Progress of newborn screening for spinal muscular atrophy

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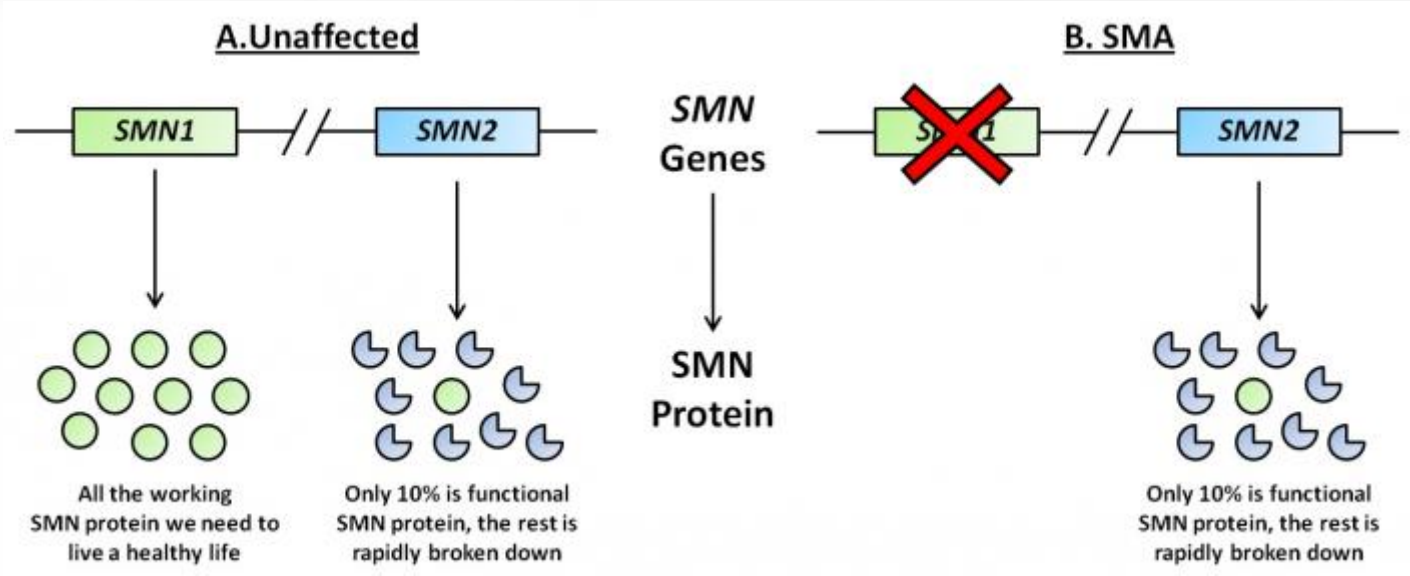
Spinal muscular atrophy (SMA)
~95% due to SMN1 deletion

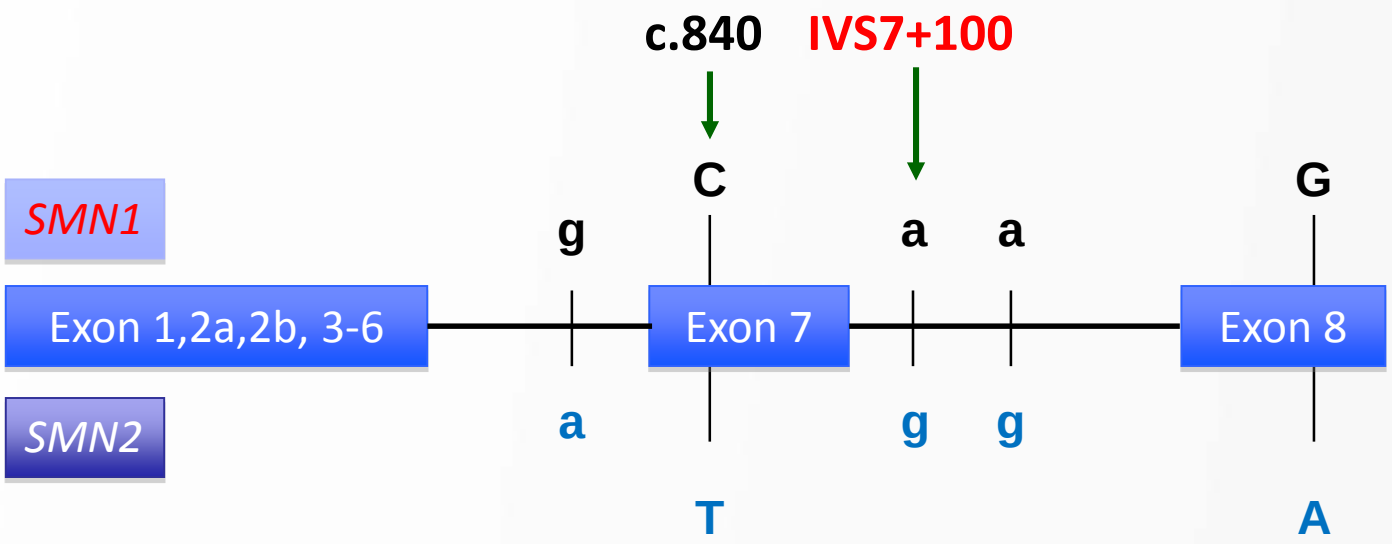


-Copy number of *SMN2* correlate with the onset age

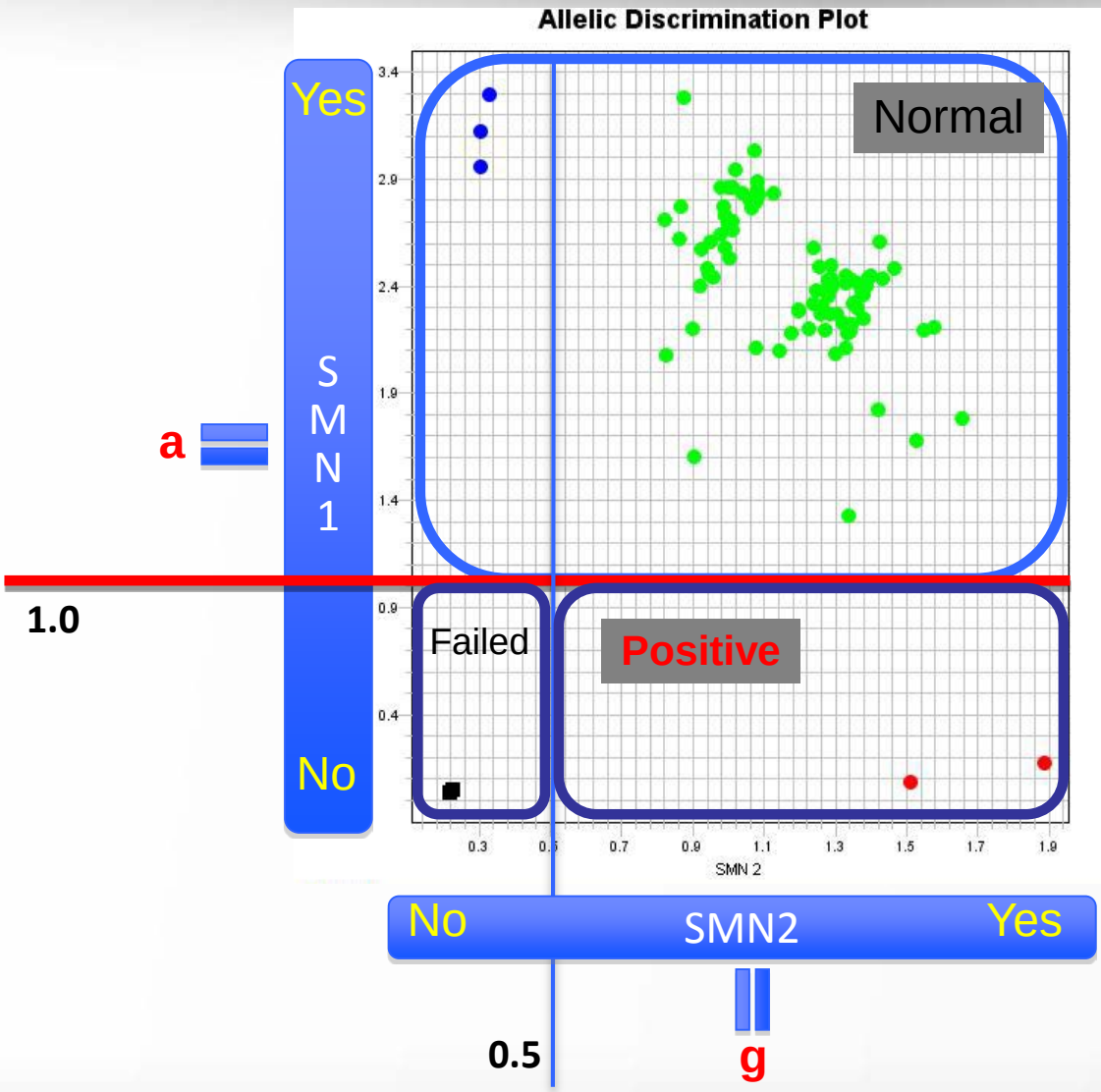
- Because of the high incidence of SMA, rapid loss of motor neurons in the spinal cord and the brainstem, and there are several promising treatment options, newborn screening for SMA is considered.

Screening principle
detect SMN1 deletion

- Lack of IVS7+100 a (first-tier test by qPCR)
- Lack of c.840 C (second-tier test by SMN-specific PCR/Sequencing and ddPCR)

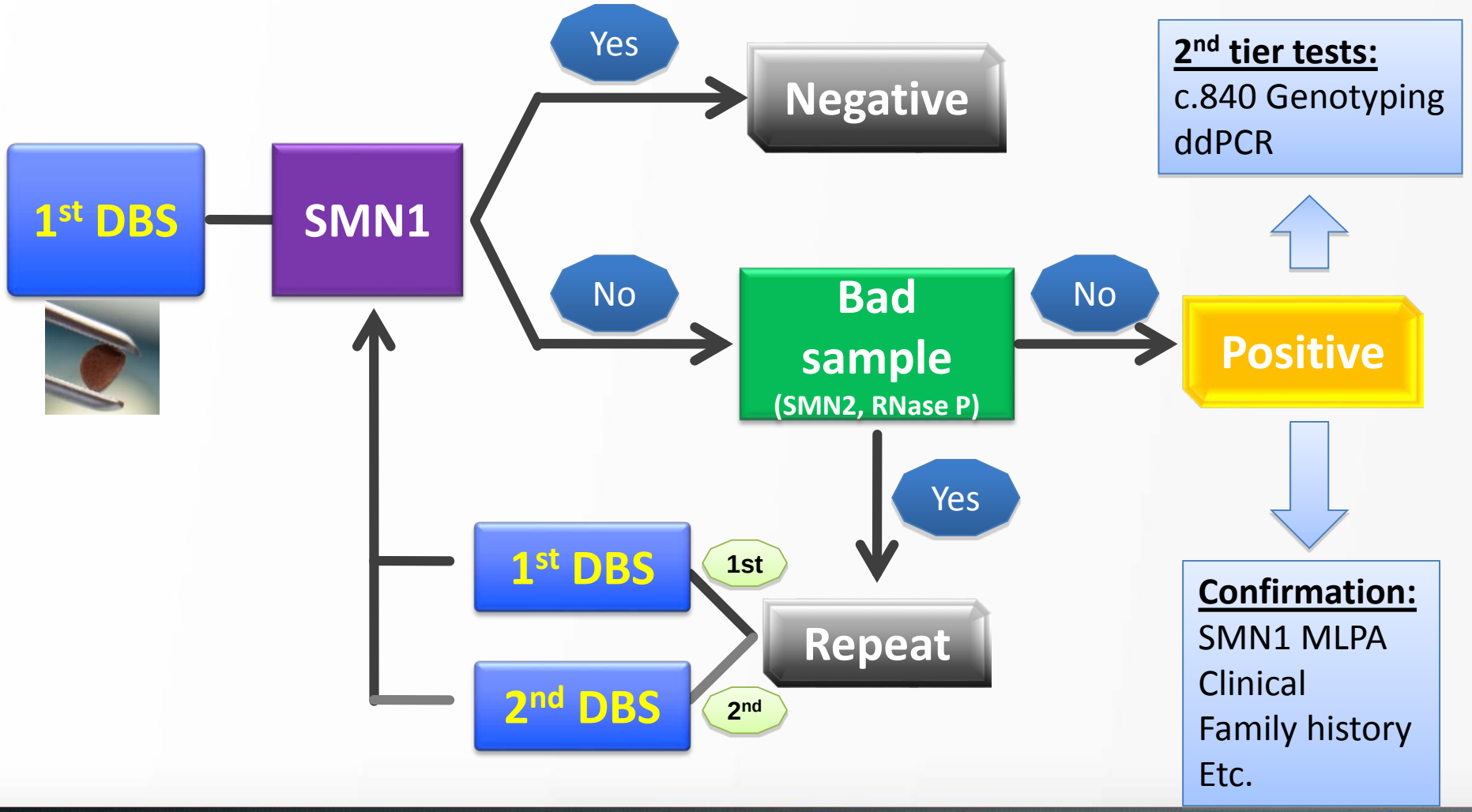


Example results



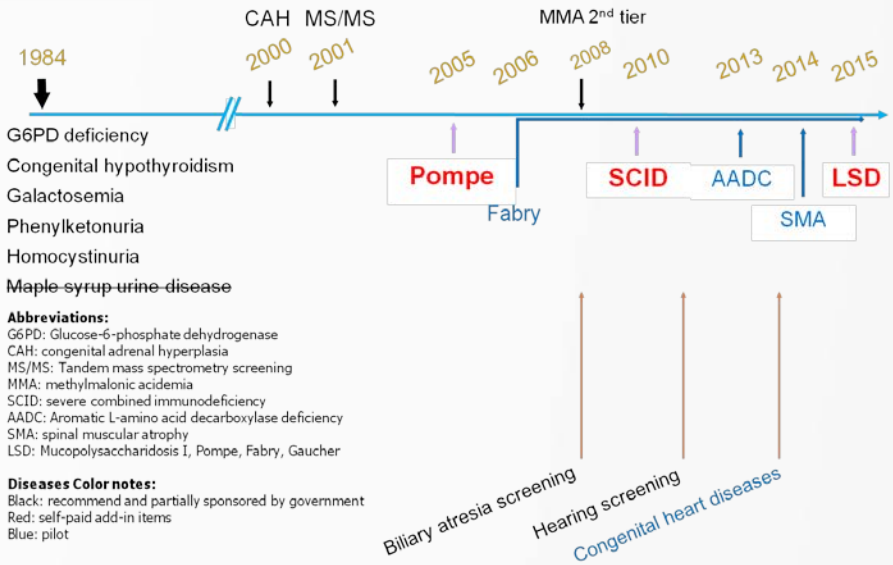
Rn: normalized reporter signal
the fluorescence of the reporter dye
divided by
the fluorescence of a passive reference dye
ΔRn:
Rn (post-PCR read) – Rn (pre-PCR read)

Algorithm

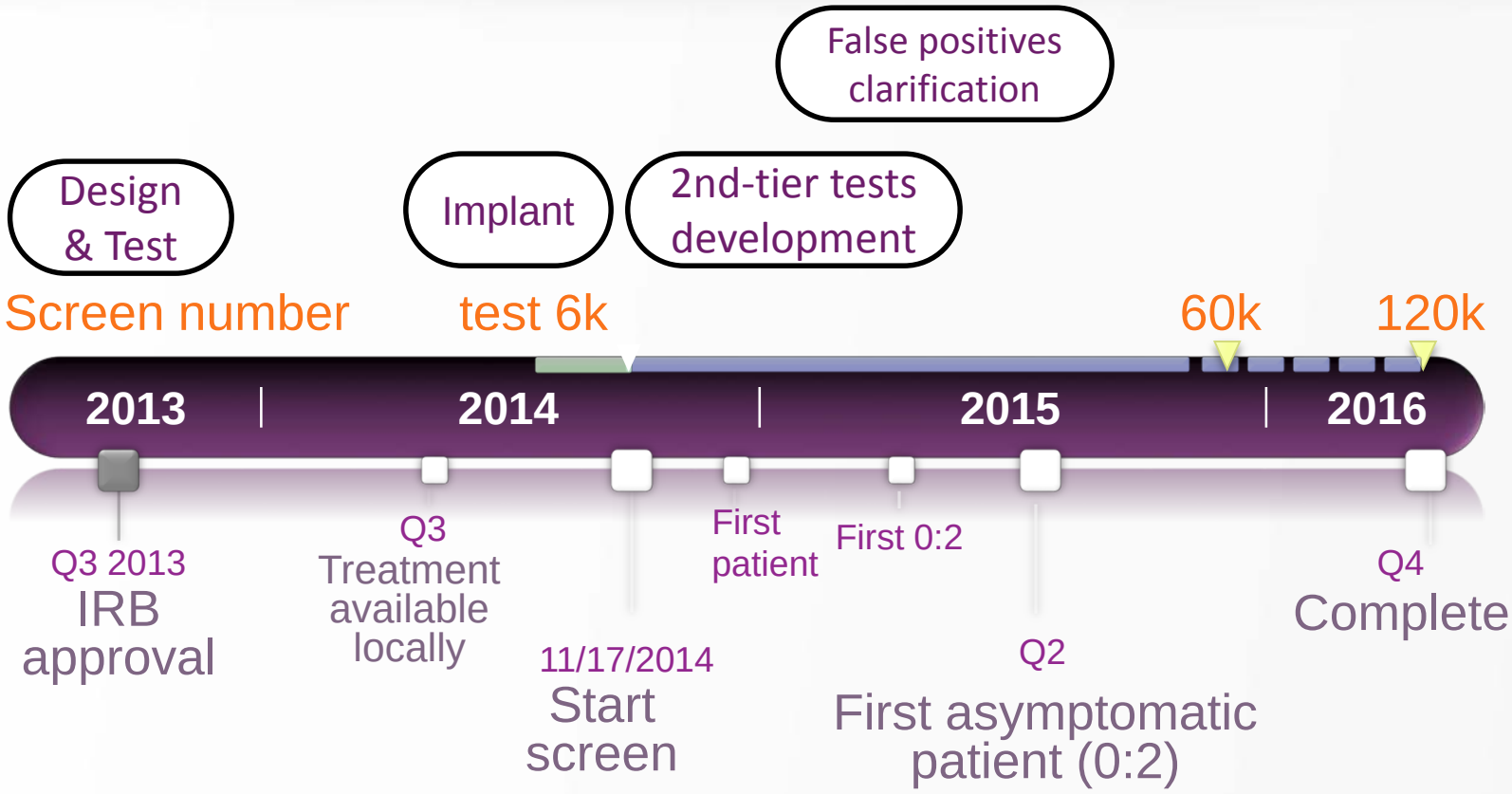

1st DBS
SMN1
Yes
Negative
No
Bad sample
(SMN2, RNase P)
No
Positive
2nd tier tests:
c.840 Genotyping
ddPCR
Yes
1st DBS
1st
2nd DBS
2nd
Repeat
Confirmation:
SMN1 MLPA
Clinical
Family history
Etc.

Pilot SMA newborn screening program

- National Taiwan University Hospital (NTUH) Screening center screened 35~37% of populations in Taiwan
- Incorporated into routine metabolic screening
  - 1st sample at 3-days-old
  - Informed consent required

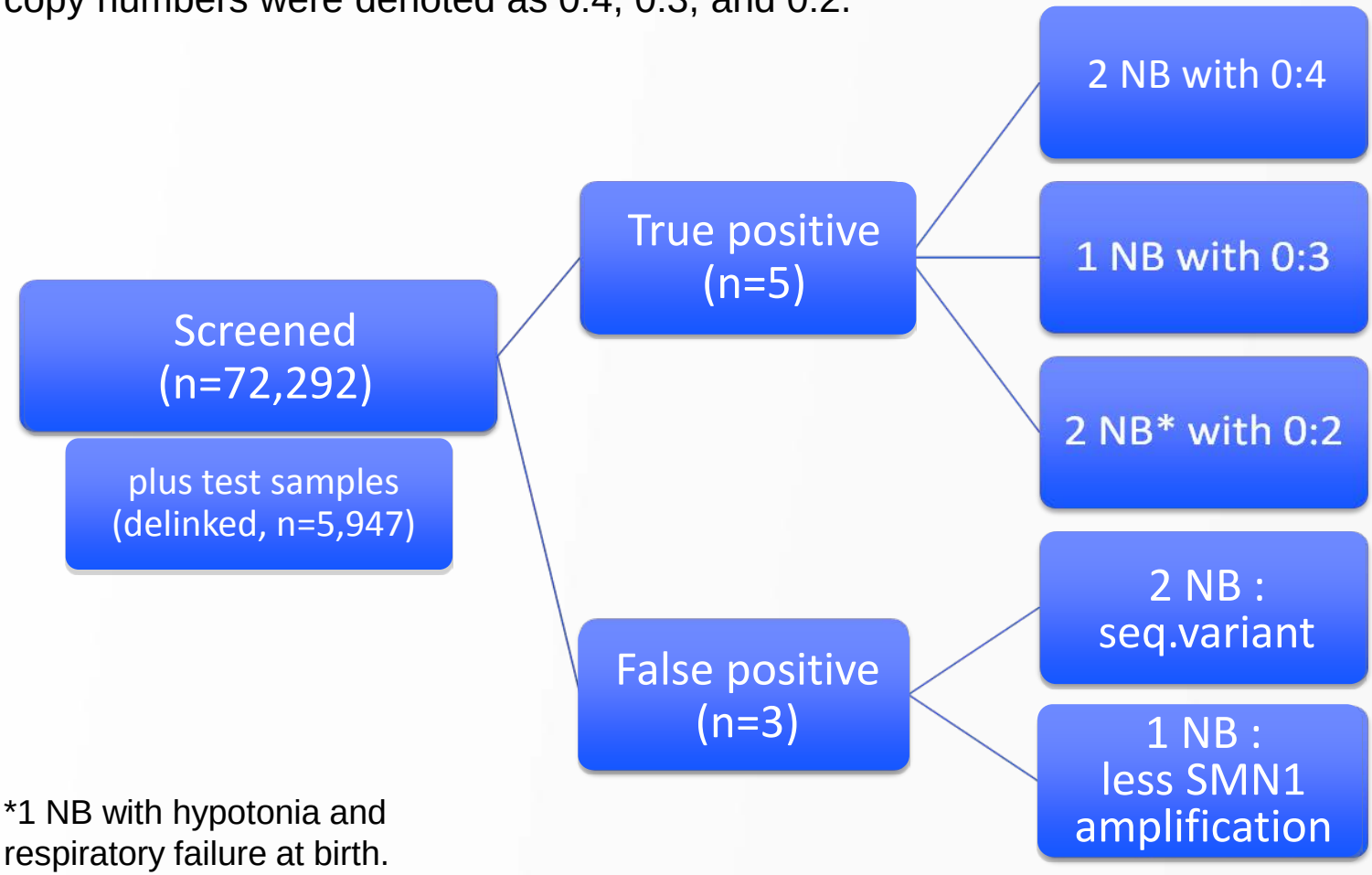


NTUH SMA NBS Timeline



Current progress
an incidence as ~1 in 14,500 live births

In true positive patients, *SMN1* gene and *SMN2* gene copy numbers were denoted as 0:4, 0:3, and 0:2.

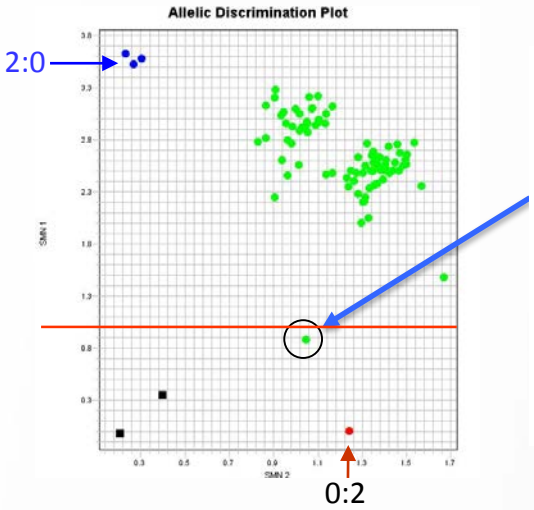


*1 NB with hypotonia and respiratory failure at birth.

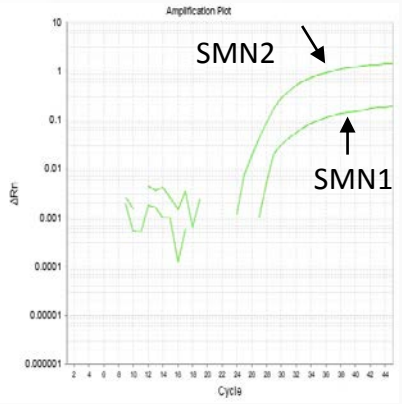
False positive in 1st- tier Screening

- One newborn with lower amplification at the target sequence (IVS7+100) in SMN1, and second-tier test showed presence of SMN1 at c.840

Amplification curve

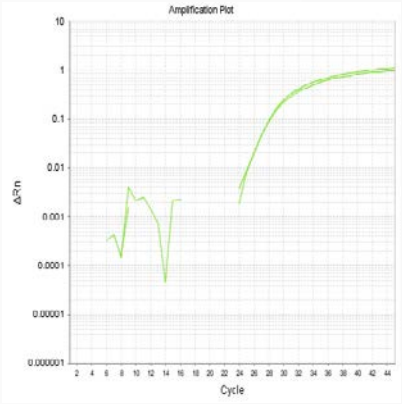


False positive case



SMN IVS7+100

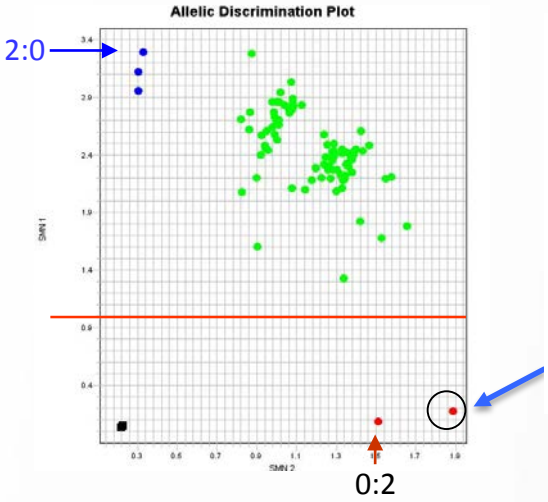
Normal control (2:2)



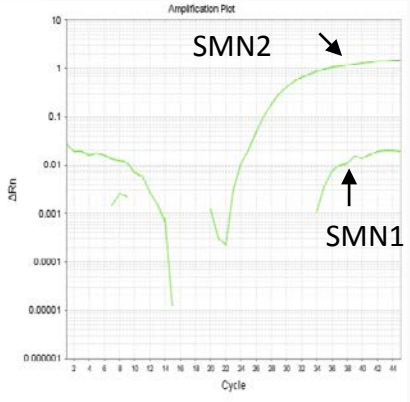
SMN IVS7+100

False positive in 1st- tier Screening

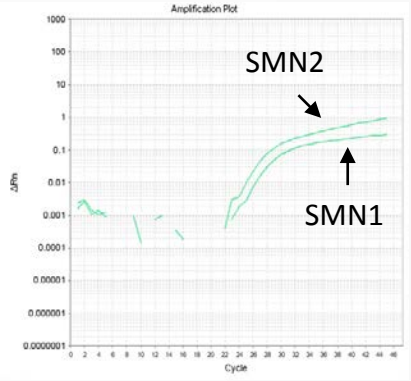
- Two newborns with a nucleotide change at IVS7+100, but second-tier test showed presence of SMN1 at c.840



Amplification curve of this false positive case



SMN IVS7+100



SMN c.840

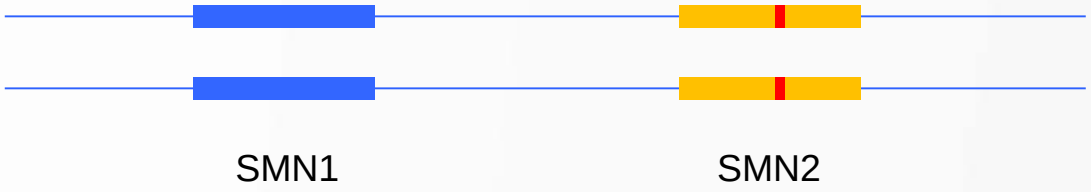
Frequency of recombination between Exon7 and Intron7

- Risk of false negative due to IVS7+100 variation is low, only when both SMN1 del homozygous and one SMN2 T-a

genotype of c.840 - IVS7+100	n
SMN1 (represent by ASP-C) (C---a)	Totally 1463 NB samples
C---a	1458
C---a/**g**	**5**
SMN2 (represent by ASP-T) (T---g)	
T---g	1401
T---**a**/g	**5**
No SMN2 PCR product	62

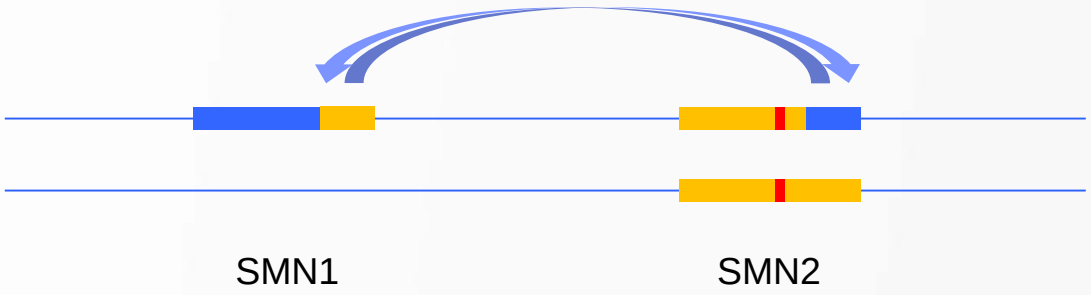
Proposed mechanism for recombination between Exon7 and Intron7 (after verifying exon8 polymorphism)

Normal



Gene conversion?

The 2 False-positive newborns



Conclusion

- The incidence of SMA in our newborn population is 1 in 14,500 (95% Confidence interval 1 in 6,176~33,849).
- Doing only 1st-tier has very low false positive, and the positive prediction rate is 62.5%.
  - Combining with 2nd-tier test, such as assay at c.840 either by Genotyping assay or ddPCR, can largely improve the screening performance
  - The theoretically false negative rate was 5% since we only detect SMN1 deletion homozygous patients.

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