Overview of the First Eight Years Of Newborn Screening For Cystic Fibrosis: The California Experience.

March 1, 2016

Tracey Bishop, BS; Juan Yang, PhD; RuiLing Liu, PhD,
Lisa Feuchtbaum DrPH, MPH;
California Department of Public Health

Newborn Screening for Cystic Fibrosis in California

- **In July 2006 legislation passed that added Cystic Fibrosis to the Newborn Screening Panel in California.**
- **Statewide screening began on July 16, 2007.**

CF Newborn Screening Development

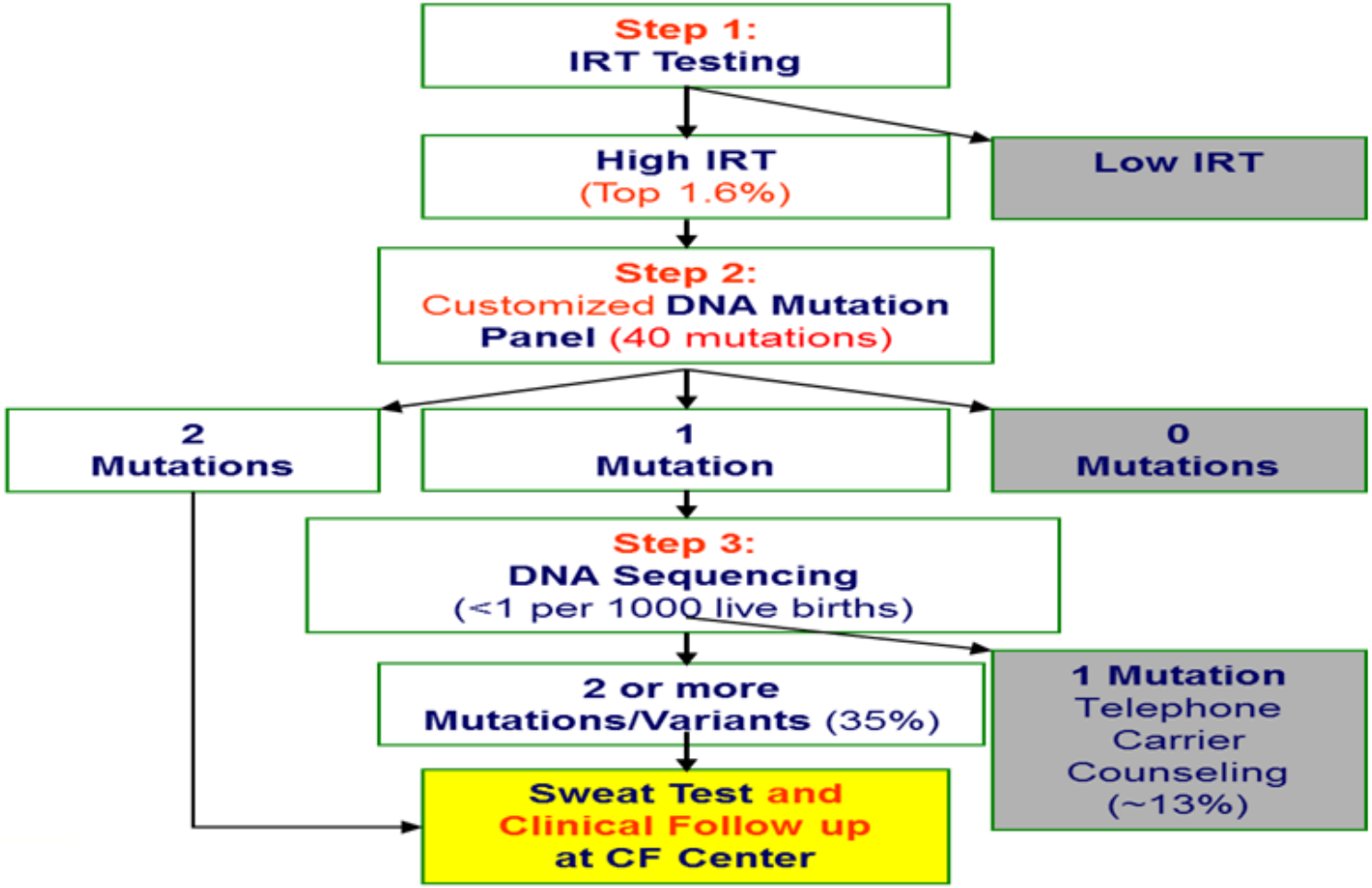
California faced specific challenges in implementing NBS for CF including:

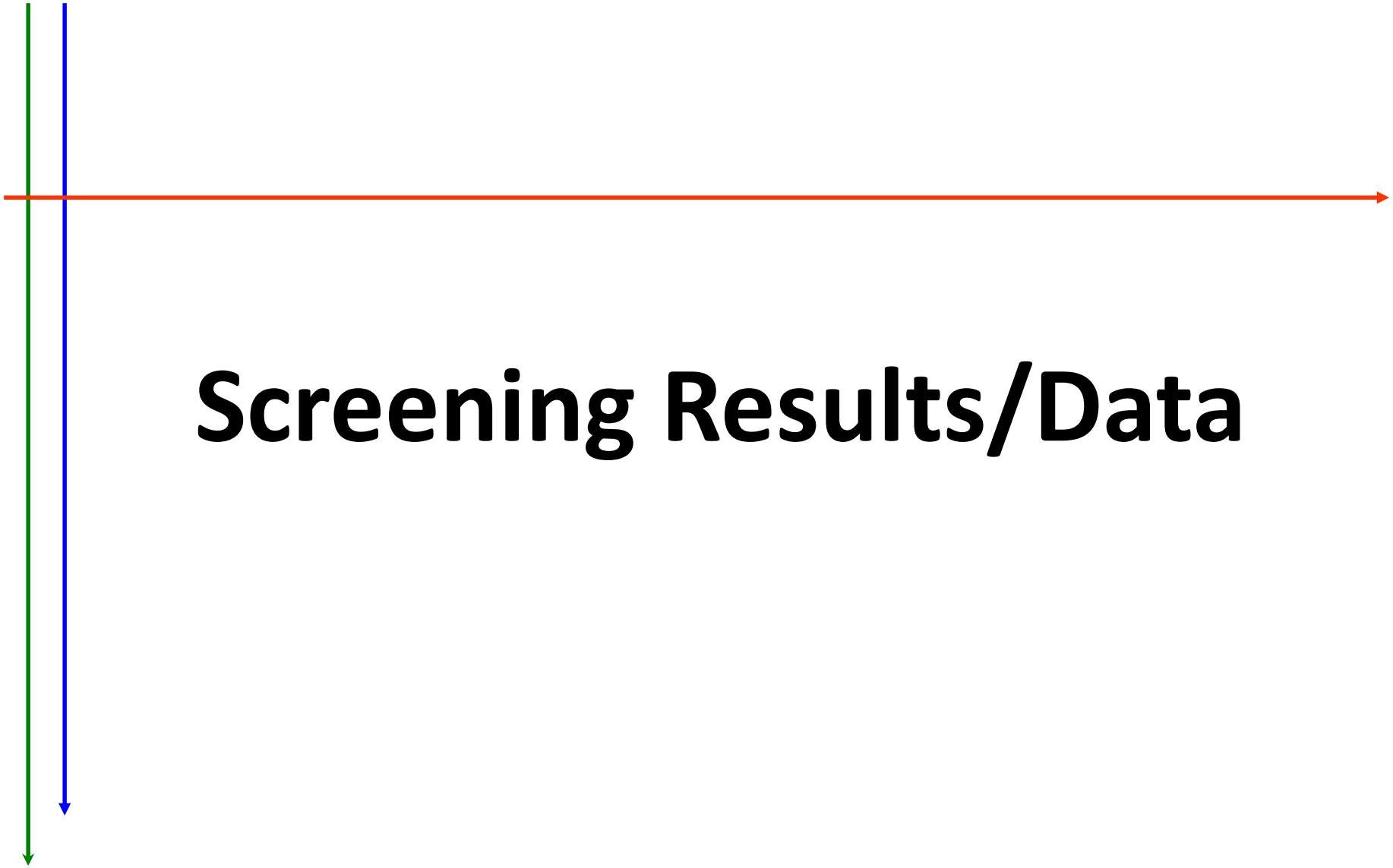
- **The large number of births (500,000/year).**
- **A poor understanding of the common CFTR mutations in our very heterogeneous population.**
- **Limited resources at CF Centers for all the positive tests that would result if we followed the standard IRT/DNA model.**

CF Newborn Screening Development

- Our goals were:
  - Maximize case detection
  - Minimize the burden of false positives, false negatives, sweat chloride tests and the associated follow-up costs
  - Identify at least 90% of all Hispanic, non-Hispanic White and Black children with CF
- A detailed study of the three main race/ethnic groups was carried out to determine the most common mutations.
- A 3-step model of immunoreactive trypsinogen (IRT)-mutation panel- DNA sequencing model for CF newborn screening was developed that would work for our program.

California 3 Step CF NBS Model

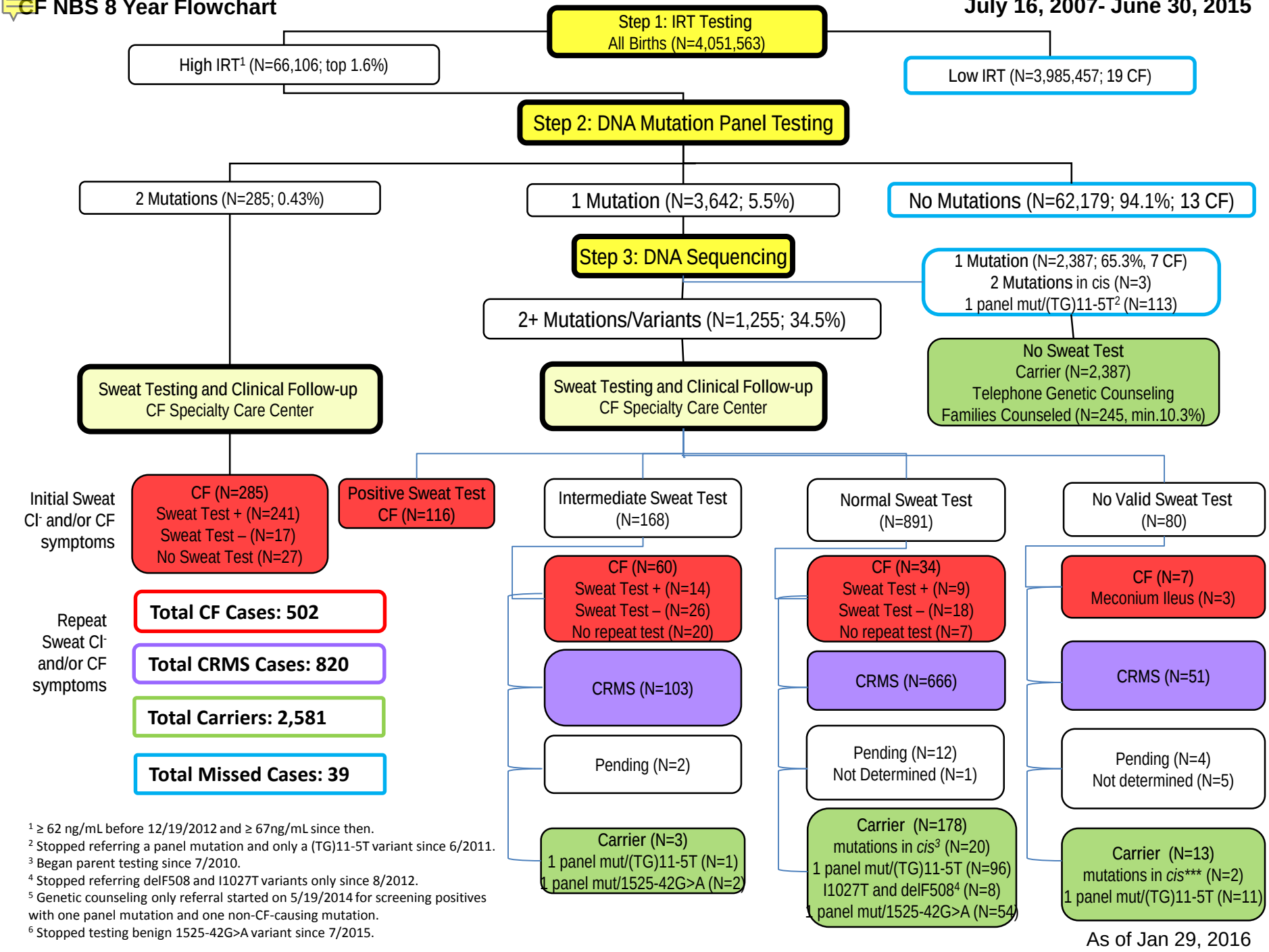




Screening Results/Data

CF NBS 8 Year Flowchart

July 16, 2007- June 30, 2015



[1] ≥ 62 ng/mL before 12/19/2012 and ≥ 67ng/mL since then.
[2] Stopped referring a panel mutation and only a (TG)11-5T variant since 6/2011.
[3] Began parent testing since 7/2010.
[4] Stopped referring delF508 and I1027T variants only since 8/2012.
[5] Genetic counseling only referral started on 5/19/2014 for screening positives with one panel mutation and one non-CF-causing mutation.
[6] Stopped testing benign 1525-42G>A variant since 7/2015.

As of Jan 29, 2016

Program Data Summary

Of 4,051,563 births in CA over the 8 years we have found:

- **502 cases of CF**
  - **285 by the panel (100%)**
  - **217 by sequencing (17% of sequenced)**
- **820 CRMS Cases**
- **2581 Carriers**

Program Data Summary

- 368 unique mutations/variants were found in newborns with positive DNA sequencing results, including 38 panel mutations.
- 200 mutations/variants were seen only once and 111 were novel.
- Of the 111 newborns with novel mutations detected, 23 (21%) were diagnosed with CF
- Of cases diagnosed with CF by DNA sequencing, only about half have an initial positive sweat test.
- The most common mutations associated with this 'progression' from CRMS to CF were IVS8 (TG)13-5T, R117H-7T and D1152H

Estimated CF Prevalence by Race/Ethnicity

Client Race/Ethnicity	Live births screened	Screen positive	Diagnosed CF	Missed CF	Prevalence 1/X
Hispanic/Latino	1,664,800	510	153	14	9,969
Non-Hispanic White	1,035,354	632	231	9	4,314
Non-Hispanic Asian	383,058	23	4	3	54,723
Non-Hispanic Black	211,199	97	18	1	11,116
Other /Multiple	757,152	278	96	12	7,011
Total	4,051,563	1,540	502	39	7,489

Modifications to Screening Algorithm

IVS8 Poly 5T/TG

- At the start of the program we initially called out all IVS8 Poly 5T with an 11, 12 or 13 TG tract
- After approximately 3 years we had a large number of 11TG-5T (over 100) and follow-up data indicated that they nearly all had negative sweat tests and no symptoms.
- Additional literature was published that confirmed these findings and reported the primary possible concern is congenital bilateral absence of the vas deferens (CBAVD) .
- On June 1, 2011 we discontinued calling 11TG-5T as positive. They are now considered ‘carriers’ but there is a small chance of CBAVD in males with 11TG-5T.

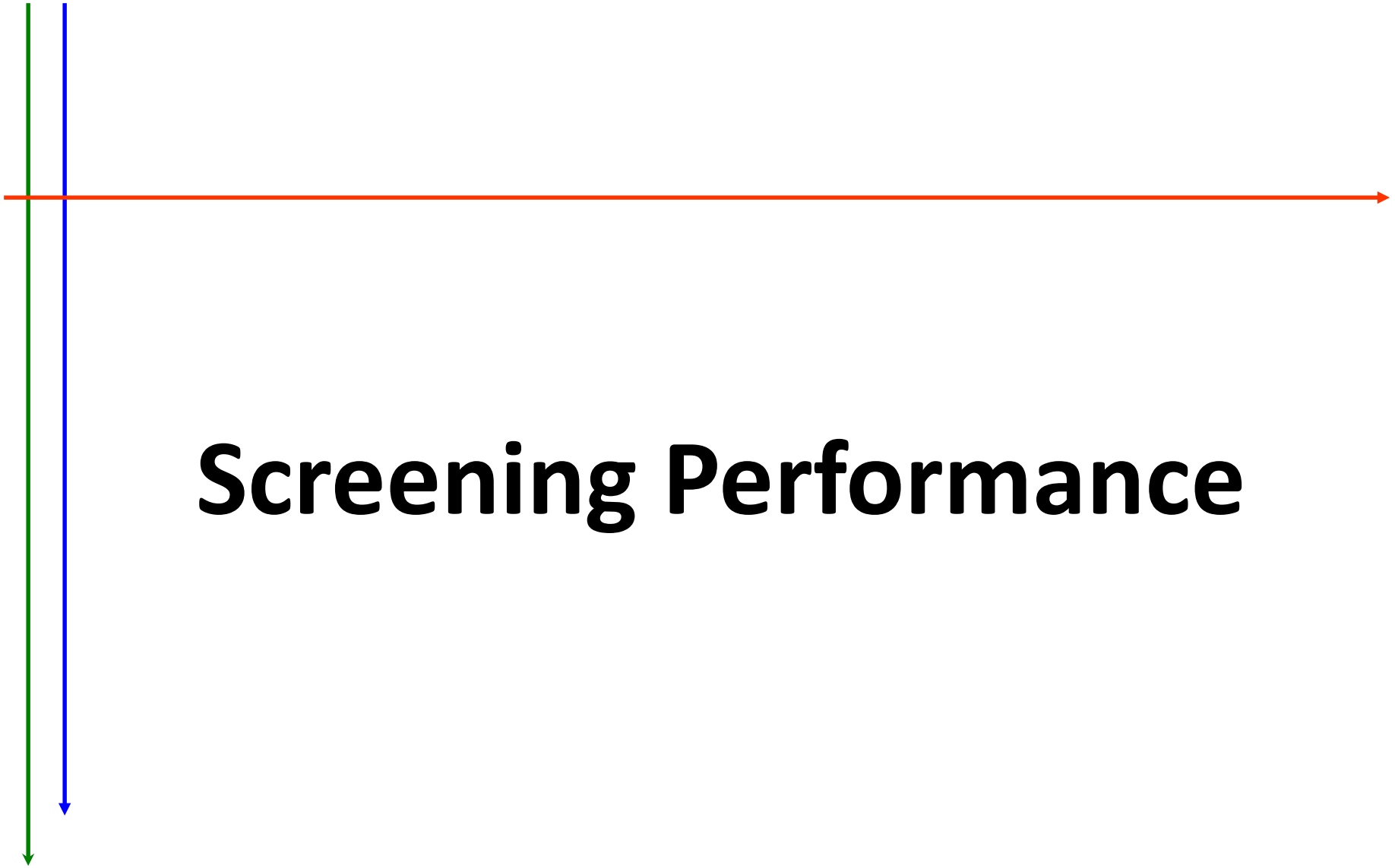
CFTR2 'Non-CF Causing Mutations'

- Based on John Hopkins-based CFTR2, there is a group of mutations called out by the California program that CFTR2 describes as 'non CF causing'.
- The mutations that CFTR2 updated are: R668C, G576A, G576A + R668C, L997F, R31C, R1162L, V754M, S1235R.
- On May 2014: continue to refer these children to the CF centers as CF screen positive, but recommend genetic counseling as the minimum follow-up.

1525-42G>A

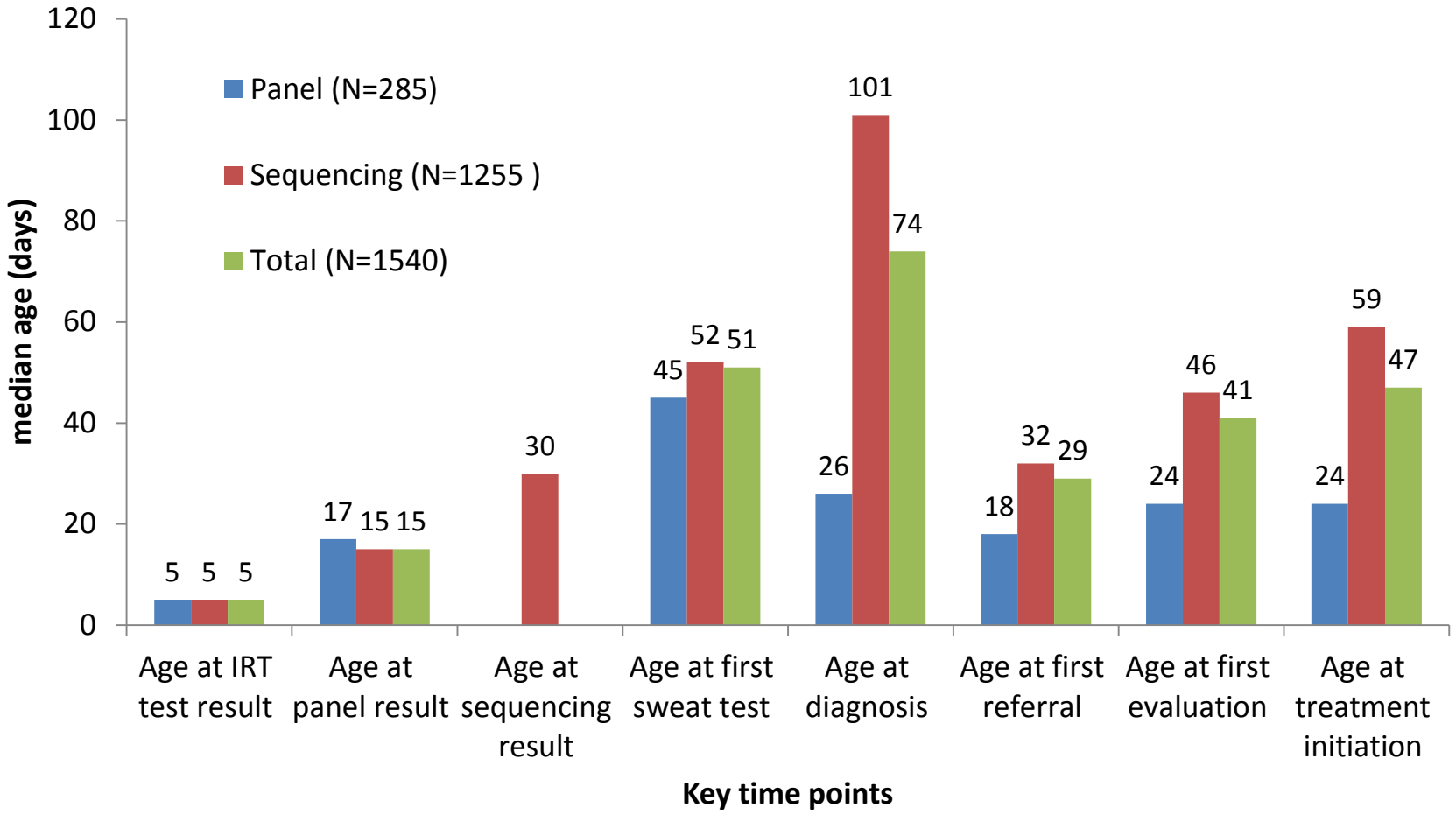
- **In Spring 2015 we stopped reporting out 1525-42G>A as a mutation through sequencing because newborns/infants with this mutation have had negative sweat tests and done well.**

- **This mutation is very common in Hispanics and is now classified as a benign polymorphism.**
  - **was detected in 4.3% of Hispanics in a study by Shrijver et al 2015.**
  - **Consistent with our findings of a frequency of 1.88% in the CA population as a whole and 4.94% in the Hispanic population.**



Screening Performance

Median Age at Key Screening Points for Screen Positive Cases: July 16, 2007–June 30, 2015



Screening Performance - Detection

- The ratio of screen positives to cases detected is 3.1 to 1. (1,540 screen positives / 502 cases detected)
- Case detection rate = 92.8%
  - 502 cases detected by screening /541 total CF cases
- Missed cases: 39 CF cases with negative screening results were reported
  - 19 by low IRT
  - 13 by negative panel
  - 7 had negative sequencing

Lessons Learned

- Including sequencing increases the time for result reporting.
- DNA Testing leads to a group who are found to have at least two CFTR mutations or variations but display no symptoms. In California we call this group CRMS.
- Even with sequencing, there are gross deletions or duplications that are not picked up as well as possible lab errors.
- It is very important that physicians know to make referrals if there are signs and symptoms consistent with CF – even with a negative screen.

In Summary

- Including sequencing greatly reduces the false positive rate.
- Sequencing provides more information for the medical team for those who are referred to a CF center.
- We have evidence that a single initial sweat chloride test may not be the best diagnostic 'gold standard'
  - 43% of diagnosed cases by sequencing did not have an initial positive sweat test (>60 mmol/L).

Acknowledgements - Thanks

- GDSP Program Evaluation Staff
- The California CF Consortium & the staff at the CF centers!

Questions

CA CF 40 Mutation Panel

#	Mutation	#	Mutation
1	1288insTA	21*	delI507
2*	1717-1G>A	22	G330X
3	1812-1G>A	23*	G542X
4	2055del9>A	24*	G551D
5	2105-2117del13insAGAAA	25*	G85E
6	2307insA	26	H199Y
7*	3120+1G>A	27*	N1303K
8	3272-26A>G	28	P205S
9	3791delC	29	Q98R
10*	3849+10kbC>T	30	R1066C
11	3876delA	31*	R1162X
12	406-1G>A	32*	R334W
13*	621+1G>T	33*	R553X
14	663delT	34	R75X
15*	711+1G>T	35	S492F
16	935delA	36	S549N
17	A559T	37	W1089X
18	CFTRdele2,3(21kb)	38	W1204X (3743G>A)
19	delF311	39	W1204X (3744G>A)
20*	delF508	40*	W1282X

* Mutation included on ACMG 23 Panel