LABORATORY STANDARDS AND PROCEDURES WORKGROUP

August 2, 2019

Co-chairs: Kellie Kelm, PhD & Susan Tanksley, PhD

Agenda

TOPIC	PRESENTER
Welcome and Roll Call (5 min)	Kellie Kelm Susan Tanksley
Next Gen Sequencing and its use in Newborn Screening	Michele Caggana (NY)
Brainstorming on gaps, topics	All
Feedback on Components: RUSP Condition Nomination & Evidence Review Process (30 min)	All
Wrap-up/Next Steps (5 min)	Kellie, Susan

Workgroup Roster

Mei Baker
Carla Cuthbert
Tricia Hall
Nathalie Lepage
Scott Shone
Stan Berberich
George Dizikes
Travis Henry
Jelili Ojodu
Michael Watson
Michele Caggana
Rosemary Hage
Amy Karger
Miriam Schachter
Holly Winslow

- Chair: Kellie Kelm
- Co-chair: Susan Tanksley
- HRSA staff: Kathryn McLaughlin

Workgroup Charge

Define and implement a mechanism for the periodic review and assessment of

1. The conditions included in the uniform panel
2. Laboratory procedures utilized for effective and efficient testing of the conditions included in the uniform panel.
3. Infrastructure and services needed for effective and efficient screening of the conditions included in the uniform panel

Project 1

- Laboratory procedures: Explore the role of next generation sequencing in newborn screening
  - Screening is currently based on phenotypic data. How do we accumulate the data to identify correlation between phenotypic & genotypic data?
  - Are there conditions for which sequencing is the only screening method?
  - What do you gain/lose from NGS?
  - Which data do you report?
    - What do you do with variants of unknown significance?
    - When do you report carrier status? Are there particular conditions where reporting carrier status is important?
  - What new infrastructure needs to be built for NGS?

Project 2

- Infrastructure and services: A portion of the timeliness initiatives fits here:
  - Review data related to testing (Timeliness 1.0)
  - What are the implications of earlier specimen collection (<24 hrs)?
  - What are the unforeseen consequences and costs of timeliness?

Project 3

- Impact of broad phenotypes on laboratories
  - Share lessons learned on identifying late onset Pompe disease, SMA cases with 2, 3, or 4 copies of SMN2, etc.
  - Use information to refine the target of the RUSP condition?

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Next Gen Sequencing and its Use in Newborn Screening

Michele Caggana, Sc.D., FACMG

July 31, 2019

NextGen Sequencing of an Entire Gene

Previously 94% of referred CF screens are false positives in NYS

Screen positive – ↑IRT and at least 1 CF causing mutation

- Most assays detect a panel of variants that cause CF
- >2000 known variants in CFTR gene

Not all CFTR mutations cause classic CF

- Will identify CF related metabolic syndrome (CRMS) or unknown variants
- Can limit sequence detection to known variants but will miss cases?
- How many missed cases are tolerable?

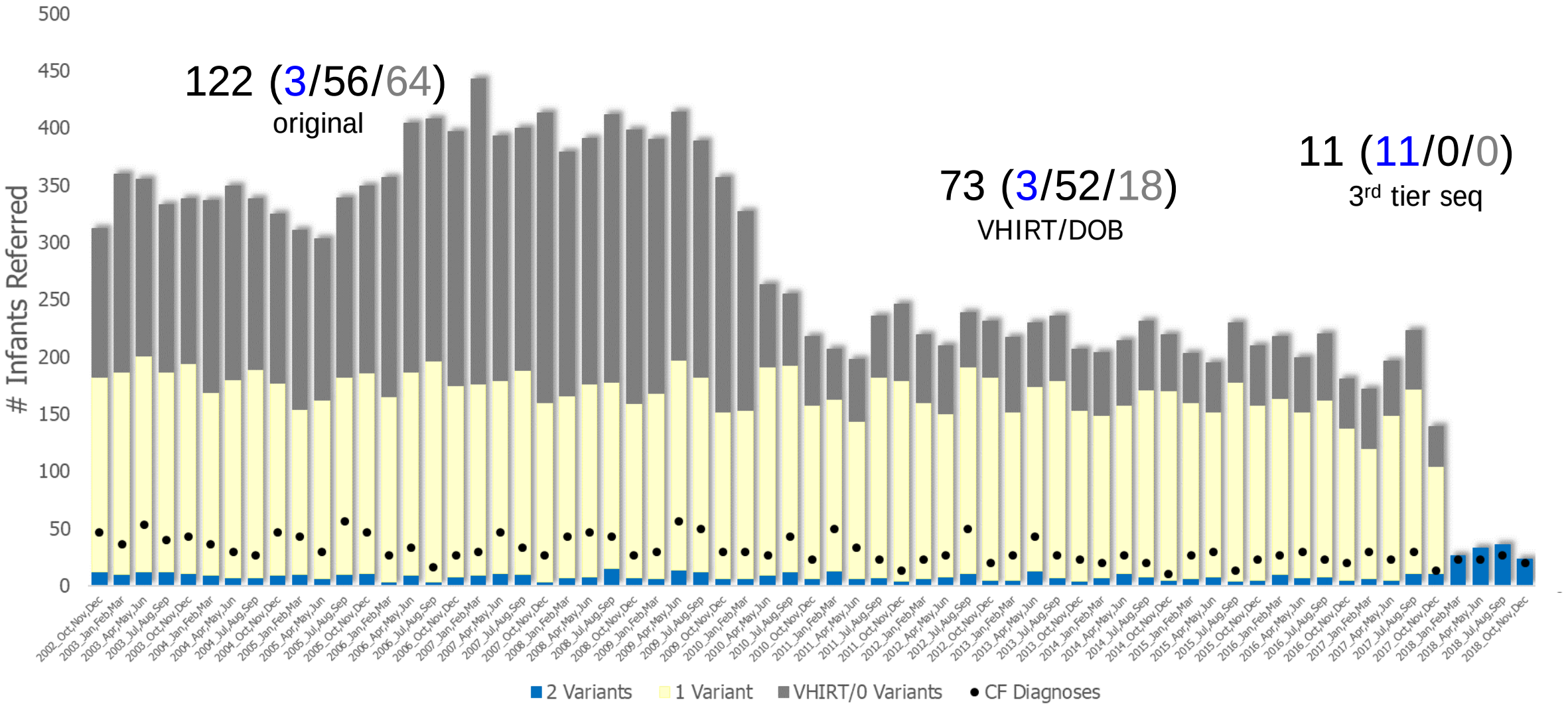
Hughes EE et al., Hum Mutat, 37:201-208

Cystic Fibrosis Newborn Screening Summary

- NY Annual birth rate: ~250,000
- 1st tier: Babies in upper 5% IRT: ~12,500
- 2nd tier: Babies with 1 or 2 *CFTR* variants or VHIRT: ~900
- 3rd tier: Babies with 2 *CFTR* variants: ~100. Only these babies are sent for diagnostic evaluation and testing

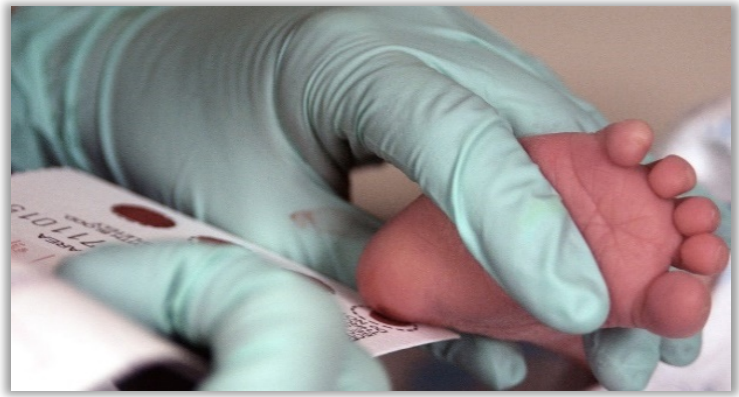
S. Cordovado, Ph.D.

CF Referrals and Diagnoses, 2002 – 2018



Conclusions & Lessons Learned *(May 2019)*

- **Referrals reduced by 83.0% (749 vs 127)**
  - **445 carriers and 177 infants negative not referred**
- **PPV increased 6.6-fold (3.8% to 25.2%)**
- **Infants with CF are promptly referred & diagnosed**
- **Challenges in variant interpretation**
  - **VOUS and VCCs detected by SEQ contribute to higher CRMS to CF ratio (2.8 to 1)**
  - **Variants may be *in cis* (6/42 phased)**
  - **Variants may be reclassified (2/90 reportable variants in 8/127 referrals)**



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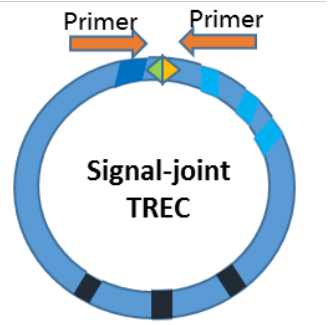
Next Gen Sequencing and SCID Newborn Screening

Issue: SCID is a spectrum of disorders that can only be differentiated by identifying causative mutations

- **Many genes involved in SCID**
- **Immunologists can provide better care when SCID causative mutations are known quickly**
- **Screening labs can provide timely mutation analysis**
- **When public health provides mutational analysis, ensures health equality**



Specific Aims

- Validate 2 platforms for 39-gene NGS immunodeficiency panel
- Evaluate Next Gen Sequencing Utility and TAT
  - Shortened time to diagnosis?
  - Fewer visits to Specialist?
  - Earlier, targeted treatment?
  - Long-term follow-up
- Create and disseminate educational materials for parents and providers to state programs

Sample	Gene	Variant (cDNA)	Variant (protein)	Variant classification	Zygosity	Number Vars Reflexed to Sanger	Gaps to Sanger (Exons)
NGS001	IL2RG	c.545G>C	p.Cys182Ser	Likely Pathogenic	Hemizygous	5	28
NGS001	MTHFD1	c.1561T>C	p.Leu521=	Uncertain Significance	Heterozygous	5	28
NGS002	DOCK8	c.971C>A	p.Ala324Asp	Uncertain Significance	Heterozygous	6	28
NGS003	No likely causative variants identified	No likely causative variants identified	No likely causative variants identified	No likely causative variants identified	No likely causative variants identified	10	30
NGS004	LIG4	c.1739G>A	p.Arg580Gln	Uncertain Significance	Heterozygous	2	29
NGS005	RAG1	c.527G>T	p.Cys176Phe	Likely pathogenic	Homozygous	2	29
NGS005	IL7R	c.28A>G	p.Met10Val	Uncertain Significance	Heterozygous	2	29
NGS006	DOCK8	c.626G>A	p.Arg209Gln	Uncertain Significance	Heterozygous	3	27
NGS006	FOXN1	c.415G>A	p.Glu139Lys	Uncertain Significance	Heterozygous	3	27
NGS006	NBN	c.2081C>T	p.Pro694Leu	Uncertain Significance	Heterozygous	3	27

Discussion of gaps, topics, etc.

- Clear case definitions of what we're screening for
- Pre-determined performance target goals (PPV)
  - Will need harmonization of terms and harmonization of data across platforms
- Making NBS conditions reportable, similar to infectious disorders that are reportable to CDC
- Second tier tests to reduce Dx testing, impact on parents, cost effectiveness
  - Regionalization
- CDC Adaptive learning – courses for community

Feedback on components:
RUSP Condition Nomination & Evidence Review Process

- Need to define the terminology for the evidence review process (e.g. what is the case definition)
- Set the case definition for the condition under consideration – allows FP/FN projections for states
- External evidence review not available for public health impact assessment survey
- Re-think the requirement of one case identified prospectively
- How is treatment defined? Approved/in trial/SOC?
- Assessment of factors impeding labs from bringing on new conditions

Feedback on components:
RUSP Condition Nomination & Evidence Review Process

- Public health system impact assessment – how can we get information from other stakeholders (non-newborn screening programs)
  - Can we utilize organizations that advise ACHDNC?
  - Information from insurance
  - Unchangeable survey because of clearance – how do we get supplementary information? Can we use other sources? NewSTEPs Readiness Tool?