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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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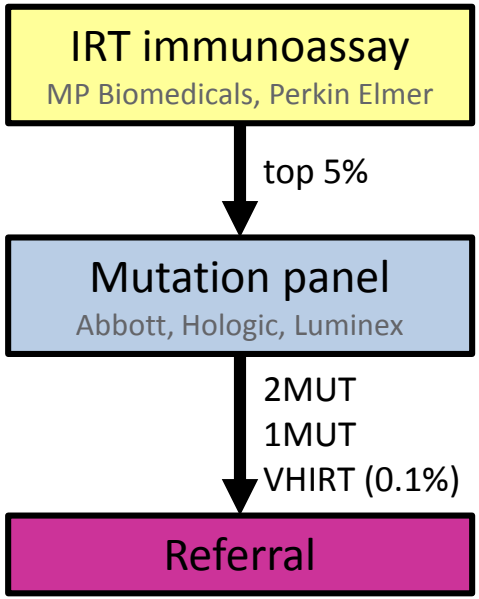
Disclosures

Illumina provided reagents for 2014 validation study and a loaned instrument.

Cystic Fibrosis (CF) NBS in New York State

IRT–DNA algorithm



2013–2016

850 infants referred annually

23–36* confirmed CF (51–59**)

PPV

Overall	3.7%* (7.2%**)
2MUT	65.2%* (100%**)
1MUT	1.7%* (4.5%**)
VHIRT	0.6%* (1.1%**)

*CF

**CF, CRMS, Possible CF, 2MUT/sweat negative

Only infants with a final diagnosis assigned are included in PPV .

The problem

Current algorithm: low PPV (high FPR)
- impact on families
- healthcare cost

Mutation panels not comprehensive

Diverse NYS *CFTR* mutation spectrum
- 439 infants with CF
  - 160 different variants
  - 95% rare/private
- need to refer 1MUT (29%), VHIRT (6%)

Sequence 1MUT, VHIRTs and refer only 2MUT
- Sanger labor intensive, expensive
- NGS???

P-089

Comprehensive CFTR Genotyping of New York State Infants with Cystic Fibrosis: Mutation Spectrum and Algorithm Change

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A possible solution?

Illumina MiSeqDx Cystic Fibrosis **C**linical **S**equencing **A**ssay (**CSA**)

- FDA-cleared IVD
- 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

illumina

MiSeqDx™ Cystic Fibrosis Clinical Sequencing Assay

FOR IN VITRO DIAGNOSTIC USE

Catalog # DX-102-1001: 6 Runs, up to 48 Samples per Kit

Intended Use

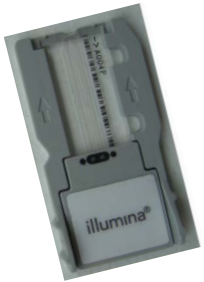
The Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay is a targeted sequencing *in vitro* diagnostic system that re-sequences the protein coding regions and intron/exon boundaries of the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene in genomic DNA isolated from human peripheral whole blood specimens collected in K_2EDTA. The test detects single nucleotide variants, and small indels within the region sequenced, and additionally reports on two deep intronic mutations and two large deletions. The test is intended to be used on the Illumina MiSeqDx Instrument.

The test is intended to be used as an aid in the diagnosis of individuals with suspected cystic fibrosis (CF). This assay is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations. The results of the test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available information including clinical symptoms, other diagnostic tests, and family history.

This test is not indicated for use for stand-alone diagnostic purposes, fetal diagnostic testing, for pre-implantation testing, carrier screening, newborn screening, or population screening.

Goals

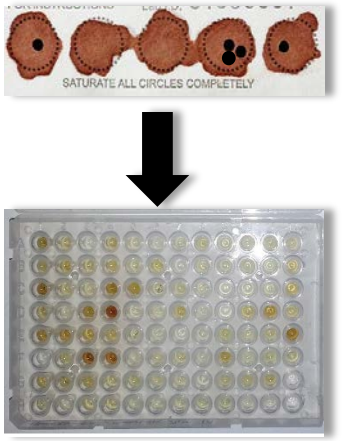
1. Validate DBS.
2. Assess clinical validity.
3. Assess logistics, potential for implementation.



Technical validation, N=266 DBS

- DNA extracted from 1 x 3-mm DBS
- Genotyped using CSA, validated using independent methods
- Sanger sequenced >10 samples (to assess false negative rate)

- 94.7% (252/266) samples w/ 100% call rate on first pass
- Median read depth = 9,550X (5–121,666X)
- Excellent (not perfect) concordance
  - 1 recurrent **false positive**/**position fail** (p.Q1035K)
  - 1 **false negative** (c.1679+1.6kbA>G)
  - 1 recurrent **reporting** issue (c.1973_1985del13ins5)
  - 1 recurrent **mutation causing sample fail** (homozygous c.1817_1900del84)



DNA	CSA Specs	DBS DNA tested
Extr Method	Any validated	Saavedra-Matiz, 2013, Clin Chem
Conc. (A260/A280)	50 ng/µl	~1 – 6 ng/µl
Amount	250 ng	~5 – 30 ng

Clinical validation

Diverse NYS *CFTR* mutation spectrum
- 160 variants among 439 CF patients

Illumina 139 Variant Assay
- Panel sensitivity = 86.0%
- Refer 2MUT only → 75.4% CF

Illumina Clinical Sequencing Assay (CSA)
- Panel sensitivity = 96.8%
- Refer 2MUT only → 94.1% CF

Comprehensive Genotyping*
- Sensitivity = 99.2%
- Refer 2MUT only → 98.6% CF

*complete Sanger, del/dup exons 1-27, gap PCRs

Cost-benefit analysis of adding supplemental assays

94.1% – 98.6%

All before taking FN due to low IRT into account

New York State IRT-DNA-SEQ algorithm

Panel: CSA–Supplemental (CSA-S)

	Variant(s)	# alleles	Method(s)
Outside coding region	c.1679+1643G>T 9-bp downstream of c.1679+1634A>G (1811+1.6KbA>G)	3	bioinformatics
Validated for del/ins ≤3 bp	c.1817_1900del84 (1949del84)	3	gap-PCR / qPCR
Large del/dup	exon 2 del/dup	5	qPCR
	exon 17b del/dup	3	qPCR
Positions missed (see FDA recall)	c.3615delC (3747delC)	1	bioinformatics

CSA sensitivity = 96.8%

Refer 2MUT only → 94.1% CF

+15 CF alleles

CSA-S sensitivity = 98.5%

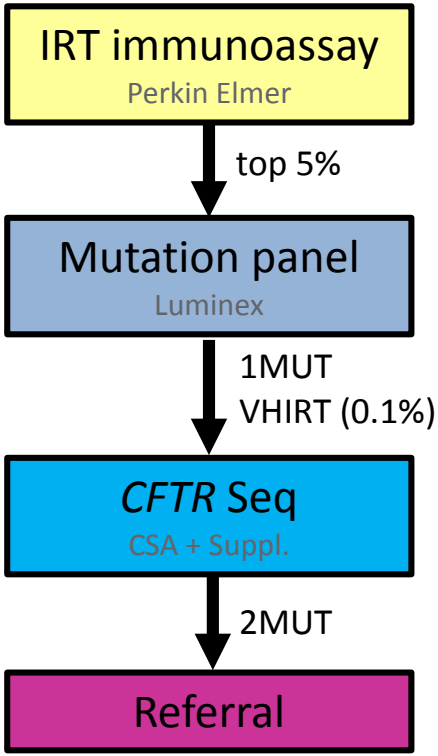
Refer 2MUT only → 97.5% CF

alleles detected using supplemental panel among 439 CF patients

Before taking FN due to low IRT into account

Strategy and significance

Fall 2017: implementation of IRT-DNA-SEQ



Third-tier genotyping labs at Wadsworth

- **CSA**: Applied Genomics Technologies Core
- **Supplemental**: Newborn screening lab

Refer *only* if 2 mutations
Carriers reported (not referred)

- Molecular dx at screening
- ↓ false positive referrals (900 → 100)
- ↓ healthcare costs
- impact on families

- Low throughput (6/run)
- Long run time (~2.5 days)
- $$$

Prospective blind pilot study

- Specimens tested, assessed and referred using current IRT-DNA CF NBS algorithm
- 1MUT and VHIRT blinded and tested using CSA-S
- Assess
  - impact on referral rate
  - infrastructure – handling, batching, testing, reporting
  - effect on turnaround time (TAT)

Prospective blind pilot study

1/23/17 – 4/03/17

IRT-DNA	
Tier 1 IRT	45,388
Tier 2 Luminex-39	2,560
2 Mutations	5
1 Mutation	93
VHIRT	42
Screen Negative	2,420
Total Referrals	140

IRT-DNA-SEQ	
Tier 1 IRT	45,388
Tier 2 Luminex-39	2,560
Tier 3 Illumina CSA-S	140
2 Variants	26
1 Variant (Carrier)	87
Screen Negative	27
Total Referrals	26

114 unnecessary referrals

81.4% reduction in referrals

Variant interpretation and reporting

Variant classification

- ACMG guidelines
- Databases used: CFTR2, SickKids, EmVClass, ClinVar, PubMed, Google/Scholar, ExAC/gnoMAD

Modifications to mailers

- Screening sensitivity
- Genetic counseling, recurrence risk, family planning

Variant Types	
CF-causing Pathogenic Likely pathogenic	
Varying clinical consequence Unknown significance Variant of uncertain significance (VOUS)	
Non CF-causing Benign Likely benign	(available upon request)
Polymorphism	(not reported)

Prospective blind pilot study

140 infants: 26 referrals, 87 carriers, 29 screen neg
59 different *CFTR* variants

15 CF-causing (on NYS panel)
6 CF-causing
1 Likely pathogenic (splicing, nonsense, del/dup/ins)

7 Varying clinical consequences
3 Unknown significance
12 VOUS (missense, synonymous, non-coding)

5 Not CF-causing
5 Benign/likely benign
5 Common polys (not reported)

22 VOUS	
p.R74W*	p.I37L
p.D1270N*	p.I807T
p.V201M*	p.R851Q
p.F640=*	p.R31L
p.R117H;7T	p.S912L
5T;13TG	p.Y1014C
5T;12TG	p.N1148K
5T;11TG	p.P1332=
p.R1070Q	p.M1354T
p.R170H	p.C1355F
p.L467F	p.D1445N

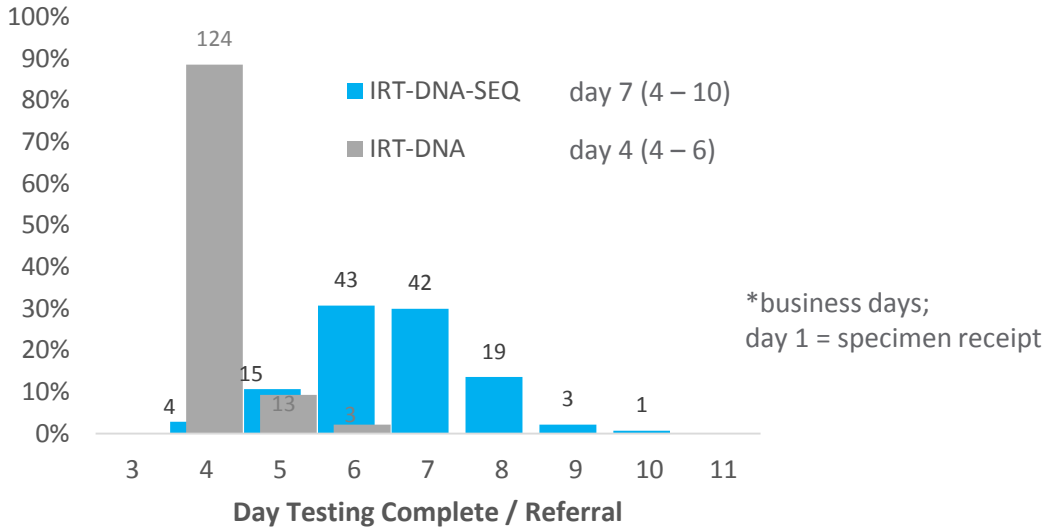
*typically *in cis*

Considerations – Turnaround time (TAT)

Blind Pilot Study
- 3 (2 – 3) runs/week
- 5 (2 – 6) samples/run

Batching schema (6 samples per run)
- 3 instruments
- 6 samples/run + pos + neg
- day, # specimens, # held

Turnaround time IRT-DNA-SEQ
- IRT days 1-2
- DNA days 2-3
- DNA confirmation days 3-4
- CSA-S days 3-6
- CSA-S confirmation days 6-7
- Report days 6-7



Confounders: batching, fails, confirmations, day, holidays

Considerations - Troubleshooting

Company can't provide guidance for **off-label use** of products (FDA)

CSA protocol modifications

- Increase DNA input volume from 5 µl to 15 µl
- Scrutinize quality metrics if cluster density <300
- Quantitate library preps using Qubit
- Triple amount pooled library (9 µl → 27 µl) sequenced if library yields <1.0 ng/µl
- Eliminate normalization step?

What's next?

Go-live mid-Fall 2017

Custom Illumina TruSeq Custom Amplicon panel

- MiSeqDx (RUO/Dx mode), MiSeq (RUO)
- *CFTR* coding, targeted non-coding, del/dup
- Higher throughput (96 vs 6 per run)
- Virtual 2nd tier (read panel CF-causing variants) → unmask 3rd tier if 1MUT or VHIRT

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