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To: [Mark Pratt](#); [Richard Chen](#); [Hugo Lam](#); [Sarah Garcia](#); [Rong Chen](#)
Subject: Draft framework for Personalis accuracy-related product development
Date: Friday, January 6, 2012 9:47:27 AM
Attachments: [Accuracy framework JW 5Jan2012.ppt](#)

All,

I am attaching some thoughts in PowerPoint about our accuracy-related product development. These slides focus primarily on Analytic Validity. We will also need to address Clinical (or functional for discovery research customers) Validity & Utility. This is just a strawman to get the ball rolling. Your feedback is welcome.

In a minute I will also email an article by an NHGRI group from last summer about human genome sequencing accuracy.

John West

Framework for assessment & improvement of accuracy in whole genome medical interpretation

**Draft Jan 5, 2012
John West**

Research Market Products

- Comprehensive medically-focused interpretation of whole human genome sequences
 - Huge # of potential results
 - Even a low % error can swamp customer & waste time trying to track down invalid hypotheses
 - If research is in a clinical setting, particularly with return of results to patients, IRB's may want "FDA-like" quality systems in place
 - Standard quality approaches
 - Standard quality terminology & reporting
 - Accuracy / quality to "near-FDA" standards can be a sales advantage in the research market
- Maximal-accuracy analysis of a genome. Personalis :
 - Takes responsibility for the sample
 - Specifies the combination of next-gen sequencing performed
 - Augments NGS with other technologies to determine key variants
 - Conducts genotyping & assesses concordance
 - Conducts follow-up genetic & non-genetic testing to validate results

Eventual clinical product

- Also focused on comprehensive medically-focused interpretation of whole human genomes
 - FDA anticipates “Class 3” (highest) level of regulatory oversight
 - Need to undo bad reputation created by DTC genotyping companies
 - Pro-active early leadership in quality systems for whole genome may create a positive reputation for Personalis at FDA

CDC Process – Established process & terminology

(Augment with FDA processes, likely to be similar)

CDC Home



Centers for Disease Control and Prevention

CDC 24/7: Saving Lives. Protecting People. Saving Money through Prevention.

Genomic Testing

ACCE Model Process for Evaluating Genetic Tests

From 2000 – 2004, CDC's Office of Public Health Genomics (OPHG) established and supported the ACCE Model Project, which developed the first publicly-available analytical process for evaluating scientific data on emerging genetic tests. The ACCE framework has guided or been adopted by various entities in the United States and worldwide for evaluating genetic tests; the CDC-supported [EGAPP™ initiative](#) builds on the ACCE model structure and experience.

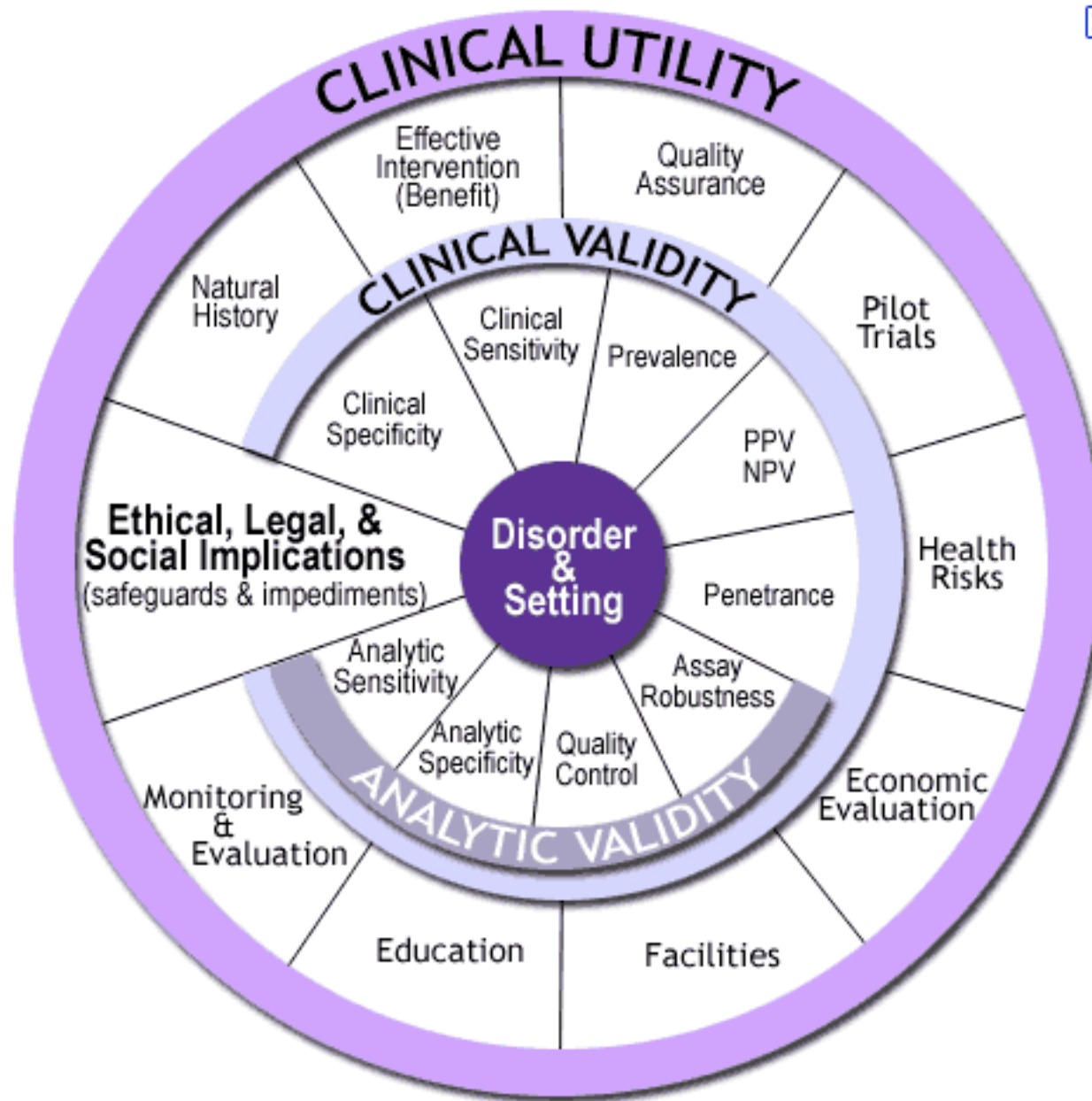
Introduction to ACCE

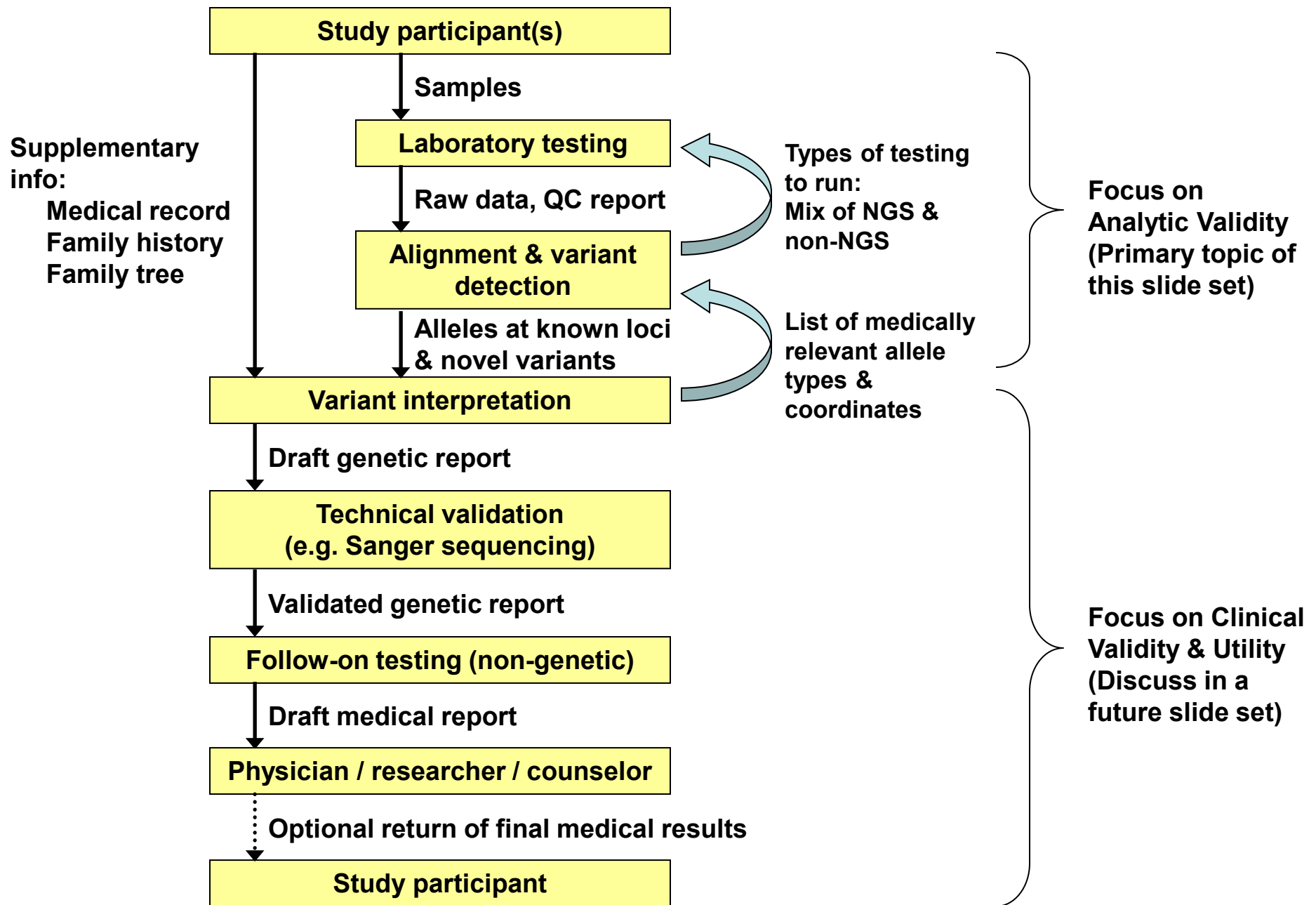
ACCE, which takes its name from the four main criteria for evaluating a genetic test — [analytic validity](#), [clinical validity](#), [clinical utility](#) and associated [ethical, legal and social implications](#) — is a model process that includes collecting, evaluating, interpreting, and reporting data about DNA (and related) testing for disorders with a genetic component in a format that allows policy makers to have access to up-to-date and reliable information for decision making. The ACCE model process is composed of a standard set of [44 targeted questions](#) (3) that address disorder, testing, and clinical scenarios, as well as analytic and clinical validity, clinical utility, and associated ethical, legal, and social issues.

An important by-product of the ACCE model process is the identification of gaps in knowledge that will help to define future research agendas. The ACCE approach builds on a methodology originally described by Wald and Cuckle (1) and on terminology introduced by the Secretary's Advisory Committee on Genetic Testing (2).

Learn more about [ACCE](#).







Elements of Analytic Validity in CDC's ACCE Model

See Appendix for additional detail

- Analytic Sensitivity : How often positive when mutation is present ?
 - One minus the false negative rate ?
- Analytic Specificity : How often negative when mutation is not present ?
 - One minus the false positive rate ?
- Assay Robustness : How often does the test fail to give a usable result ?
- Repeatability :
 - On the same sample
 - Within & between labs
 - Between sample types & other process variables
- Confirmatory testing to resolve false positives ?
 - Genetic validation tests
 - Non-genetic tests (molecular, imaging, physiological)
- Quality Control: Internal QC program defined & externally monitored ?

Crucial to develop a model which links analytic validity results to monitor-able process variables

- Allele determination performance is known to vary widely between loci
- With millions of known loci, many quite rare, it is impractical to determine the assay performance for each as an independent system.
- Novel loci, inherently, cannot be validated in advance
- Proposed approach:
 - Create a quantitative process model of allele determination
 - Incorporate known process variables (sample type, storage & preparation, coverage, read length, repeat structure, algorithm parameters, etc)
 - Augment with models of process variability & failure mechanisms
 - Instrument the model with QC observables, related to normal & failure modes
 - Compare model predictions with real data & characterize differences
 - Iterate until model can confidently predict analytic validity metrics for both known & novel alleles, including in combination
 - System performance metric should prioritize medically interpretable alleles
 - Use insights from experimentally assessed error model to :
 - Evaluate / prioritize process improvements
 - Select disease-associated alleles which can be assayed with NGS⁺

Genetic variant types to include in model

- SNP's
- InDel's
- Larger deletions
- STR / VNTR's
- Structural variants
- Copy Number Variation
- Trisomies
- Combinations:
 - Any of these can be heterozygous
 - Compound heterozygosity
 - Small variants within larger het deletions or other CNV's
 - Clusters of closely spaced variants, which might interfere with each others' detection

Examples of known error mechanisms

Many more likely to follow

- Locally low / no coverage due to:
 - GC bias of biochemistry
 - Alignment degeneracy (repeats)
 - Clusters of variant alleles exceed alignment mismatch allowance
 - Alignment poor due to problems with reference sequence
- Reads align, but incorrectly
 - InDels missed, or inconsistently placed in homopolymers
 - Misplacements lead to :
 - Phantom het SNP's
 - Apparent tri-allelic loci
 - Apparent triple haplotype structure
- Raw read errors, random or systematic
- Allelic imbalance of reads due to:
 - Random extremes of expected binomial distribution, esp where low coverage
 - Allelic biases - biochemical or bioinformatic (e.g. clusters of SNP's in LD)
- Read length too short to quantify length of VNTR, or VNTR embedded in other repeat structures

Examples of process variability

Many more likely to follow

- Sample source (blood, saliva, tissue, cell culture)
- Sample preservation, culturing & prep for sequencing
- DNA sequencing platform, lab & operator(s)
- Coverage
- Read length
- Paired-end insert length (a distribution)
- Cluster creation & Sequencing chemistry versions
- Cluster density
- Variation in data processing algorithms, input parameters, reference sequence
- DNA contamination or incomplete filtering of primer-related artifacts
- Availability of supplementary data from the same sample (e.g. genotyping)
- Inconsistent supplementary information from / about the individual sequenced

QC Readouts, to monitor the process

(Particularly given that it may be impractical to standardize all process variables completely, over multiple years)

- Paired-end insert length distribution vs expected
- Coverage non-uniformity vs % GC content; relative to a standard
- Coverage histogram in regions selected to avoid repeats & %GC variation
- Total coverage ratios of chromosomes
- Raw read Q-score %-iles vs position along a read; relative to a standard
- Allelic distribution of reads vs expected binomial, at a “diagnostic” set of het SNP loci
- Sex, ethnicity, admixture and family relationships between samples vs expected from supplementary data provided
- Raw read error rates (monitored at homozygous loci), systematic / random
- Mendelian inheritance errors (family genome data sets)
- Clusters of variants which are close / dense enough to predict higher probability of errors
- Two alleles / haplotypes in single sex chromosomes, vs a standard
- Three alleles / haplotypes in diploid chromosomes , vs a standard
- Amount and location of autozygosity , vs a standard
- Screening for non-human DNA
- Concordance with genotyping data, vs a standard

Categories of experimental data to assess error types, mechanisms, frequencies

- Repeatability :
 - Same person twice (or more ?), trying to keep everything else constant
 - Alternatives: Genomes of identical twins, or children where identical
- Controlled process experiments :
 - Same person sequenced multiple times, each time varying just one parameter of the process (many of these can be done computationally)
- Concordance of other technology platforms on the same sample
- Compare process QC metrics from many sources of raw genome data, even low coverage (e.g. 1,000 Genomes)
- Mendelian inheritance errors (MIE's) determined from family genome sets
- Absolute accuracy :
 - Genomes of individuals known to have clear Mendelian diseases
 - Sequencing of complex synthesized oligo sets (synthetic genome)
 - Targeted modifications of DNA from a naturally occurring genome

Lists of problematic regions in the genome

Capture all in one place, unify, & link to underlying error mechanisms of the model

- Mapability / alignability assessments (some on Santa Cruz browser site)
- Genes with known CNV's
- Genes with known pseudogenes
- List of problematic genes for NGS (e.g. VWF)
- Evan Eichler's list of problematic regions
- 1,000 Genomes list of regions with top 0.1% of coverage
- List of compressions
- MIE's & MIE-cluster regions from family data
- Difficult regions from phasing of a family
- Regions of high raw read error rates (monitor at homozygous loci)
- Loci apparently tri-allelic in the genome of a healthy individual, or 3 haplotypes
- Regions of allelic bias
- Regions of no or low aligned coverage, determined experimentally
- Segmental duplications & other repeat structures

List of Variants to Optimize Against

- Medically interpretable variants (not just SNP's !):
 - Mendelian “Top 200”
 - Specific loci / variant types, where known
 - Genes in which to identify novel variants
 - PharmGKB
 - Curated & novel variants
 - Alleles in top Risk-O-Grams
 - Diseases
 - Molecular or physiologic medically relevant phenotypes
 - Blood & tissue typing
- Functional elements (broad interest for discovery research)
 - RefSeq genes
 - Linc-RNA
 - Regulome elements
 - eQTL loci

Proposed Initial Priorities

- Literature review related to genome sequencing accuracy, followed by discussion with SAB
- Develop an initial list of top priority medically interpretable variants to optimize against
- Create / collect lists of problematic regions of the genome
- Screen target medical variant list against problematic regions to get 1st cut scale & composition of the accuracy problem
- Develop initial quantitative model of allele determination, including known error mechanisms. Predict & characterize accuracy & repeatability levels
- Obtain existing experimental data, reprocess with current version algorithms, and conduct 1st cut repeatability & accuracy assessment. Compare with model on a genome-wide basis and at the medically interpretable loci.
- Launch program to obtain long-lead-time experimental data, including prototype of “maximally accurate genome” product (based on NGS augmented by other technologies).

Appendix

**US Centers for Disease Control (CDC)
ACCE Model Process for Evaluating Genetics Tests**

**Potentially useful framework & terminology for moving towards FDA
approval of genome-based diagnostics**



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Genomic Testing

ACCE Model List of 44 Targeted Questions Aimed at a Comprehensive Review of Genetic Testing

Element	Component	Specific Question
Disorder/Setting		1. What is the specific clinical disorder to be studied? 2. What are the clinical findings defining this disorder? 3. What is the clinical setting in which the test is to be performed? 4. What DNA test(s) are associated with this disorder? 5. Are preliminary screening questions employed? 6. Is it a stand-alone test or is it one of a series of tests? 7. If it is part of a series of screening tests, are all tests performed in all instances (parallel) or are only some tests performed on the basis of other results (series)?

Genomic Testing

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Element	Component	Specific Question
Analytic Validity		8. Is the test qualitative or quantitative?
	Sensitivity	9. How often is the test positive when a mutation is present?
	Specificity	10. How often is the test negative when a mutation is not present?
		11. Is an internal QC program defined and externally monitored? 12. Have repeated measurements been made on specimens? 13. What is the within- and between-laboratory precision? 14. If appropriate, how is confirmatory testing performed to resolve false positive results in a timely manner? 15. What range of patient specimens have been tested? 16. How often does the test fail to give a useable result? 17. How similar are results obtained in multiple laboratories using the same, or different technology?

Genomic Testing

ACCE Model List of 44 Targeted Questions Aimed at a Comprehensive Review of Genetic Testing

Element	Component	Specific Question
Clinical Validity	Sensitivity	18. How often is the test positive when the disorder is present?
	Specificity	19. How often is the test negative when a disorder is not present?
		20. Are there methods to resolve clinical false positive results in a timely manner?
	Prevalence	21. What is the prevalence of the disorder in this setting?
		22. Has the test been adequately validated on all populations to which it may be offered? 23. What are the positive and negative predictive values? 24. What are the genotype/phenotype relationships? 25. What are the genetic, environmental or other modifiers?

Genomic Testing

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Element	Component	Specific Question
Clinical Utility	Intervention	26. What is the natural history of the disorder?
	Intervention	27. What is the impact of a positive (or negative) test on patient care?
	Intervention	28. If applicable, are diagnostic tests available?
	Intervention	29. Is there an effective remedy, acceptable action, or other measurable benefit?
	Intervention	30. Is there general access to that remedy or action?
		31. Is the test being offered to a socially vulnerable population?
	Quality Assurance	32. What quality assurance measures are in place?
	Pilot Trials	33. What are the results of pilot trials?

Genomic Testing

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Element	Component	Specific Question
	Health Risks	34. What health risks can be identified for follow-up testing and/or intervention?
		35. What are the financial costs associated with testing?
	Economic	36. What are the economic benefits associated with actions resulting from testing?
	Facilities	37. What facilities/personnel are available or easily put in place?
	Education	38. What educational materials have been developed and validated and which of these are available?
		39. Are there informed consent requirements?
	Monitoring	40. What methods exist for long term monitoring?
		41. What guidelines have been developed for evaluating program performance?

Genomic Testing

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Element	Component	Specific Question
ELSI	Impediments	42. What is known about stigmatization, discrimination, privacy/confidentiality and personal/family social issues?
		43. Are there legal issues regarding consent, ownership of data and/or samples, patents, licensing, proprietary testing, obligation to disclose, or reporting requirements?
	Safeguards	44. What safeguards have been described and are these safeguards in place and effective?