depth (median sequencing depth)*	*N.A*	*250x*
	Reference sample depth (median sequencing depth)	*N.A*	*209x*
SNV and INDEL detection	*Reference sample sensitivity (SNVs)*	*>98%*	*99.7%*
	Reference sample sensitivity (1–10 bp INDELs)	*N.A*	*96.0%*
	Reference sample sensitivity (11–20 bp INDELs)	*N.A*	*98.8%*
	Reference sample sensitivity (21–50 bp INDELs)	*N.A*	*100.0%*
CNV detection	*Test sample sensitivity (5 exon)*	*>98%*	*98.8%*
	Reference sample sensitivity (5 exon)	*N.A*	*99.8%*
Sequencing yield	*Test sample sequencing yield (number of reads)*	*N.A*	*174M*
	Reference sample sequencing yield (number of reads)	*N.A*	*150M*

CNVs, copy-number variations; INDEL, insertions and deletions; Del/Dup, deletions and duplications.

*Average from 376 production samples.

Proficiency testing

To continuously monitor our performance, Blueprint Genetics participates in external quality assessment schemes provided by external organizations such as the College of American Pathologists (CAP) and European Molecular Quality Network (EMQN). The proficiency testing providers send proficiency testing samples to Blueprint Genetics and after we analyzing them, we submit the results to the organizer for grading.

Blueprint Genetics participates in nine Next-Generation Sequencing proficiency testing schemes provided by CAP and in a NGS scheme provided by EMQN. CAP schemes are targeted for specific genetic conditions/diseases and the tested samples are sent twice a year. CAP sends the results from the graded challenges directly to the US Centers of Medicare & Medicaid Services (CMS).

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