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

IRT–DNA
2002 – 2017
IRT immunoassay
MP Biomedicals, Perkin Elmer
top 5%
Mutation panel
Abbott, Hologic, Luminex
2MUT
1MUT
VHIRT
Referral

IRT–DNA–SEQ
2017 – present
IRT immunoassay
Perkin Elmer
top 5%
Mutation panel
Luminex
1MUT
VHIRT (0.1%)
CFTR sequencing
Illumina CSA + Suppl.
2MUT
Referral
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
Referrals

After 3rd Tier:
127 referrals
445 carriers
177 negative

83.0% reduction in referrals

IRT immunoassay
top 5%

Mutation panel
2MUT 26
1MUT 512
VHIRT 211

CFTR seq + suppl

Sweat test
2MUT 101

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*
- 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)

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

6.4-fold increase in PPV
Diagnoses

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

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

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

6.4-fold increase in PPV

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**

<table>
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<tr>
<th>Group</th>
<th>Lab Turnaround (Receipt to Referral, Business Days)</th>
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<tr>
<td>Overall (N=127)</td>
<td>8 (3 - 12)</td>
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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 \textit{in cis} (6/39 phased)
- Variants may be reclassified (2/90 reportable variants in 8/127 referrals)
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