Improved Algorithm for Cystic Fibrosis Screening in the State of New Jersey

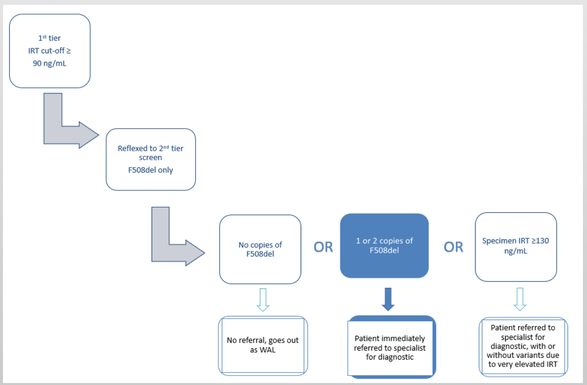
Marie-Line A. Kam, Caitlin Russo, Miriam Schachter, Mary Carayannopoulos

New Jersey Department of Health

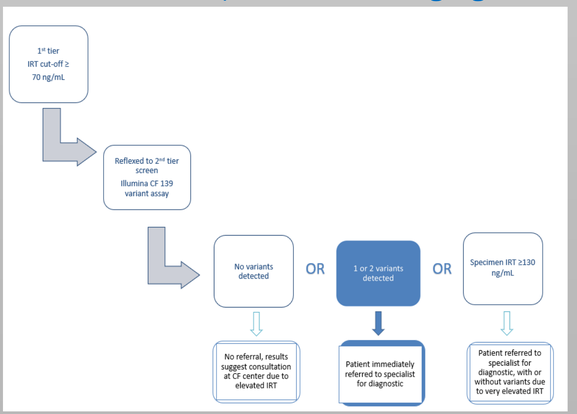
INTRODUCTION

Cystic fibrosis (CF) is a progressive inherited disorder caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CTFR). A malfunctioning CFTR can lead to a decline in lung function and premature death. New Jersey's Screening algorithm is comprised of immunoreactive trypsinogen (IRT) and DNA. Prior to 2018, the algorithm did not meet national standards due to insensitivity of the IRT cutoff and limited detection of CFTR variants. In 2018, NJ received a grant from the CF Foundation to adjust the screening algorithm to meet national standards, which concurrently led to the step-wise optimization of the CF screen.

Original screening algorithm



Optimized screening algorithm



METHODS

1st-tier: To improve the sensitivity of the first-tier screen, the IRT cutoff was lowered from ≥ 90 ng/mL to ≥ 70 ng/ml.

2nd-tier: The sensitivity of the second-tier screen was refined by increasing the number of variants detected from 1(F508del only) to 139 using the Illumina MiSeqDx™ CF 139-Variant Assay.

To evaluate the impact of these changes on our screening outcomes, we reviewed the number of screen positive and confirmed cases to identify the number of patients that would have been missed prior to the implemented changes.

RESULTS

Phase 1 Results
Lowering IRT

April 2, 2018– July 26, 2022	
Infants Screened	420,619
IRT ≥ 70 ng/ml	6,426 (1.5%)
IRT 70-89 ng/ml	4,434 (1.1%)
IRT ≥ 90 ng/ml	1,992 (0.47%)
IRT ≥ 130 ng/ml	438 (.10%)
1 copy of (F508del) (IRT 70-89)	123 80
2 copies of F508 del	11
Number of confirmed cases	36
Confirmed cases IRT 70-89 ng/mL	5
CF cases that would have been missed with former algorithm	5
Specimen with 2 variants found on Illumina CF 139 Assay	N/A

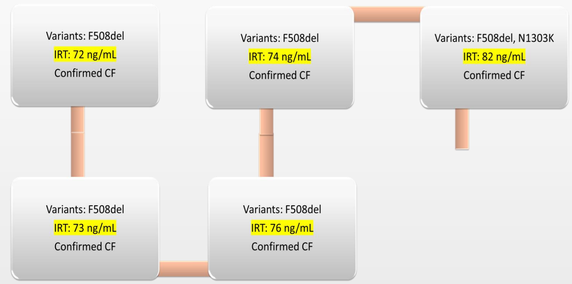
Phase 2 Results
Implementation of CF139-Variant Assay

July 27,2022 – July 28, 2023	
Infants Screened	99,866
IRT ≥ 70 ng/ml	1,711 (1.7%)
IRT 70-89 ng/ml	1,194 (1.2%)
IRT ≥ 90 ng/ml	517 (0.52%)
IRT ≥ 130 ng/ml	130 (0.13%)
1 variant (F508del) (IRT 70-89 ng/ml)	51(22)
1 variant (F508 del) IRT ≥ 90 ng/ml	37 (19)
2 variants (IRT 70-89 ng/ml)	1
2 variants ≥ 90 ng/ml	6
Number of confirmed cases	7 *2 awaiting confirmation
CF cases that would have been missed with former algorithm	2
Specimen with 2 variants found on Illumina CF 139 Assay	7 * 2 specimen had 2nd variant determined by Specialist

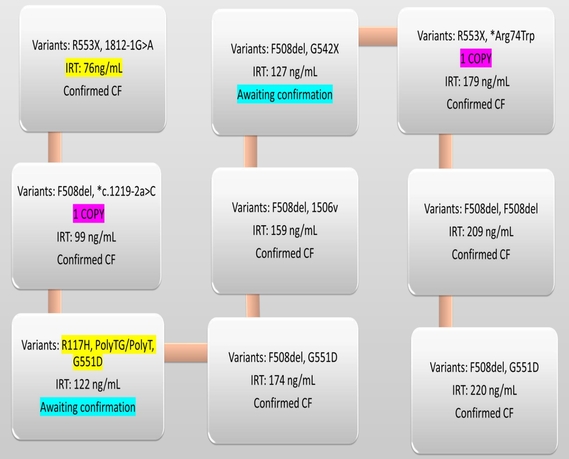
- 6,426 specimens (1.5%) had IRT levels ≥ 70 ng/mL and were reflexed to DNA analysis (only testing for F508del)
- 4,434 specimens (1.1%) had IRT levels 70-89 ng/mL (population that would have been missed with the former algorithm)
- 1,992 specimens (0.47%) had IRT levels ≥ 90 ng/mL (only population that would have been reflexed to DNA analysis in former algorithm)
- 438 specimens (0.10 %) had IRT levels ≥ 130 ng/mL (very elevated IRT population that would have been referred with or without F508 del)
- 80/123 specimens had IRT values 70-89 ng/mL and 1 copy of F508del
- From April 2, 2018-July 26, 2022 there were 36 confirmed cases of CF
- 5/36 confirmed cases (13.9%) had IRT values between 70-89ng/mL (population that would have been missed with former algorithm)
- With the former algorithm only reflexing specimens with IRT values ≥ 90 ng/mL, 5 cases would have been missed

- 1,711 specimens (1.7%) had IRT levels ≥ 70 ng/mL and were reflexed to DNA analysis (testing for 139 variants on the Illumina panel)
- 1,194 specimens (1.2%) had IRT levels 70-89 ng/mL (population that would have been missed with the former algorithm)
- 517 specimens (0.52%) had IRT levels ≥ 90 ng/mL (only population that would have been reflexed to DNA analysis in former algorithm)
- 130 specimens (0.13%) had IRT levels ≥ 130 ng/mL (very elevated IRT population that would have been referred with or without F508 del)
- 51 specimens with IRT 70-89 ng/mL had 1 variant detected, 29 specimens in this population would not have been referred (due to not having F508del)
- 37 specimens with IRT ≥ 90 ng/mL had 1 variant found, 18 specimens in this population would not have been referred (due to not having F508del)
- There was one specimen with 2 variants (IRT 70-89 ng/mL), and neither of the variants were F508del – this specimen would have been missed with the former algorithm based on DNA and IRT analysis
- There were 7 confirmed cases, there are currently still 2 pending cases with 2 pathogenic variants detected
- 2 cases in Phase two would have been missed due to formerly only looking at specimens with IRT levels ≥ 90 ng/mL or very elevated IRT 130 ng/mL and F508del
- While cases are still pending, 7 specimens were found to have 2 variants detected solely from the Illumina panel. There are 2 specimens that had only 1 variant detected, and the second was determined by a CF specialist.

Phase 1 results: confirmed cases that would have been missed



Phase 2 results: pending & confirmed cases



Yellow = cases that would have been missed with former screening algorithm
Pink = 1 copy confirmed cases that had 2nd variant determined by Specialist
Blue = two pathogenic variants detected, awaiting CF confirmation from Specialist

CONCLUSION

New Jersey's screening algorithm was adjusted to be in line with national standards and resulted in the identification of 36 confirmed cases in Phase 1, and 7 confirmed cases in Phase 2.

- In total, there are 7 cases that would have been missed with the former screening algorithm (5 from Phase 1, 2 from Phase 2).
- Implementation of the CF 139 Variant Assay has tremendously optimized the ability to identify carriers for the disease. With the CF-139 Variant Assay, we were able to identify 47 carriers. This is beneficial for diagnosis within families.
- Next steps include switching from a set IRT cutoff to a floating cutoff, reflexing the top 4% of specimens presented with elevated IRT levels to the second-tier screen. A floating cutoff will better control for variability in IRT levels based on the time of year, as well as assay variability.

Acknowledgements

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