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Implementing Next Generation Sequencing as a Third-Tier Newborn Screen for Cystic Fibrosis in New York State

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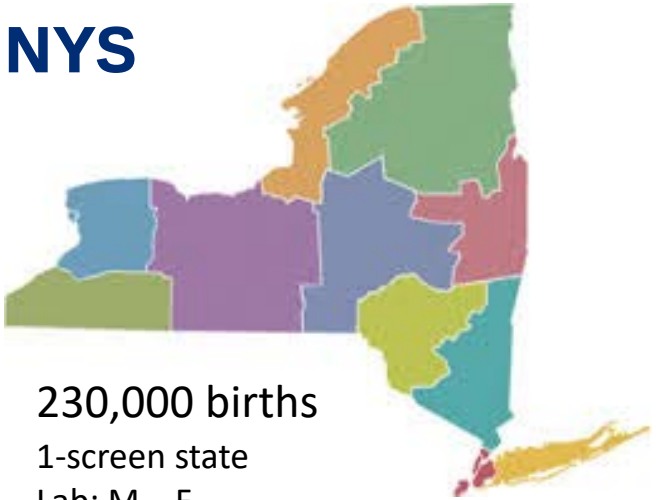
Overview: Year 1 IRT-DNA-SEQ in NYS

- CF referrals
- *CFTR* variants
- Turnaround times
- Diagnoses
- Lessons learned
- Conclusions

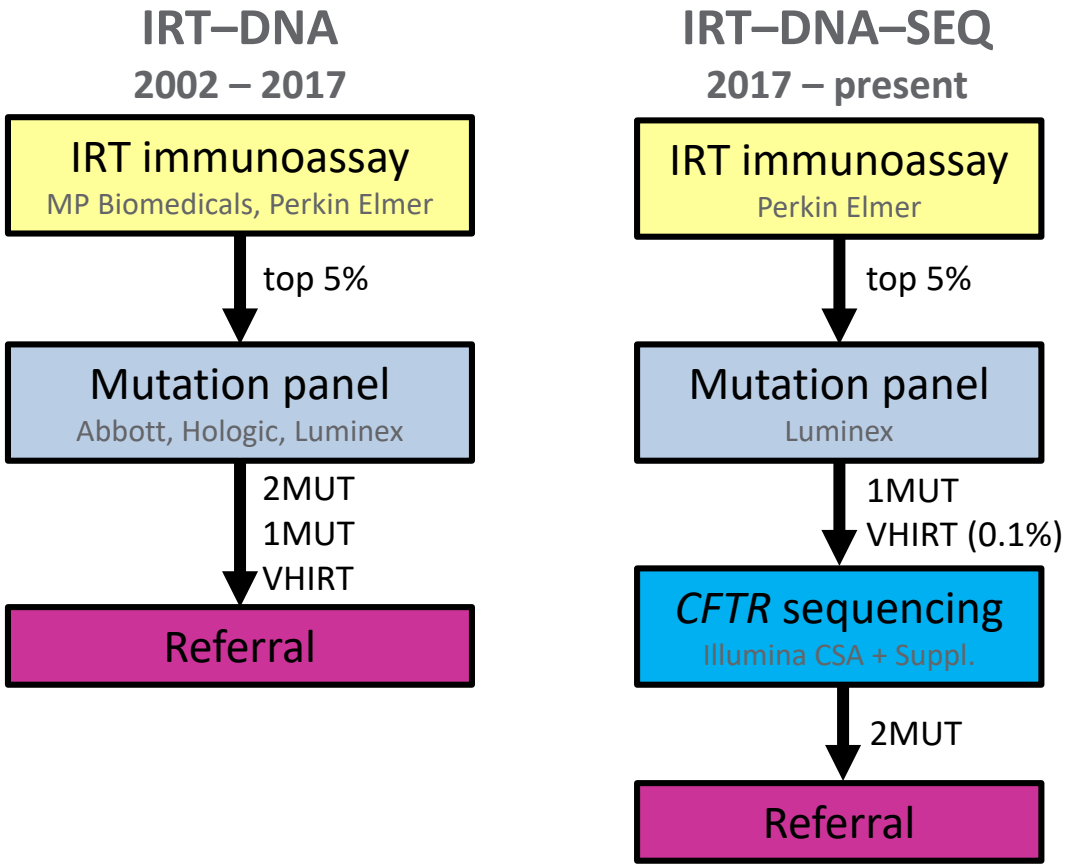


230,000 births

1-screen state

Lab: M – F

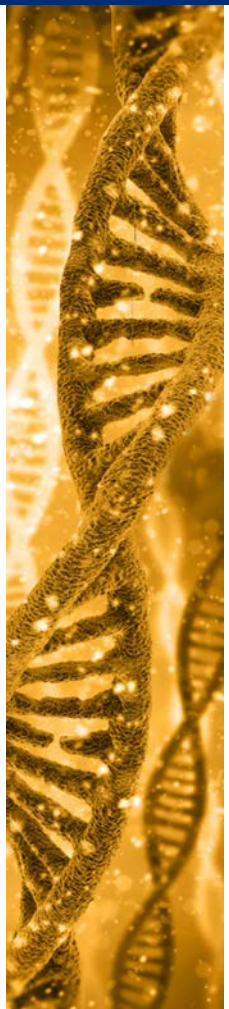
Cystic Fibrosis (CF) NBS in New York State



Why IRT-DNA-SEQ?

- Decrease false positives
- Refer only if 2 variants
- Carriers reported, not referred
- Molecular dx at screening
- Decrease healthcare cost
- Decrease family anxiety

- Newer technology (NGS)
- $$$
- Longer run time
- Low throughput
- Detection of *CFTR* variants of uncertain significance (VOUS)
- Frequency of CRMS / CFSPID



CRMS= *CFTR*-related metabolic syndrome
CFSPID= CF screen positive / inconclusive diagnosis

December 1, 2017

November 30, 2018

NYS 3rd Tier

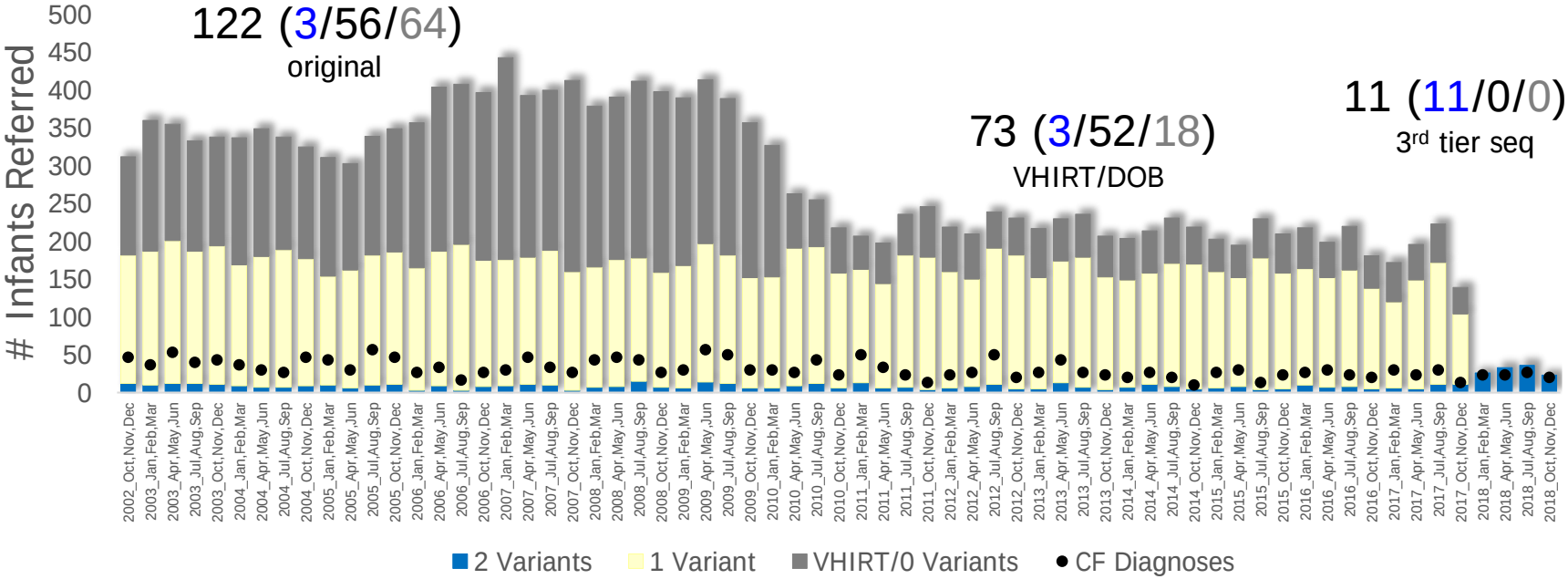
DNA from 1 x 3-mm DBS punch

Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay (CSA)

- FDA-cleared IVD (for whole blood)
- amplicon-based
- next generation sequencing assay
- 27 *CFTR* exons, intron/exon boundaries, 2 deep intronic
- point mutations, small ins/del, 2 large del, intron 8 polyTG/T

Supplemental deletion assays – exons 2, 13, 17b

CF Referrals and Diagnoses, 2002 – 2018



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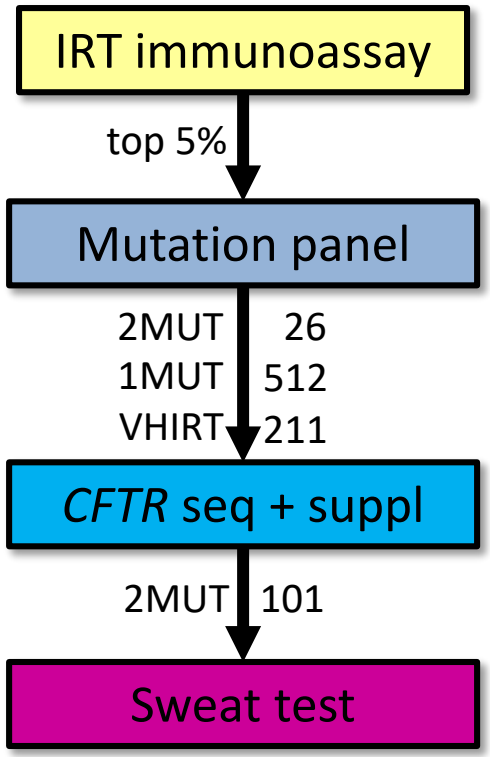
Referrals

After 3rd Tier:
127 referrals
445 carriers
177 negative

83.0 % reduction in referrals



262,273 specimens screened

13,559 to DNA

723 infants sequenced

127 referred

CFTR Variants

90 unique reportable variants among 572 infants (127 referrals and 445 carriers)

- 47 CF-causing, pathogenic, likely pathogenic (540 alleles)
- 12 varying clinical consequence (VCC; 103 alleles)
- 44 variants of uncertain significance (VOUS; 65 alleles)

NYS CFTR variant classification

- Variants classified using ACMG guidelines (Richards, 2015, Genet Med). CFTR2 database considered gold standard. Other evidence: CF and population genetic databases, literature, functional data, *in silico* predictions, etc.
- Benign/likely benign not reported, with one exception: intron 8 poly T/TG 5T-11TG *reported* as VCC/likely benign, since CFTR2 classifies as a VCC but we consider likely benign due to very low penetrance (Salinas, 2016, Genet Test Mol Biomarker). 5T-11TG doesn't prompt referral (unless 2 other reportable variants detected).

Diagnoses: CF (N=30)

Sweat Chloride
- 25 sweat chloride ≥ 60 mmol/L
- 4 sweat chloride 30 – 59
- 1 sweat chloride N/A

CFTR Variants
- 21 w/ 2 panel variants
- 8 w/ 1 panel and 1 rare variant
- 1 w/ 2 rare variants

CFTR Variant Types
- 59 P/LP and 1 VCC

IRT-DNA, 2013 – 2017:
PPV=3.8% (144/3,785)

6.4-fold increase in PPV

IRT-DNA-SEQ, 12 months:
PPV=24.4% (30/123)

Diagnoses

127 infants referred
- 30 CF
- 86 CRMS / CFSPID
- 6 carriers
- 5 pending/other

IRT-DNA, 2013 – 2017:
PPV = 3.8 % (144/3,785)

6.4-fold increase in PPV

IRT-DNA-SEQ, 12 months:
PPV = 24.4 % (30/123)

CRMS= *CFTR*-related metabolic syndrome
CFSPID= CF screen positive / inconclusive diagnosis

Diagnoses: CRMS / CFSPID (N=86)

3 with 2 P/LP variants meet CF criteria

- 1 sweat chloride 40 – 59
- 2 sweat chloride < 30

7 sweat chloride 30 – 59

- 2 w/ 1 VOUS
- 5 w/ 1 VCC

75 sweat chloride < 30

- Each w/ 1 – 2 VCC or VOUS

1 sweat chloride N/A

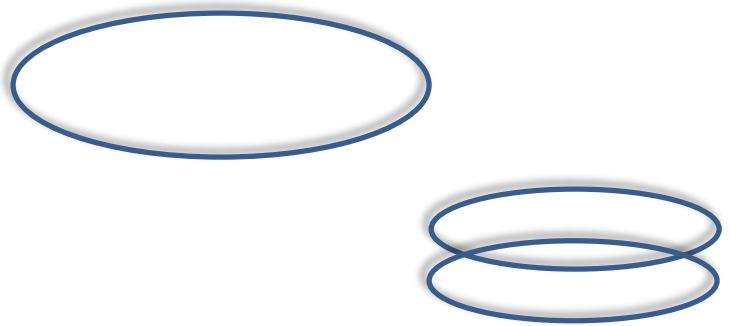
- 1 VCC

2.9 CRMS : 1 CF

- Will some 'convert' to CF?
- Can others be released from follow-up?
- Impact on families?

Turnaround Times

Group	Lab Turnaround (Receipt to Referral, Business Days)
Overall (N=127)	8 (3 - 12)



93.1% (27/29) CF cases w/ initial exam or ST within 30 days

Conclusions & Lessons Learned

Referrals reduced by 83.0% (749 vs 127)

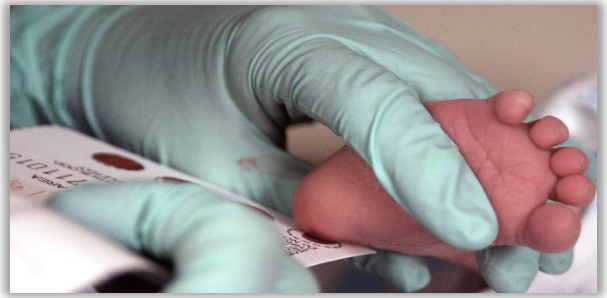
- 445 carriers and 177 negative not referred

PPV increased 6.4-fold (3.8% to 24.4%)

Infants with CF are promptly referred & diagnosed

Challenges in variant interpretation

- VOUS and VCCs detected by SEQ contribute to higher CRMS to CF ratio (2.9 to 1)
- Variants may be *in cis* (6/39 phased)
- Variants may be reclassified (2/90 reportable variants in 8/127 referrals)



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- Long Island Jewish
- Albany Medical Center
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- NYU Langone
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