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 pipeline, 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.*

Sequencing and Del/Dup (CNV) panels

Blueprint Genetics utilizes a proprietary targeted sequencing technology, Oligonucleotide-Selective Sequencing (OS-Seq), that was developed in Stanford University (2). Our data analysis pipeline has been validated for detection of single nucleotide variants (SNVs), insertions and deletions (INDELs) (**Table 2**), as well as deletions and duplications (DELDUPs) (**Table 3**).

37 reference samples with confirmed SNVs and INDELs, 283 reference samples with confirmed DELDUPs, and the golden standard reference sample (NA12878) were applied in the validation.

Table 2 Analytic validation of the Sequencing panels.

Performance metric	Value	Approach
Accuracy (SNVs) *Sensitivity (SNVs)* *Specificity (SNVs)* *Positive predictive value (SNVs)*	*99.9%* *99.3%* *99.9%* *99.3%*	*TN: 17,387,846* *TP: 10,393* *FP: 70* *FN: 74*
Sensitivity (1–10 bp INDELs)	*96.1%*	*TP/FN: 1,494/61*
Sensitivity (11–20 bp INDELs)	*88.5%*	*TP/FN: 904/118*
Sensitivity (21–30 bp INDELs)	*66.8%*	*TP/FN: 245/122*
Sensitivity (31–46 bp INDELs)	*19.8%*	*TP/FN: 41/166*
Nucleotides with >15x sequencing depth *Mean sequencing depth at nucleotide level*	*99.60%* *234x*	
Reportable range (SNVs) *Reportable range (INDELs)*	*Hom, Het* *0–46 bp*	
Repeatability	*99.4%*	
Reproducibility	*99.8%*	
Intended use (blood samples with >98% of nucleotides with >15x sequencing depth)	*100%*	
Intended use (saliva samples with >98% of nucleotides with >15x sequencing depth)	*100%*	

SNVs, single nucleotide variants; INDELs, insertions and deletions; TN, true negative; TP, true positive; FP, false positive; FN, false negative.

Table 3 Analytic validation of Del/Dup panels.

Performance metric	Value	Approach
Sensitivity (1 exon) *Specificity (1 exon)*	*71.5%* *100%*	*TP/FN: 89,692/35,769* *TN/FP: 1,782,470/545*
Sensitivity (2 exon) *Specificity (2 exon)*	*95.2%* *100%*	*TP/FN: 59,837/2,998* *TN/FP: 1,782,672/343*
Sensitivity (3 exon) *Specificity (3 exon)*	*99.0%* *100%*	*TP/FN: 41,544/431* *TN/FP: 1,782,751/264*
Sensitivity (4 exon) *Specificity (4 exon)*	*99.9%* *100%*	*TP/FN: 31,430/34* *TN/FP: 1,782,823/192*
Sensitivity (5 exon) *Specificity (5 exon)*	*99.9%* *100%*	*TP/FN: 25,225/28* *TN/FP: 1,782,866/149*
Clinical sensitivity *(proportion of pathogenic Del/Dups detected*)*	*92.42%*	
Clinical sensitivity *(proportion of target genes covered**)*	*97.01%*	*29,992/30,916 exons*
Reportable range (deletions)	*317 bp and larger*	
Reportable range (duplications)	*545 bp and larger*	
Repeatability	*99.7%*	
Reproducibility	*99.3%*	
Intended use (blood samples with >98% of nucleotides with >15x sequencing depth)	*100%*	
Intended use (saliva samples with >98% of nucleotides with >15x sequencing depth)	*100%*	

Del/Dups, deletions and duplications; TP, true positive; TN, true negative; FP, false positive; FN, false negative.
* Estimated proportion of patients with Del/Dups that will get a diagnosis with our assay. Analysis has been performed based on sensitivity to detect Del/Dups of different sizes and estimated proportion of different sized pathogenic Del/Dups in LDLR, FBN1, BRCA1 and DMD based on literature review and HGMD.
** The human genome contains regions that are affected by pseudogenes, repeats and extreme GC-content that are not reproducibly analyzed using short read sequencing.

Whole Genome Del/Dup (CNV)

We utilize low-coverage whole genome sequencing and a proprietary genome segmentation algorithm to detect large Del/Dups.

28 reference samples with 34 confirmed chromosomal aberrations and the golden standard reference sample (NA12878) were applied in the validation **(Table 4).**

Table 4 Analytic validation of Whole Genome Del/Dup (CNV).

Performance metric	Value	Approach
Sensitivity (chromosomal aberrations, >100 kb)	*97.0%*	*TP/FN: 33/1*
Sensitivity (Del/Dups) *10–25 kb* *25–50 kb* *>50 kb*	 *6%* *76%* *99%*	 *TP/FN: 16/273* *TP/FN: 143/44* *TP/FN: 101/1*
Clinical sensitivity (proportion of genes that are analyzed)*	*94.2%*	*130,284 out of 138,259 segments used*
Reportable range	*10 kb and larger Del/Dups*	
Repeatability	*86%*	
Reproducibility	*85%*	
Intended use (blood samples where common CNVs are detected)	*100%*	
Intended use (saliva samples where common CNVs are detected)	*100%*	

Del/Dups, deletions and duplications; CNVs, copy-number variations; TP, true positive; FN, false negative.
* Coding regions of the human genes contain regions that are affected by pseudogenes, repeats, and extreme GC-content that are not reproducibly analyzed using short read sequencing.

Whole Exome Sequencing

Whole Exome Sequencing and a highly optimized data analysis pipeline are applied for efficient detection of SNVs and INDELs.

10 reference samples with confirmed SNVs and INDELs and the golden standard reference sample (NA12878) were applied in the validation **(Table 5).**

Table 5 Analytic validation of Whole Exome Sequencing.

Performance metric	Value	Approach
Sensitivity (SNVs) *Specificity (SNVs)* *Positive predictive value (SNVs)* *Accuracy (SNVs)*	*99.5%* *99.9%* *99.4%* *99.9%*	*TN: 326,571,803* *TP: 152,827* *FP: 896* *FN: 791*
Sensitivity (1–10 bp INDELs)	*97.2%*	*TP/FN: 6,033/169*
Sensitivity (11–20 bp INDELs)	*95.7%*	*TP/FN: 356/16*
Sensitivity (21–30 bp INDELs)	*97.0%*	*TP/FN: 162/5*
Sensitivity (31–35 bp INDELs)	*100.0%*	*TP/FN: 10/0*
Nucleotides with >15x sequencing depth *Mean sequencing depth at nucleotide level*	*98.86%* *149x*	
Reportable range (SNVs) *Reportable range (INDELs)*	*Hom, Het* *0–35 bp*	
Repeatability	*99.4%*	
Intended use (blood samples with >98% of nucleotides with >15x sequencing depth)	*100%*	
Intended use (saliva samples with >98% of nucleotides with >15x sequencing depth)	*100%*	

SNVs, single nucleotide variants; INDELs, insertions and deletions; TN, true negative; TP, true positive; FP, false positive; FN, false negative.

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 6**).

Table 6 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.

Quantitative PCR

Quantitative PCR is applied to confirm pathogenic and likely pathogenic Del/Dup variants from probands as well as for screening of disease-causing mutations in the family members.

Validation of Del/Dup confirmation assay was performed using 15 well-characterized reference samples and 32 Del/Dup assays (**Table 7**).

Table 7 Analytic validation of quantitative PCR assay.

Performance metric	Value	Approach
Sensitivity *Specificity* *Positive predictive value* *Accuracy*	*95.4%* *92.0%* *91.3%* *93.6%*	*TP: 21* *TN: 23* *FP: 2* *FN: 1*
Repeatability	*100.0%*	
Reproducibility	*100.0%*	

TP, true positive; TN, true negative; FP, false 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 15 bases within intronic sequences. Our panels offer high sensitivity to detect different types of genetic variants, e.g. SNVs, INDELs, and Del/Dups.

Wide variety of accepted sample types

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

Rapid turnaround time

Test results are provided in 21 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

Blueprint Genetics result interpretation team consists of internationally recognized experts in the field of human genetics and clinical medicine. The average number of peer reviewed publications per team member is 55. This level of experience guarantees an unmatched quality of interpretation.

Similarly, Blueprint Genetics has a team of clinical consultants with expertise covering all medical specialties. The clinical consultation team consists of 4 professors and 10 assistant professors from top universities, a former president of the European Society of Human Genetics, the President of The Scandinavian Society for Immunology and the President of The European Complement Network. All consultants are MD, PhD and have specialist degree in their medical field of expertise.

CLINT and databases

Blueprint Genetics has developed an efficient big data solution called **CLINT** (CLinical INTerpretation), for clinical interpretation of genetic tests and management of clinical genetic information. This is one of the first implementations of **IBM's Watson** technology into genetic data interpretation in a clinical setting (3).

CLINT 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.

CLINT 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 8**) in real-time, and access is provided to our in-house curated knowledge base. CLINT 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.

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

Table 8 Databases applied in the clinical interpretation.

Performance metric
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 fulfills all requirements provided in the **ISO 15189, CAP,** and **CLIA** standards.

The clinical statement contains detailed case-specific information about testing performance in each patient that allows clinicians to accurately estimate the diagnostic procedure. 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 online 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 (4). 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 were applied to identify pathogenic base substitutions, insertions, and deletions in seven genes associated with pulmonary arterial hypertension (PAH) (5). 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 (6). 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% (7).

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.

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 9**).

Table 9 Quality metrics and acceptance criteria for clinical testing.

Diagnostic tests	Quality metrics	Acceptance criteria	Actual*
Sequencing Panel	*Test sample coverage (nucleotides with >15x sequencing depth)*	*>98%*	*99.4%*
	In-process reference sample sensitivity	*>98%*	*99.3%*
Del/Dup (CNV) Panel	*Test sample sensitivity (5 exon)*	*>98%*	*99.3%*
Whole Genome Del/ Dup (CNV)	*In-process reference sample sensitivity (>50 kb)*	*>80%*	*100.0%*
	Number of mapped reads	*>39M*	*58.2M*
Whole Exome Sequencing	*Test sample coverage (average)*	*>100x*	*158x*

CNVs, copy-number variations; Del/Dup, deletions and duplications.
* 3-month average.

Proficiency testing

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