Technical and Logistical Approaches to Genomic Screening

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Summary: Optimizing High Throughput Genomic Screening: Lessons Learned

Consistent DNA source and quality

Optimize and Simplify workflow with automation

Stable Process for Library Automation

Cost efficient

Continual upgrade process with testing, validation, implementation for both laboratory protocols and software upgrades

Metrics to produce a quality product and monitoring of production workflow

Semi-automated analysis pipeline

Clear communication of reporting practices

General Requirements to Launch Clinical Test (LDT)

- Performed in CLIA certified laboratory, CAP accreditation
- Indication for testing, requisition, required elements
- Specimen requirements
- SOP, validated reagents, instruments, vendors
- Technical limitations determined and disclosed
- Assay interpretation
- Validation studies: Rationale used for validation
- Clinical reporting criteria
- Limitations of testing and reporting
- Post - launch evaluation

Clinical Test Validation

Analytical validation

- Accuracy
- Precision
- Sensitivity/Specificity
- Reproducibility
- Limit of Detection
- Choice of validation samples

Clinical Validation

- Purpose of test
  - Newborn screening – actionable disorders of early childhood
  - Family planning – carrier screening
  - Wellness – hereditary cancer, CDC Tier 1, ACMG 59, PGX
- Evidence
- Actionability

Clinical Laboratory Improvement Amendments

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

NEW YORK STATE Department of Health

FDA U.S. FOOD & DRUG ADMINISTRATION

Stages of Validation of Clinical NGS Test

- Test Development and Optimization
  - Indication for testing, test scope, samples types accepted, TAT
  - Wet lab workflow, automation, QA metrics and performance
  - Establish analytical pipeline, versioning, additional modules for challenging regions
  - Variant confirmation approach
  - Validation strategy
- Test Validation
  - Measure test performance through analytical validation - sensitivity (recall, positive percent agreement), specificity (negative percent agreement), precision, reproducibility (comparison across instruments and operators), repeatability (technical replicates under same condition)
  - Stress test performance to determine limitations - difficult regions, mosaicism detection level
- Test Performance Monitoring / Quality Management
  - QA metrics established and tracked
    - Sample identity, contamination, GB>Q30, 20x%

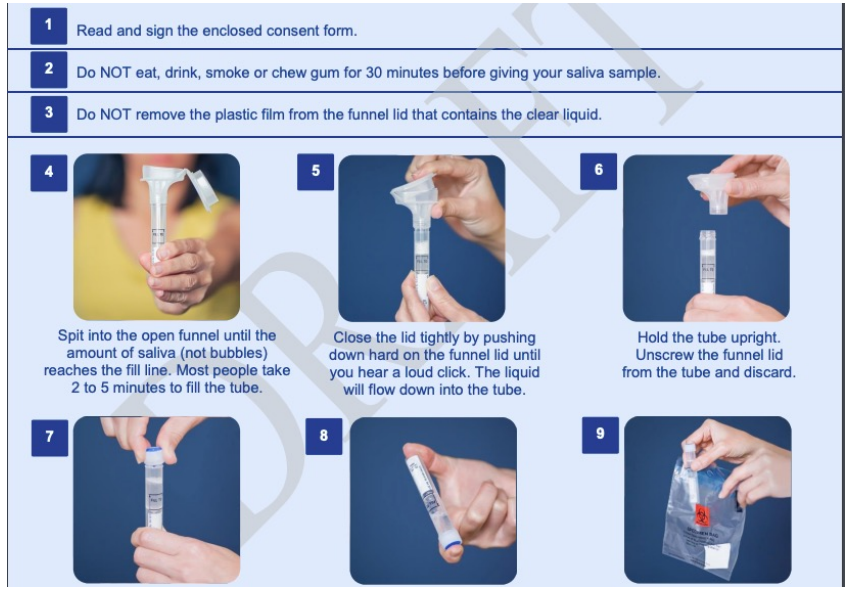
Marshall et al, Best practices for the analytical validation of clinical whole genome sequencing intended for the diagnosis of germline disease, NPJ Genomic Medicine 23 Oct 2020

Sample Collection Considerations

- Participant convenience
- Kitting, detailed instructions for self-collection
- Bullet-proof labeling and downstream matching to contact information
- Cost
- Ability to automate DNA extraction
- Sample stability – during shipment and time to processing
- Failure rate of chosen method
- Potential ability to store DNA long-term (Biorepository)

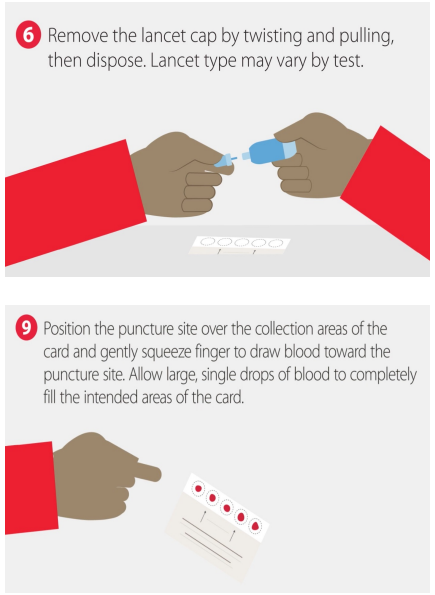
Sample Collection Options

Saliva / Mouthwash/ Buccal



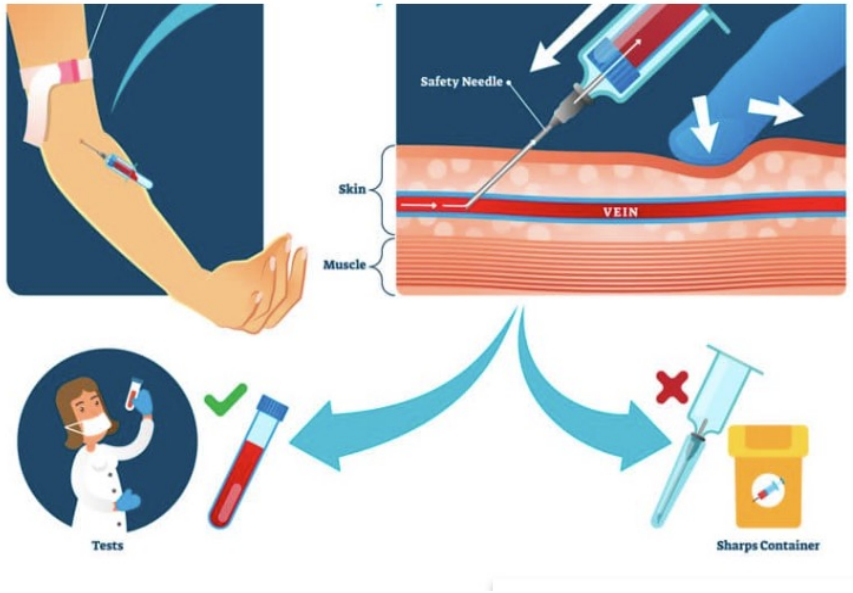
Source:www.cdc.gov/ncbddd

Dried Blood Spot



Source: Cardinal Health

Whole Blood



Source: Arizona College of Nursing

Non Invasive

Invasive

Sample Workflow

O'Brien et al., Population screening shows risk of inherited cancer and familial hypercholesterolemia in Oregon, AJHG 110:1249, 2023

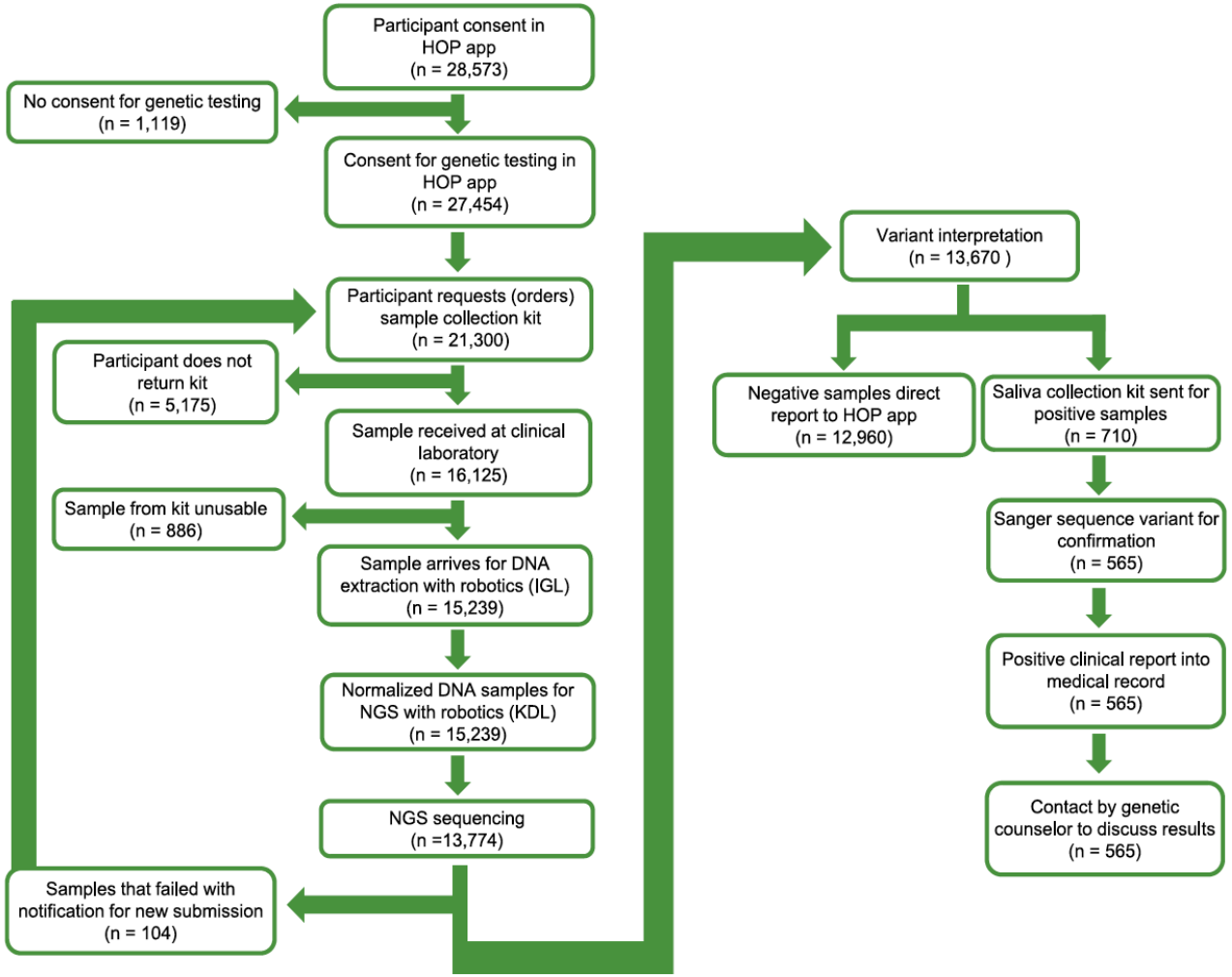
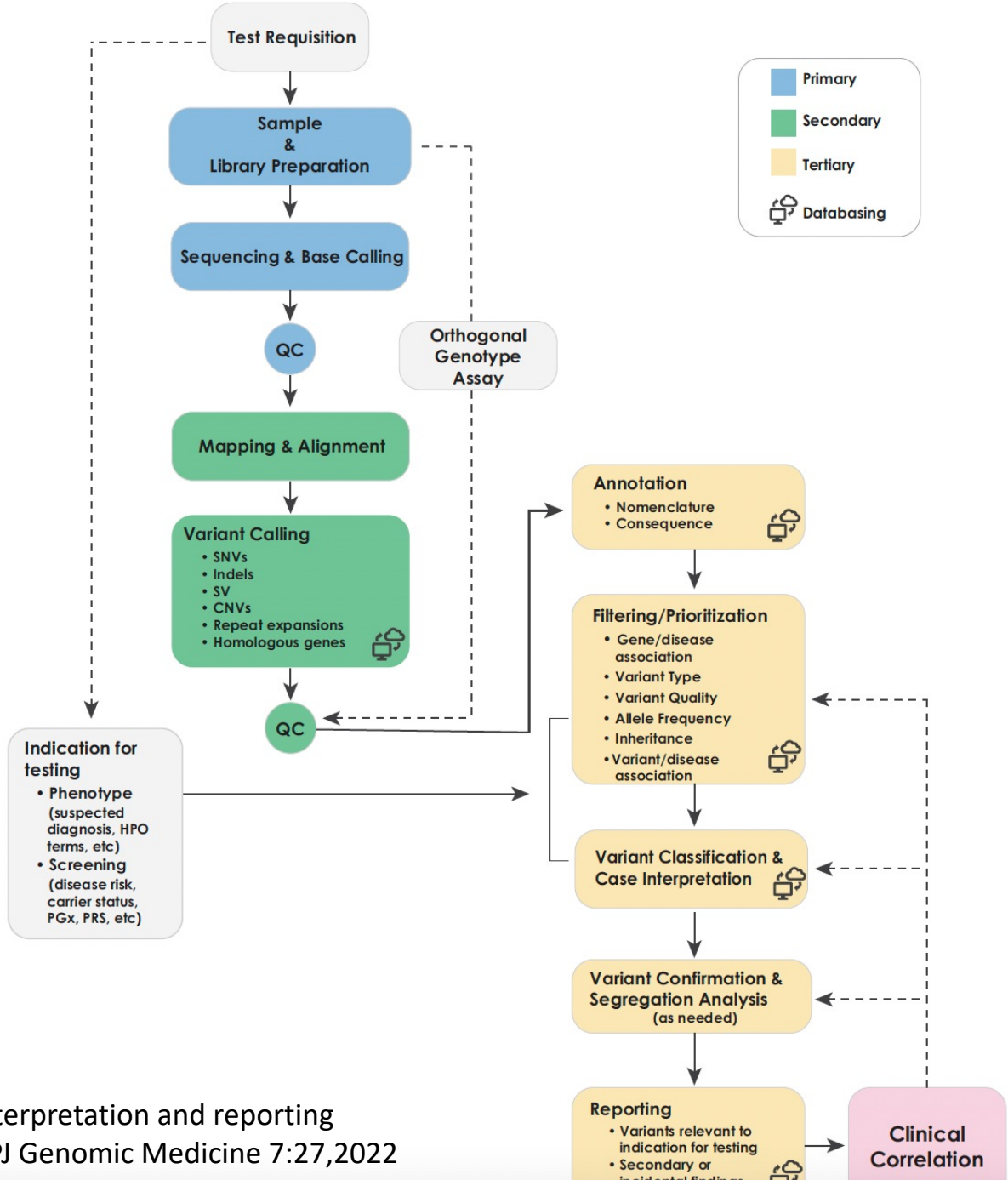


Figure 1. Pipeline for the enrollment of HOP participants and sample workflow
HOP participants sequenced as of April 15, 2022. Differences in numbers are reflective of the fluid pipeline and time it takes samples to process from consent in the HOP app through next-generation sequencing (NGS) and analysis. IGL, Integrated Genomics Laboratory, core laboratory; KDL, Knight Diagnostic Laboratories, clinical laboratory.

Choice of Platform: Planning for High Throughput Testing

- Genotyping array
  - Example: UK Biobank Array - 800,000 markers
    - Exome, GWAS, PGX, HLA
    - Samples screened at Biobanks, genome centers, core labs
  - GDA array - All of US, ancestry, concordance
  - Arrays cost effective, high throughput, low failure rate, less flexibility after design
- Targeted NGS panel
  - Example: universal carrier screening panels, hereditary cancer panels
  - Less data produced, higher coverage, better CNV detection, less flexibility after design
- WES
  - Less data, less cost than WGS, ability to re-analyze, missing regions important for PGX, PRS
- WGS
  - Hypothesis-free, ability to re-analyze, highest cost and data
- Hybrid design - low pass WGS with 30-40X WES

NGS Workflow



- Technical production
- Identification of DNA variants
- Annotation, prioritization, interpretation, reporting

Austin-Tse et al, Best practices for the interpretation and reporting of clinical whole genome sequencing, NPJ Genomic Medicine 7:27,2022

Analysis / Variant Classification

- Variant calling via bioinformatic pipeline
- Annotation, application of filters, database searches, prediction of functional effect, population frequency
  - clinical significance in ClinVar
  - classification in Human Gene Mutation Database (HGMD)
  - variant type, and population frequency
- Automated search against established database
- Manual curation of novel variants

Quality Metrics

There are three main areas where QC can be applied to next-generation sequencing (NGS)

On the starting nucleic acid samples	
After library preparation	• Insert size
Post-sequencing (Pass/Fail)	• Sample identity and contamination • Gb>Q30 • Mean coverage • % Callability
Post Sequencing Monitoring	• Performance metrics • Positive controls, periodic reference standard • Periodic review of positivity rates (%positive calls by gene)

Carrier Screening Panel Example

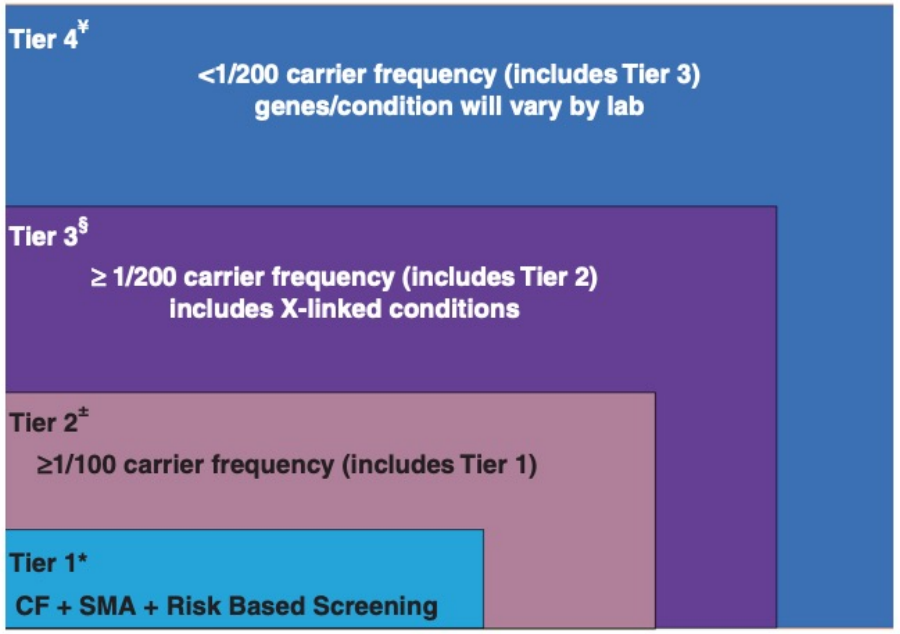


Fig. 1 The Euler diagram shows an overlapping tiered approach to carrier screening. *Recommended by the American College of Medical Genetics and Genomics (ACMG)[17,18] and American College of Obstetricians and Gynecologists (ACOG).[19] ±Recommended by ACOG.[2] §Supported by literature.[49,50] ¥Offered by molecular testing laboratories; the list of genes/conditions may vary by the laboratory. *CF* cystic fibrosis, *SMA* spinal muscular atrophy.

Gregg et al, Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG) GenetMed 23:1793, 2021

(Expanded Tier 4; 445 Gene) Carrier panel

- Exceeds current ACMG and ACOG recommendations
- Inherited X-linked carrier status identified for females
- 14 day TAT
- NGS panel with larger gene library than reported genes
- Detects SNV, indel, CNV, Path and LP variants reported
- Difficult to sequence genes (FMR1, FXN, CYP21A2) require separate assays and joining of data for reporting
- Orthogonal confirmations for specific genes

WGS-Specific Validation and Reporting Considerations

- Determine which variant types will be reported
  - SNV, indel, CNV, SV, trinucleotide repeat, mitochondrial genome
  - Gather appropriate positive controls* (common, rare)
  - Stress the system by selecting variants in challenging regions (high homology, pseudogenes)
  - Clearly define limits of detection
- Determine coverage level - depth of coverage, base quality, mapping quality
- Strategy for clinically important variants in difficult regions - additional bioinformatic modules, manual inspection of raw data, orthogonal methods, confirmation
- Tiered reporting
  - All of US - ancestry, hereditary disease risk, PGX
  - Subscription model

Return of Results

- Critical to clearly communicate the result parameters to providers and participants
- Screening usually limited to Pathogenic or Likely Pathogenic
- Affected status (AD, AR, X-linked)
- Carrier status?
- Management guidance for positives
- Does negative result require counseling?
- Re Contact / Re Analysis / Subscription
  - Clear communication / expectation regarding “living report”

Summary

- Overview of current state of clinical methods for population genomic screening
- Clear distinction between reporting of diagnostic vs screening genomic tests
- Laboratory quality measures are not distinct
- High throughput requirements can present unique challenges to the laboratory