An Evolution of Michigan's SCID Algorithm

A qualitative approach for the T cell receptor excision circle (TREC) assay for the detection of primary immune deficiency syndromes (PIDS) demonstrates better sensitivity and specificity versus a quantitative approach

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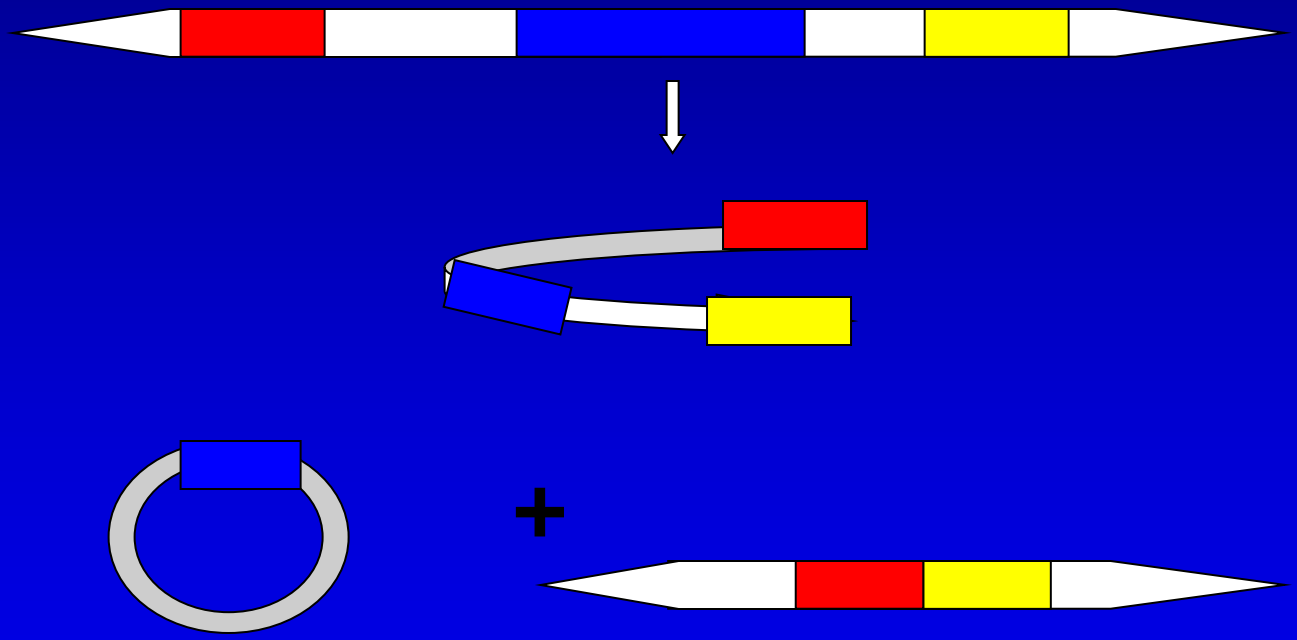
How To Screen For SCID And Other PID's?

Real time polymerase chain reaction (PCR) of

T cell receptor excision circles (TREC's) and reference gene (β-actin).

TREC's are small pieces of DNA that are formed during the differentiation of T cells in the thymus as a result of the rearrangement of the T cell receptor genes.

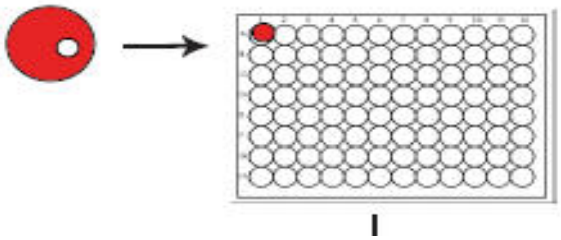
TREC Formation



This process doesn't work properly in PID's

Overall Analysis Scheme

Punch DBS into 96 well plate.



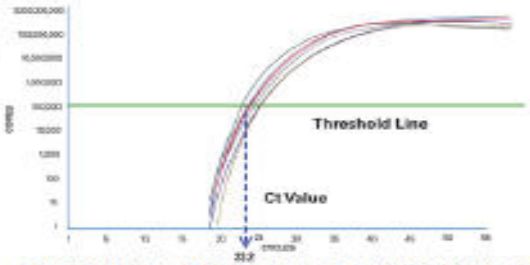
3.2 mm DBS is punched into a 96 well plate

Automated DNA Extraction

http://dna.uga.edu/image/sized/images/page-images/epMotion-5075

Partially automated DNA extraction using an Eppendorf epMotion 5075

Quantitative PCR

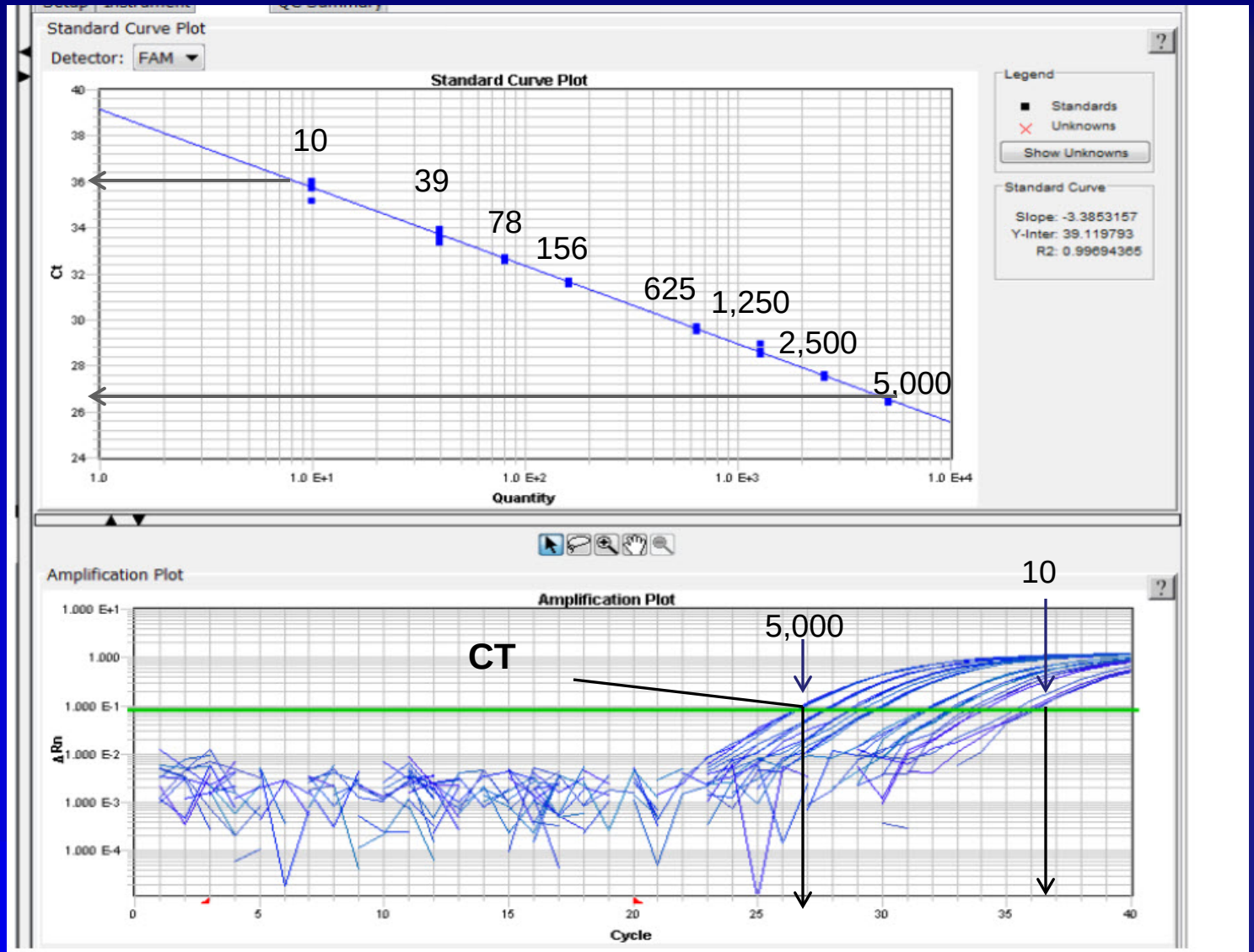


http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/real-time-pcr/real-time-pcr-vs-traditional-pcr.html

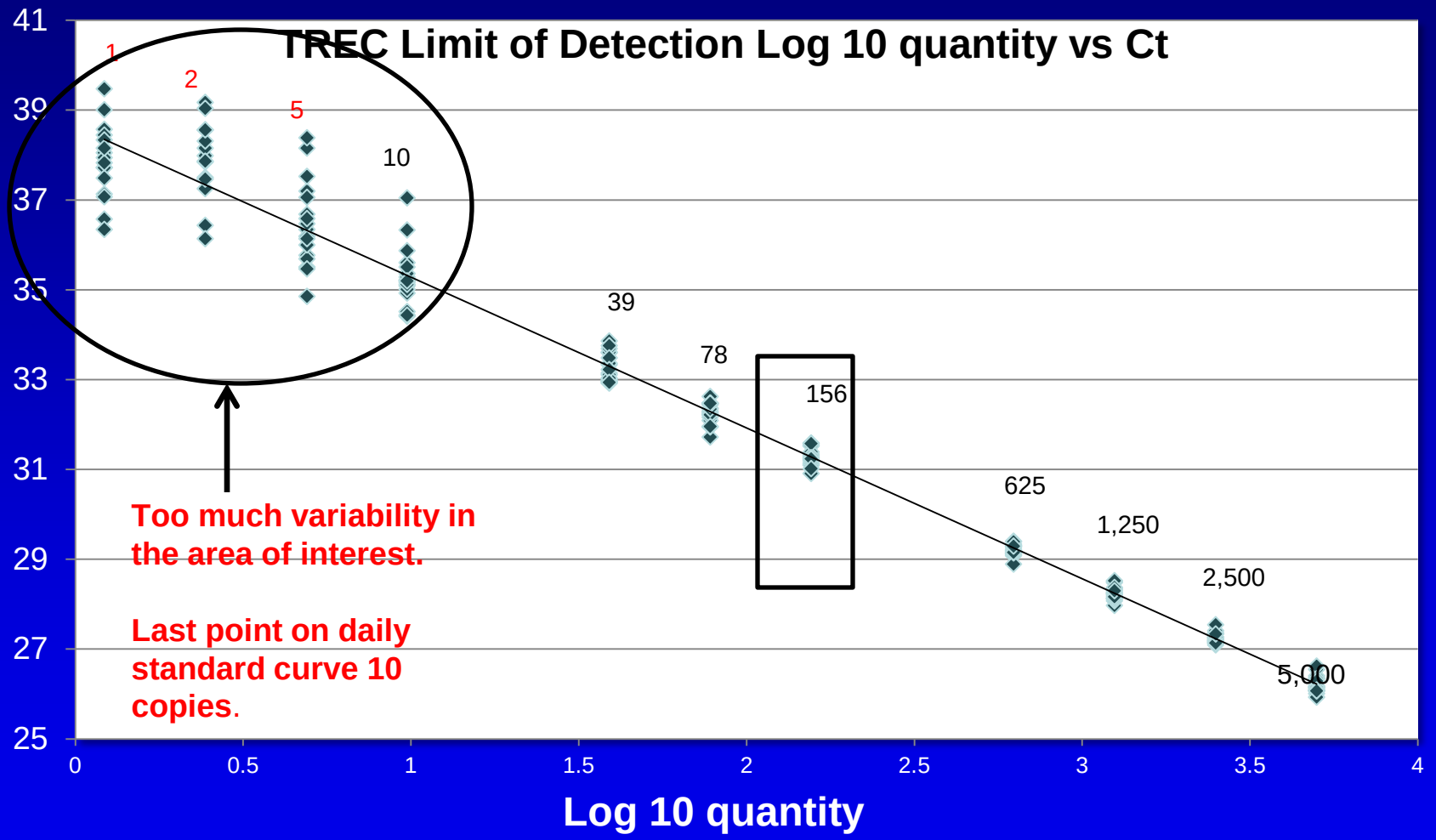
Automated set up of real time qPCR in a 384 well format using the epMotion 5075

Duplex qPCR amplification and analysis (TREC and β actin) on a 7900HT.

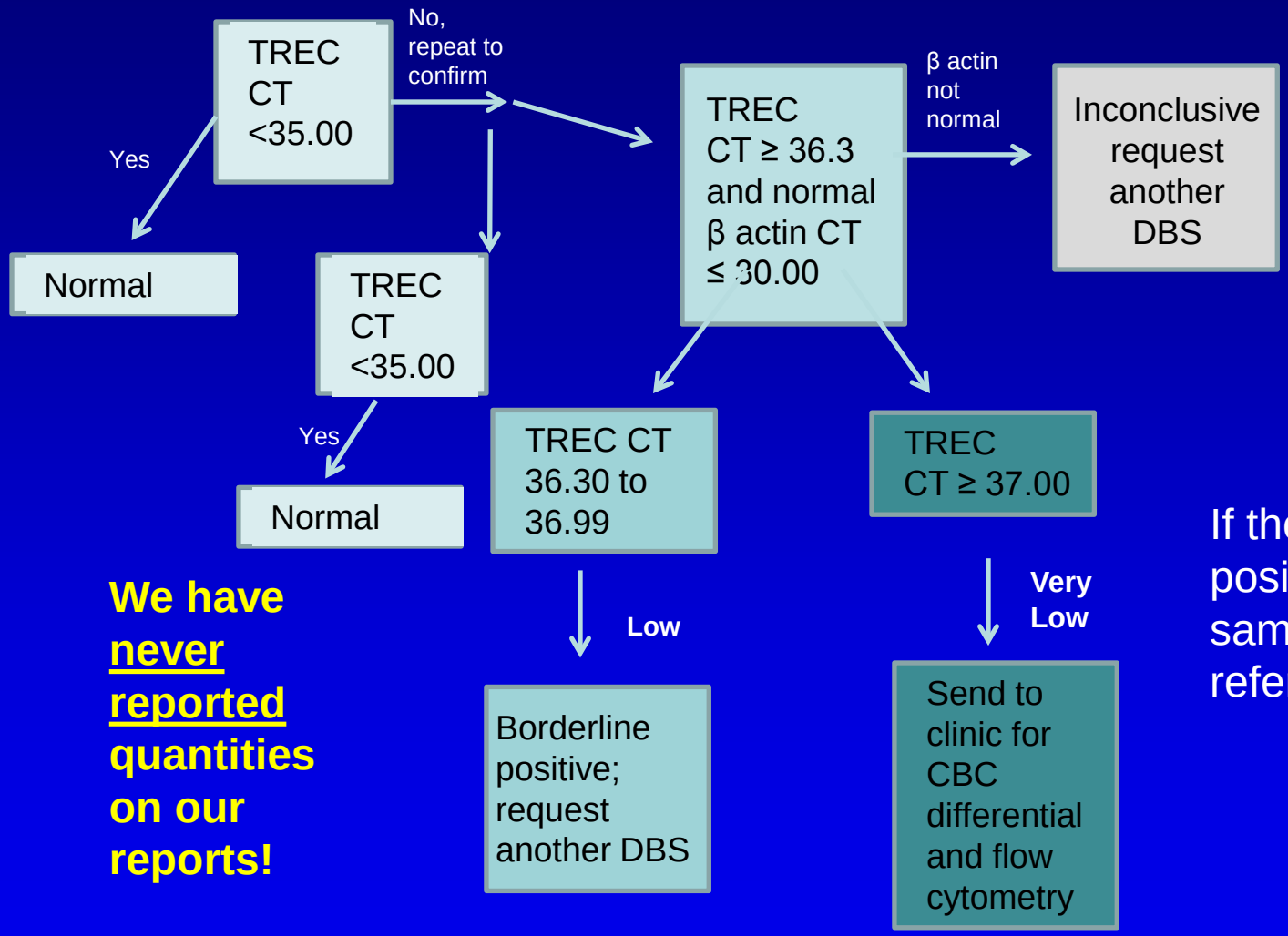
A Typical TREC Plasmid Standard Curve



TREC Plasmid Curve



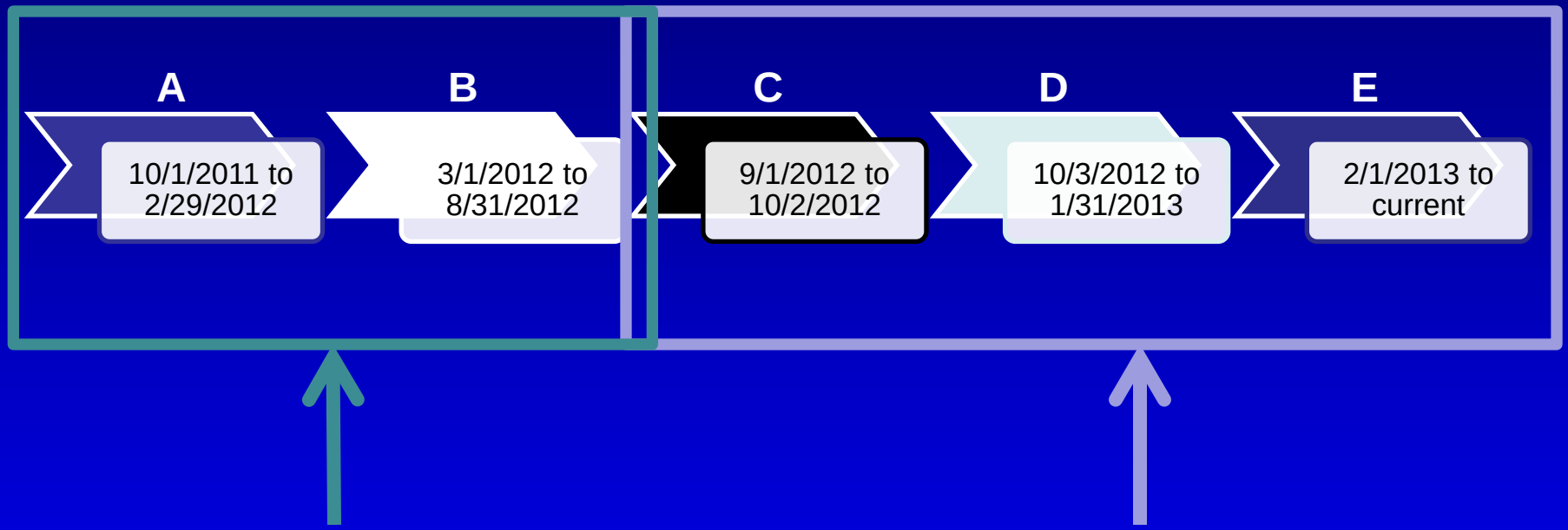
Current Qualitative Algorithm



We have <u>never</u> <u>reported</u> quantities on our reports!

If there are 2 borderline positive or 3 inconclusive samples in a row patient is referred for flow cytometry

Timeline of algorithms



Initial Michigan Method

New and Improved Michigan Method

Algorithm A (10/1/2011 to 2/29/2012)

Normal and low birth weight babies	TREC quantity copies per µl of blood	B actin Quantity copies per µl of blood	Result
	<30	≥4000	Strong positive
	<30	<4000	Inconclusive

Time= ~5 months
32 cases per month
N= 44,712
Total PPV= 18.58% Total FPR= 0.21%

Algorithm B (3/1/2012 to 8/31/2012)

	TREC quantity copies per µl of blood	B actin Quantity copies per µl of blood	Result
Normal birth weight ≥2500 grams	<30	≥8000	Strong positive
Low birth weight <2500 grams	<20	≥8000	Strong positive
Low birth weight <2500 grams	20 to <30	≥8000	Borderline positive
All babies	<30	<8000	Inconclusive

Time= ~6 months
36 cases per month
N= 57,023
Total PPV= 25.0% Total FPR= 0.09%

Algorithm C (9/1/2012 to 10/2/2012)

	TREC quantity copies per µl of blood	B actin CT value	Result
Normal and low birth weight babies	<20	≤30.00	Strong positive
	20 to <30	≤30.00	Borderline positive
	<30	>30.00	Inconclusive

Time= ~1 month

***85 cases**

N= 9,054

Total PPV= 10.59% Total FPR= 0.83%

Algorithm D (10/3/2012 to 1/31/2013)

Normal and low birth weight babies	TREC quantity copies per µl of blood	B actin CT value	Result
	≤10	≤30.00	Strong positive
	11 to 20	≤30.00	Borderline positive
	≤20	>30.00	Inconclusive

Time= ~3 months
53 cases per month
N= 36,728
Total PPV= 8.81% Total FPR= 0.39%

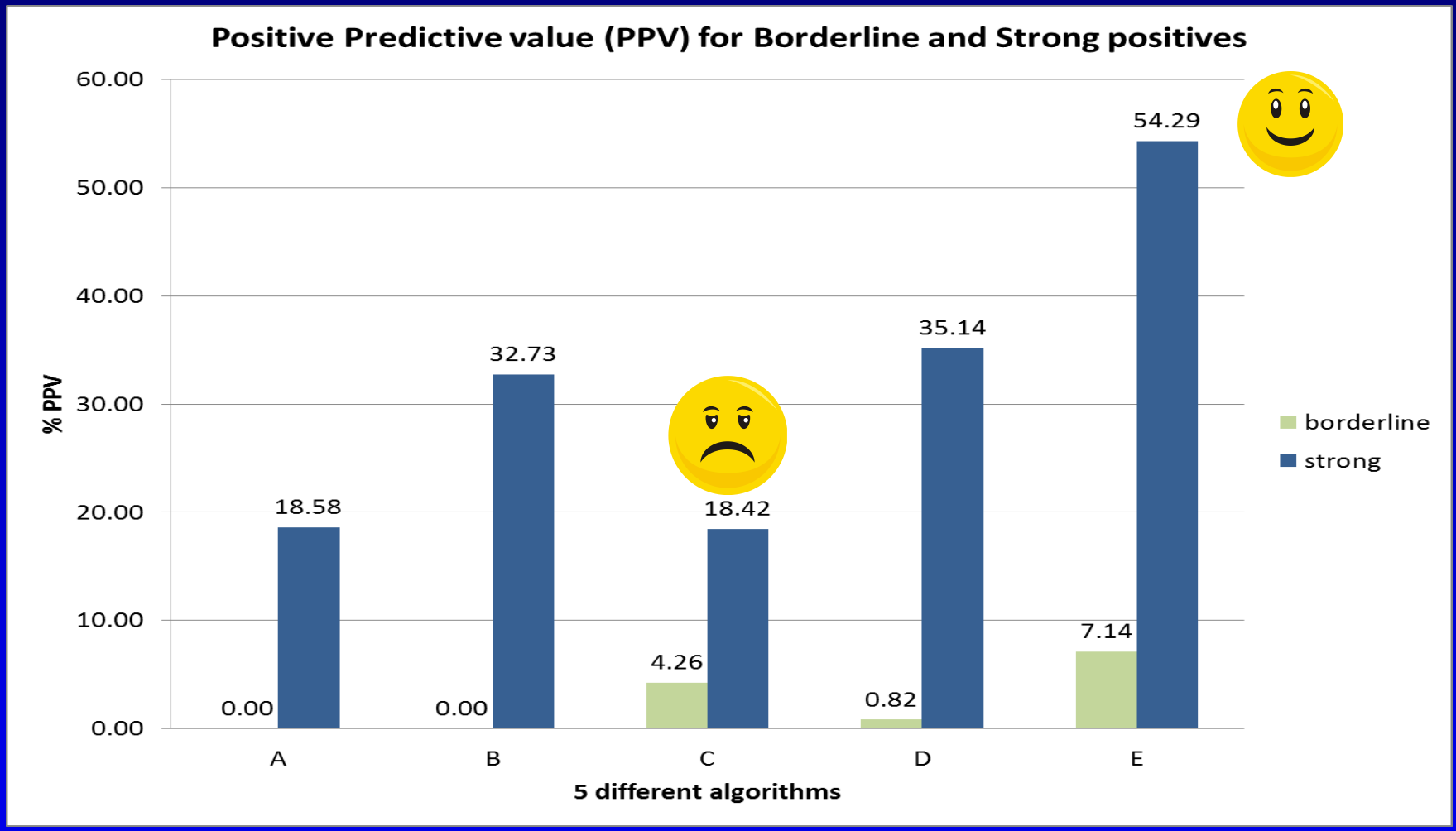
Algorithm E (2/1/2013 to current)

	TREC CT value	B actin CT value	Result
Normal and low birth weight babies	≥37.00	≤30.00	Strong positive
	36.30-36.99	≤30.00	Borderline positive
	≥36.30	>30.00	Inconclusive

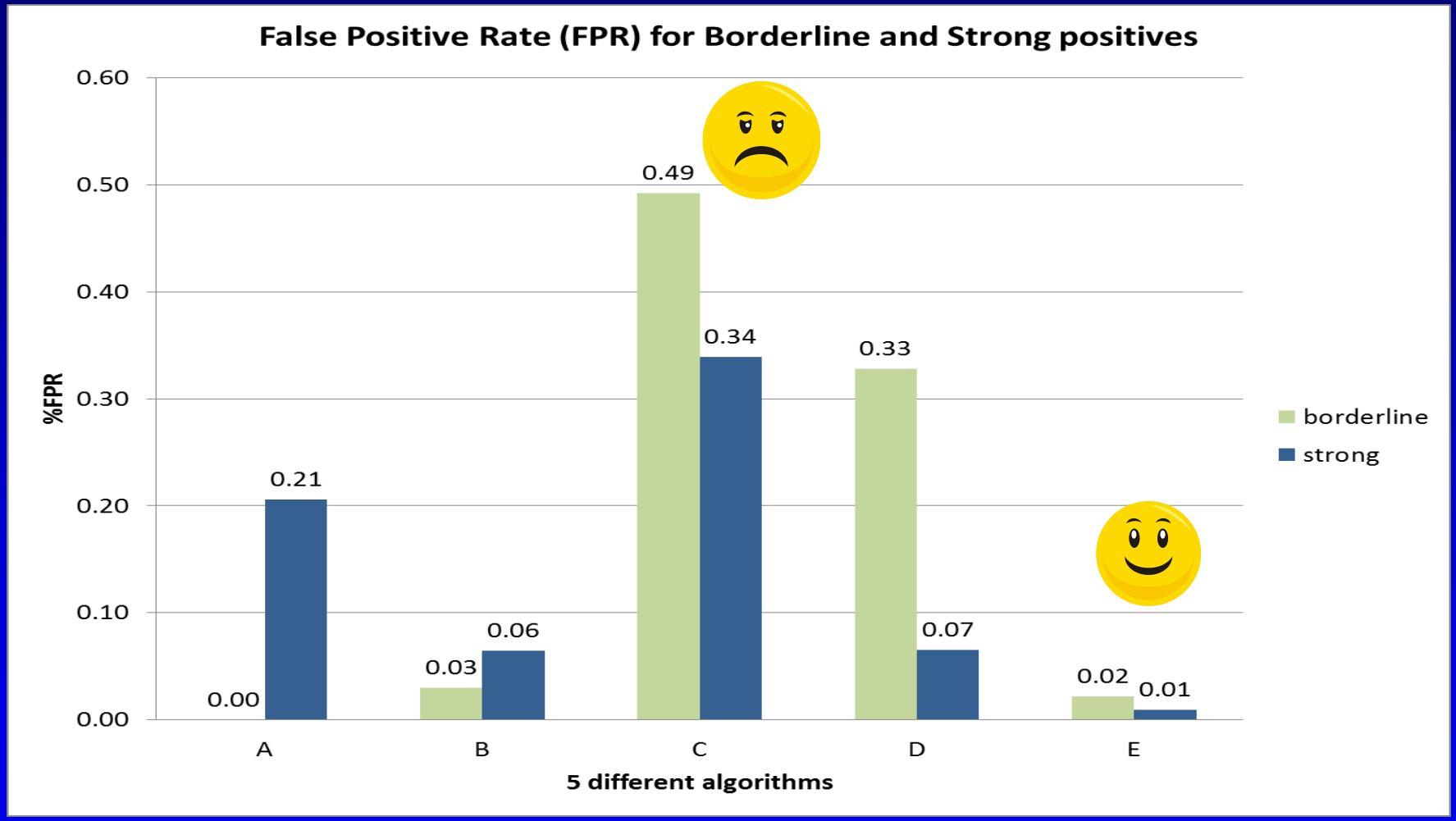
CT value	TREC quantity based on idealized standard curve
35.00	27
36.30	11
36.99	7
37.00	7

Time= ~17 months
4 – 5 cases per month
N= 178,682
Total PPV= 28.57% Total FPR= 0.03%

Positive Predictive Values



False Positive Rate

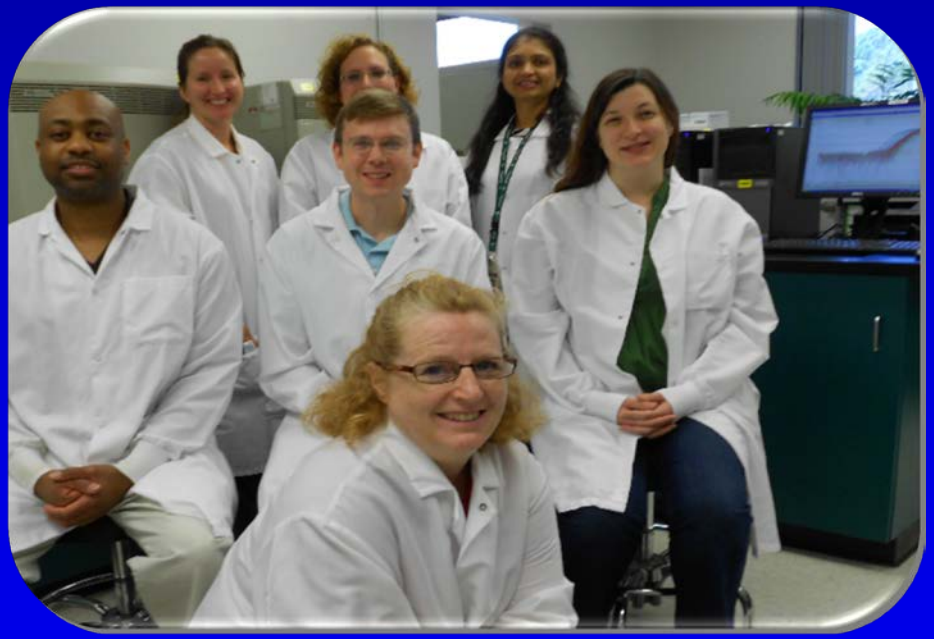


How Many Michigan Babies Have Been Screened For PID's?

- 326,041 Michigan babies have been screened from 10/1/2011 to 8/31/2014
- Using clinical categories from Region 4
  - 2 SCID
  - 3 Leaky SCID
  - 18 Syndromes with T cell impairment
  - 21 Non-preterm secondary T cell lymphopenia
  - 29 Lymphopenia

Acknowledgements

- Michigan Newborn Screening Program
- This work was partially funded by a research cooperative agreement from CDC (Grant # 01EH000936) and does not represent the official view of CDC.



TREC Plasmid Curve

	TREC Ct values							
	S8=5000	S7=2500	S6=1250	S5=625	S4=156	S3=78	S2=39	S1=10
N	1051	1058	1057	1060	1060	1047	1010	886
Mean	26.88	27.94	28.94	29.96	31.91	33.01	33.94	35.97
SD	0.41	0.41	0.44	0.43	0.46	0.48	0.51	0.69
-2SD	26.05	27.13	28.06	29.09	30.99	32.04	32.93	34.58
+2SD	27.70	28.76	29.83	30.83	32.83	33.98	34.96	37.35