What is an Investigational Device in the Context of Genomic Medicine Research? The CMH/RCIMG NSIGHT Experience

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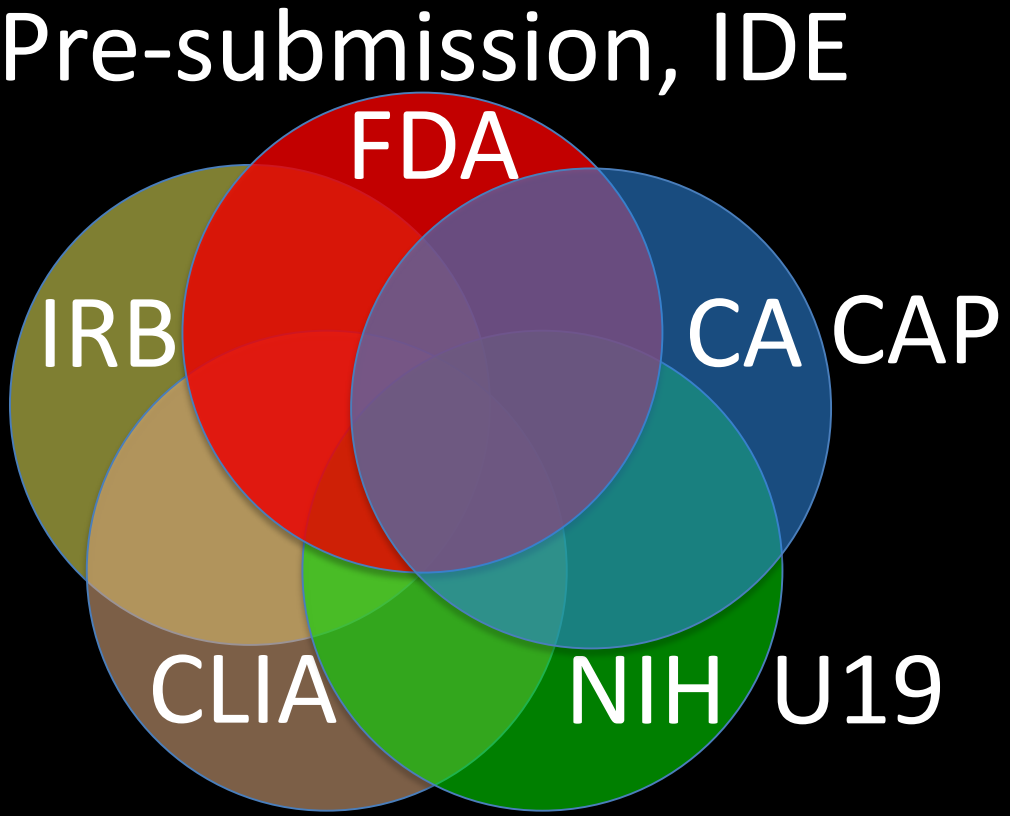
Regulatory Oversight of Genomic Medicine Research



Hypotheses: Rapid Genome Sequencing In NICU / PICU Infants with Likely Single Gene Diseases:

Increases rate of diagnosis

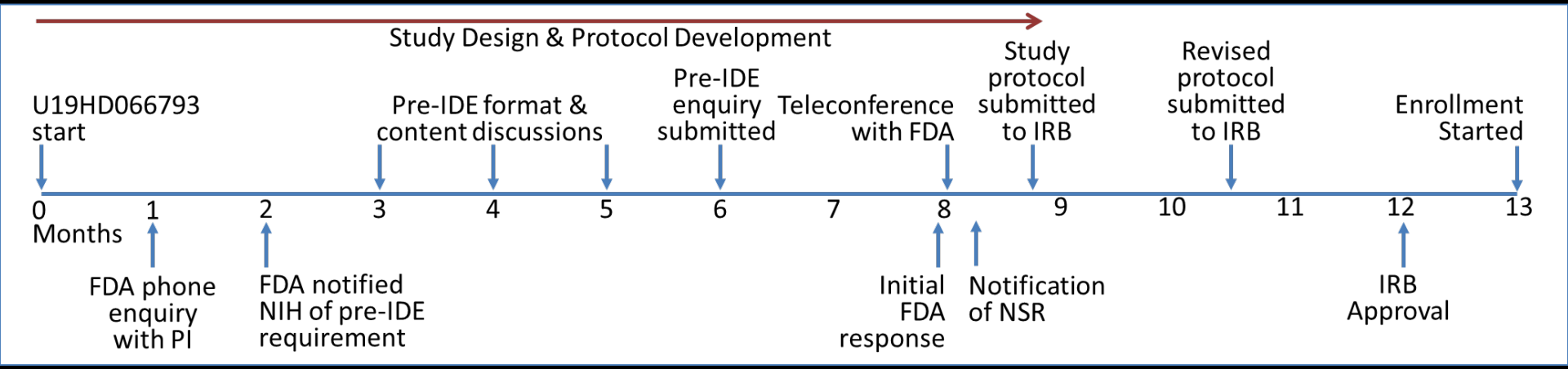
Decreases time to diagnosis

Improves the precision of acute management

- 8,240 known or suspected single gene diseases; ↑ by 20/month
- Leading cause of death in NICU, PICU, infants
- Conventional testing = NBS, chromosomal microarray, directed genetic testing

Rady Children's Institute Genomic Medicine

Pre-submission Timeline



Pre-Submission Process

13 page document with the following items:

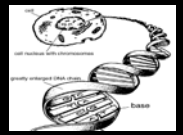
Table of Contents

A. Cover Letter
B. Table of Contents
C. Device Description
D. Proposed Intended Use/Indications for Use
E. Previous Discussions or Submissions
F. Overview of Product Development
G. Specific Questions
H. Mechanism for Feedback

References

Patient Ascertainment & Enrollment

Sample collection



DNA extraction

Library prep

Illumina HiSeq 2500

REDCap

Clinical Feature Entry

SSAGA

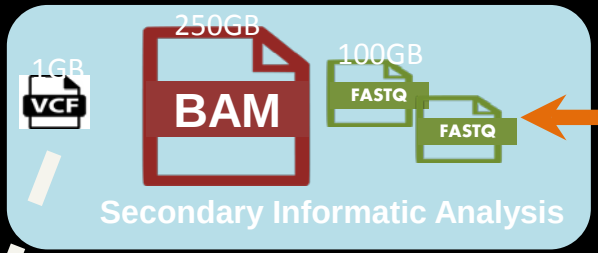
High-performance compute cluster

10 Gbps network

10 Gbps network

DataDirect NETWORKS

High performance Network Storage



RUNES

Variant database

dbGAP

QIAGEN INGENUITY SYSTEMS

VIKING

Variant Interpretation and Reporting

Verbal Report

Confirmatory Assay

Written Report in Electronic Medical Record

LIMS – Custom

Pre-Submission Process

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Design: Randomized, controlled, prospective trial

t_0

Perinatal Ascertainment

Inclusion criteria
- Likely genetic disease
- Genetic test order
- Congenital anomalies
- Poor response

Exclusion criteria
- >4 months old
- Chromosome anomaly
- Known molecular diagnosis

National Human Genome Research Institute

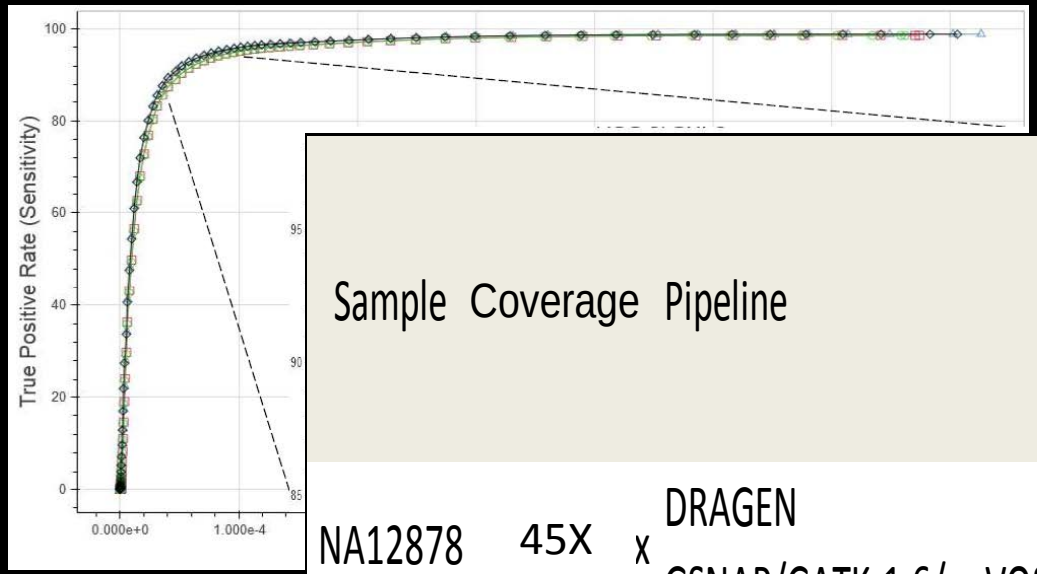
NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

Children's Mercy KANSAS CITY

Rady Children's Hospital San Diego

Analytic performance of Device

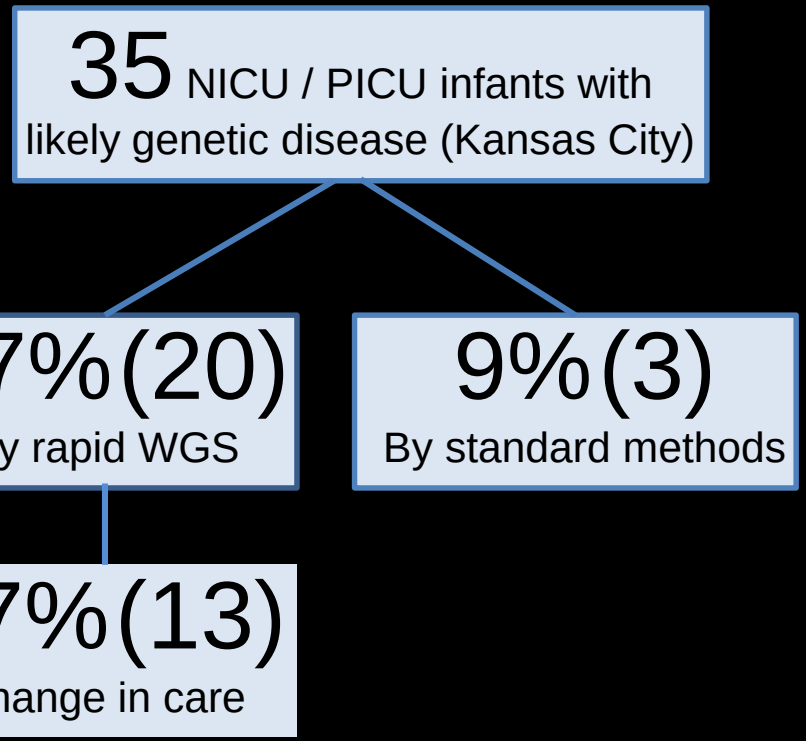
Miller NA, *et al. Genome Med.* October 2016



Sample	Coverage	Pipeline	Analytic Sensitivity	Analytic Specificity
NA12878	45X	DRAGEN	99.93%	99.87%
		GSNAP/GATK-1.6/noVQSR	99.54%	98.57%
NA12878	20X	DRAGEN	99.42%	99.46%
		GSNAP/GATK-3.2/noVQSR	97.29%	95.35%

Diagnostic Performance of Device

Willig LK, *et al. Science Trans. Med*. April 2015



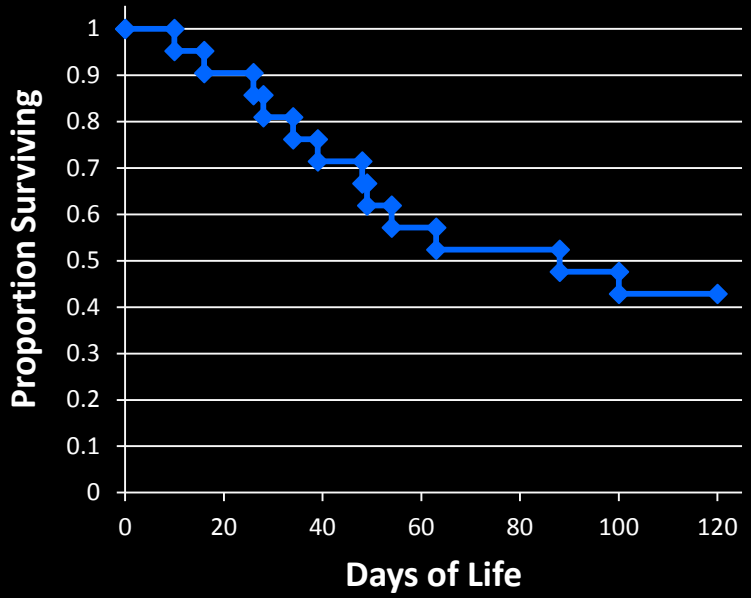
Molecular Diagnosis Enrollment ***DOL 26***; Time-to-Dx **23 days**

Rady Children's Institute
Genomic Medicine

Clinical Utility of Device

Number (% Tested)	Kansas City
Diagnoses	20 (57%)
Actionability of diagnosis	13 (37%)
Palliative Care Guidance	6 (17%)
Medication Change	4 (11%)
Life-saving treatment	1 (3%)
NICU stay decreased by >1 month	1 (3%)
Major morbidity avoided	3 (9%)
Parent or sibling diagnosed	1 (3%)
Procedure Change	3 (9%)
Diet Change	2 (6%)

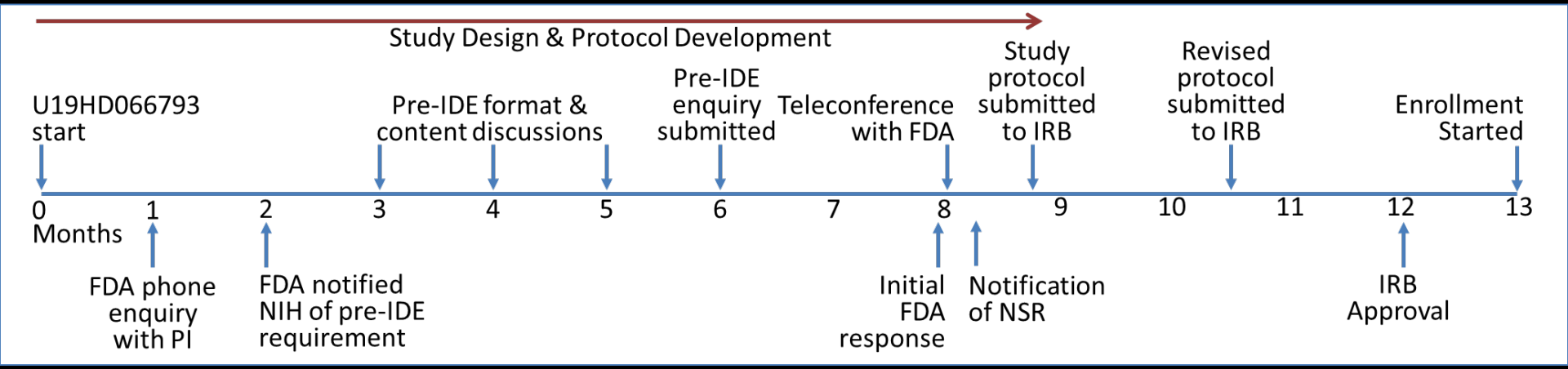
Risk Determination Context: Relative risk of adverse outcome from delayed diagnosis as a result of confirmatory testing versus risk of false positive diagnosis



Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings *Lancet Respir Med* April 28, 2015

Laurel K Willig, Josh E Petrikin, Laurie D Smith, Carol J Saunders, Isabelle Thiffault, Neil A Miller, Sarah E Soden, Julie A Cakici, Suzanne M Herd, Greyson Twist, Aaron Noll, Mitchell Creed, Patria M Alba, Shannon L Carpenter, Mark A Clements, Ryan T Fischer, J Allyson Hays, Howard Kilbride, Ryan J McDonough, Jamie L Rosterman, Sarah L Tsai, Lee Zellmer, Emily G Farrow, Stephen F Kingsmore

Pre-submission Timeline



Grant Number: 1U19HD077693-01

Principal Investigator(s):
Stephen F Kingsmore, MB

Project Title: Clinical and Social Implications of 2-day Genome Results in Acutely Ill Newborns

RE: **Q140271**

DEVICE: **Illumina HiSeq 2000/2500, NextSeq 500**

DATED: **March 4, 2014**

RESPONSE: **April 28, 2014**

Further clarifying information for FDA presubmission teleconference, May 1, 2014

Page 3: Clarification 1

While your protocol states that confirmation of results by Sanger sequencing will be performed in most cases, it does allow for disclosure of results to clinicians *prior to* Sanger sequencing in cases that involve "…identification of a life-threatening, treatable condition

- Confirmatory testing will be performed in all cases prior to return of written results.
- A verbal provisional result will be disclosed to the physician of record only in cases where testing identifies high-likelihood, acutely actionable, diagnostic variants for a life-threatening, treatable condition in an acutely ill neonate in whom the risk of a delay in reporting significantly exceeds the risk of disclosure prior to Sanger sequencing (i.e. may result in patient death or serious harm).

Definition: High Likelihood Disease Causing Variants

- Occur in ONE established genetic disease gene (e.g. defined by OMIM as #) AND
- The features of that OMIM disease fit those of the acute illness present in the patient AND
- Having an allele frequency less than 1% in local population AND
- Are either category 1 variants with literature support of pathogenicity OR cat
- For

Category	Description	Criteria
1	Previously reported, recognized cause of the disorder	HGMD variant type of 'Disease Mutant' dbSNP Snp Clinical Significance of 'pathogenic'
2	Novel, of a type expected to cause the disorder	loss of initiation premature stop codon disruption of stop codon whole transcript deletion frameshifting in/del disruption of splicing through deletion causing CDS/intron fusion overlap with splice donor or acceptor sites.
3	Novel, may or may	non-synonymous substitution

Process for determining whether verbal disclosure of a provisional result to the physician of record is warranted

- The Laboratory Director (Carol Saunders PhD FACMG) and her team review:
  - The quality and quantity of the genome sequence and read alignment information at that nucleotide position(s)
  - The support for the 5 criteria for being High Likelihood Disease Causing Variants
  - The literature support for a diagnosis being acutely "actionable" (i.e. likely to result in a material change in acute management of that disease)
  - The likelihood of death or serious adverse outcome if no disclosure occurs until Sanger confirmation is completed

Process for verbal disclosure of a provisional result to the physician of record

- The Laboratory Director (Carol Saunders PhD FACMG):
  - Requests confirmatory Sanger sequencing
  - Informs the physician of record verbally of
    - The putative diagnosis
    - The support for that diagnosis
    - The timeline for confirmatory testing
    - The potential, significant, acute "action" that prompted provisional reporting (i.e. a material change in the acute management of that disease)
  - Places a standard note in that patients Medical Record as follows:

"Whole sequencing research was performed on peripheral blood DNA from this patient and his/her parents on DD/MM/YYYY under Children's Mercy Hospital IRB Protocol XXXX for diagnosis of an acute neonatal disease. Testing disclosed acutely actionable information that was disclosed verbally to the physician of record prior to confirmation of results. For further information, please contact the Study Principal Investigator Dr. Stephen Kingsmore (816-854-0882, sfkingsmore@cmh.edu)."

Page 3: Clarification 2

While your protocol states that confirmation of results by Sanger sequencing will be performed in most cases, it does allow for disclosure of results to clinicians *prior to* Sanger sequencing in cases that involve "…identification of a life-threatening, treatable condition [and] novel variants of uncertain clinical significance" (p13).

- No results will be disclosed to clinicians prior to Sanger sequencing in cases that involve variants of uncertain significance.

Page 3: Clarification 3

The protocol also appears to leave open the possibility that return of results without confirmation may occur in other, undefined situations.

- No results will be returned without confirmation in any other situation.

Page 3: Clarification 4

Finally, the protocol states that for negative study results a statement about the testing will be placed in patients' medical records. We are uncertain what kinds of results would be considered "negative" for this purpose.

- A negative case is one in which testing does not yield a diagnostic result.
- Upon completion of analysis of whole genome sequences of the familial trio, in the absence of a diagnostic genotype, a standard note will be placed in that patients Medical Record as follows: "Whole genome sequencing research was performed on peripheral blood DNA from this patient and his/her parents on DD/MM/YYYY under Children's Mercy Hospital IRB Protocol XXXX for diagnosis of an acute neonatal disease. Testing did not disclose the cause of this disease. For further information, please contact the Study Principal Investigator Dr. Stephen Kingsmore (816-854-0882, sfkingsmore@cmh.edu)."

Page 3: Clarification 5

Furthermore, we cannot make a determination that the blood collection does not pose added risk to study subjects. To make this determination, we will require information on volume when encountering conditions such as anemia.

1-3 ml of blood will be collected from neonates and parents at time of enrollment following the Children's Mercy Hospital Research Guidelines for blood draws.

Children's Mercy Hospital Guidelines for Blood Sampling Related to Research

Body Wt (Kg)	Body Wt (lbs)	Total blood volume (mL)	Maximum allowable volume (mL) in one blood draw (= 2.5% of total blood volume)	Total volume (clinical + research) maximum volume (mL) drawn in a 30-day period	Minimum Hgb required at time of blood draw if subject has respiratory/CV compromise
1	2.2	100	2.5	5	9.0 -10.0
2	4.4	200	5	10	9.0-10.0
3	6.3	240	6	12	9.0-10.0
4	8.8	320	8	16	9.0-10.0
5	11	400	10	20	9.0-10.0
6	13.2	480	12	24	9.0-10.0
7	15.4	560	14	28	9.0-10.0
8	17.6	640	16	32	9.0-10.0
9	19.8	720	18	36	9.0-10.0
10	22	800	20	40	9.0-10.0
11-15	24-33	880-1200	22-30	44-60	9.0-10.0
16-20	35-44	1280-1600	32-40	64-80	9.0-10.0
21-25	46-55	1680-2000	42-50	64-100	9.0-10.0
26-30	57-66	2080-2400	52-60	104-120	9.0-10.0
31-35	68-77	2480-2800	62-70	124-140	9.0-10.0
36-40	79-88	2880-3200	72-80	144-160	9.0-10.0
41-45	90-99	3280-3600	82-90	164-180	9.0-10.0
46-50	101-110	3680-4000	92-100	184-200	9.0-10.0

Page 3: Clarification 6

encountering conditions such as anemia. Moreover, your protocol also provides for the possibility of collection of blood, urine, and tissue for future unspecified purposes, and it is unclear whether this would include invasive sampling outside of standard of care.

- Collection of blood, urine, and tissue for future unspecified purposes will NOT include invasive sampling outside of standard of care.
- Blood or tissue retains from procedures performed as part of standard of care will be scavenged.

Do we require an IDE submission?

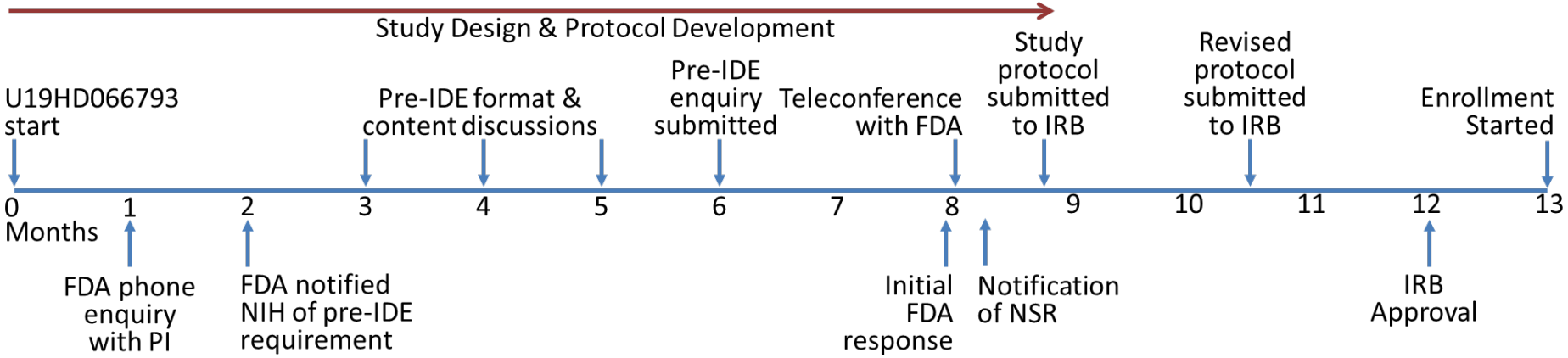
As such, your study appears to be significant risk, requiring the approval of an IDE submission unless you are able to provide further clarifying information or modifications to the protocol that allow for confirmation of all results with Sanger sequencing or an FDA cleared or approved test and, if necessary, allow for alternatives to any sample collection that is determined to pose a significant risk to subjects.

2) *What modifications/details in the protocol are recommended by the FDA prior to IDE submission if such submission is deemed necessary?*

FDA Response: The FDA does not have specific modifications to suggest.

- A verbal provisional result will be disclosed to the physician of record before Sanger sequencing only in cases where testing identifies high-likelihood, acutely actionable, diagnostic variants for a life-threatening, treatable condition in an acutely ill neonate in whom the risk of a delay in reporting significantly exceeds the risk of disclosure prior to Sanger sequencing (i.e. may result in patient death or serious harm).

Pre-submission Timeline



Newsweek

Europe Edition

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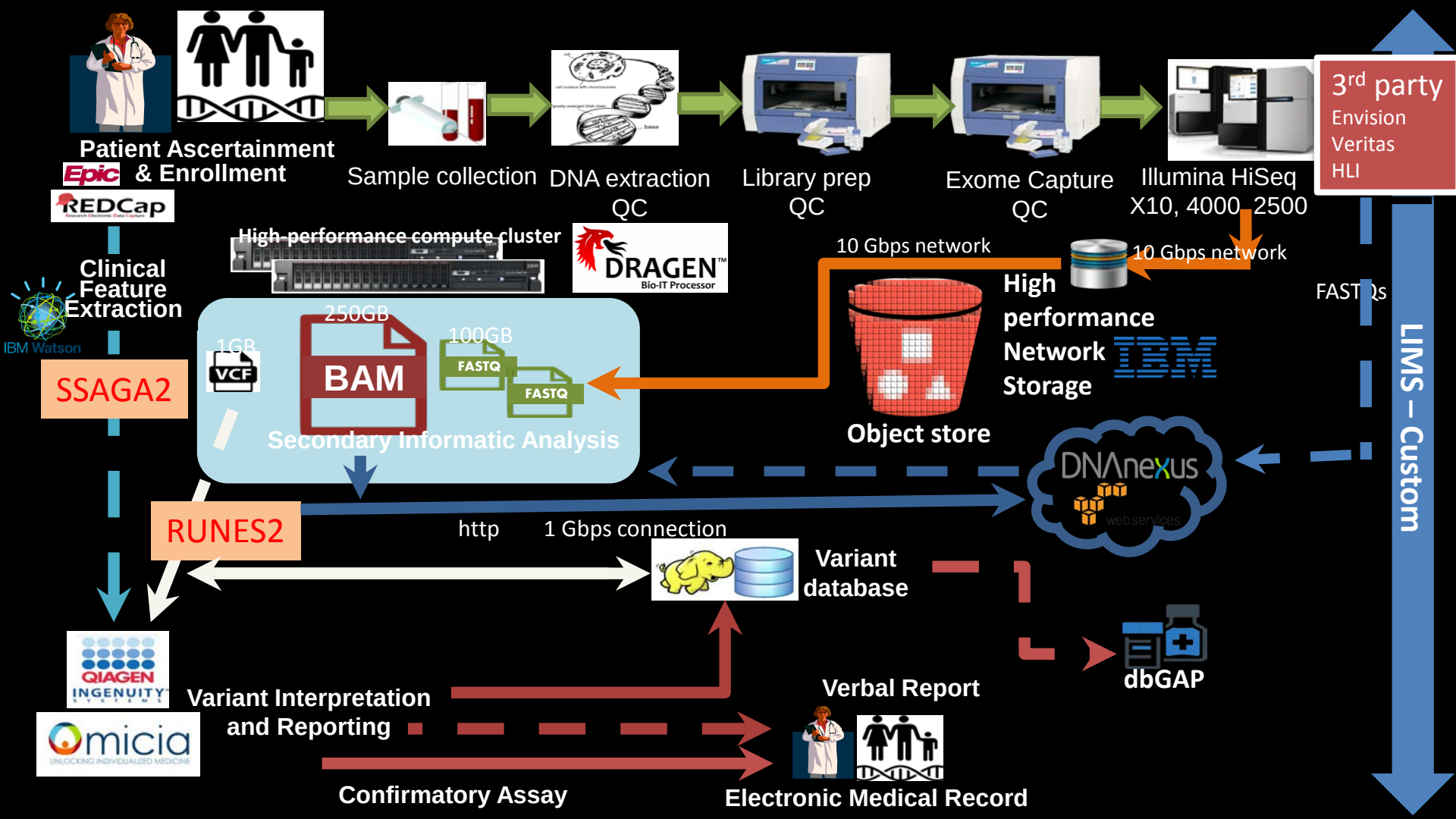
SCIENCE

Whole Genome Sequencing Time Cut to Just 26 Hours

By Conor Gaffey 10/1/15 at 9:31 PM

Whole genome sequencing can be the difference between life and death for newborns suffering from genetic diseases. A new sequencing procedure cuts down the time involved from 50 hours to just 26. Michael Dalder/Reuters


Patient Ascertainment & Enrollment
Epic
REDCap
Sample collection
DNA extraction QC
Library prep QC
Exome Capture QC
Illumina HiSeq X10, 4000, 2500
3rd party
Envision
Veritas
HLI
Clinical Feature Extraction
IBM Watson
SSAGA2
High-performance compute cluster
DRAGEN™ Bio-IT Processor
10 Gbps network
10 Gbps network
High performance Network Storage
IBM
Object store
FASTQs
LIMS – Custom
1GB
VCF
250GB
BAM
100GB
FASTQ
FASTQ
Secondary Informatic Analysis
DNAnexus
web services
RUNES2
http
1 Gbps connection
Variant database
QIAGEN
INGENUITY
omicia
UNLOCKING INDIVIDUALIZED MEDICINE
Variant Interpretation and Reporting
Verbal Report
dbGAP
Confirmatory Assay
Electronic Medical Record

Systematic Evaluation of Sanger Validation of Next-Generation Sequencing Variants. Beck TF et al. *Clin Chem.* 2016 62:647-54.

BACKGROUND:
Next-generation sequencing (NGS) data are used for both clinical care and clinical research. DNA sequence variants identified using NGS are often returned to patients/participants as part of clinical or research protocols. The current standard of care is to validate NGS variants using Sanger sequencing, which is costly and time-consuming.
METHODS:
We performed a large-scale, systematic evaluation of Sanger-based validation of NGS variants using data from the ClinSeq® project. We first used NGS data from 19 genes in 5 participants, comparing them to high-throughput Sanger sequencing results on the same samples, and found no discrepancies among 234 NGS variants. We then compared NGS variants in 5 genes from 684 participants against data from Sanger sequencing.
RESULTS:
Of over 5800 NGS-derived variants, 19 were not validated by Sanger data. Using newly designed sequencing primers, Sanger sequencing confirmed 17 of the NGS variants, and the remaining 2 variants had low quality scores from exome sequencing. Overall, we measured a validation rate of 99.965% for NGS variants using Sanger sequencing, which was higher than many existing medical tests that do not necessitate orthogonal validation.
CONCLUSIONS:
A single round of Sanger sequencing is more likely to incorrectly refute a true-positive variant from NGS than to correctly identify a false-positive variant from NGS. Validation of NGS-derived variants using Sanger sequencing has limited utility, and best practice standards should not include routine orthogonal Sanger validation of NGS variants.