

SMA screening in Utah: One year update

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- Cost benefit relationship
- Screening allows pre-symptomatic treatment

SMA/TREC Assay Method

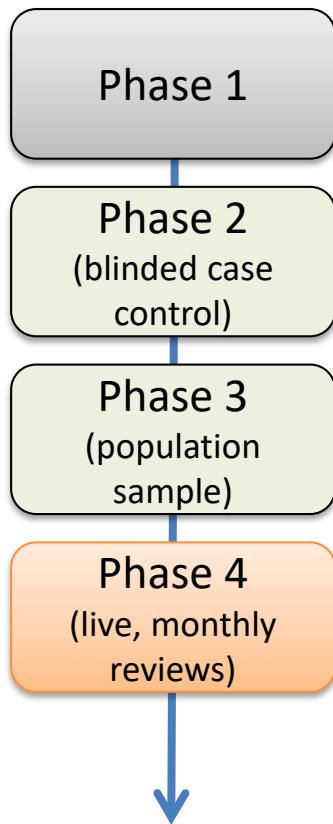
- PCR-Based Triplex Assay: modified CDC protocol
 - SMN1 – Deletion of exon 7 of SMN1 gene (SMA)
 - TREC – T-cell receptor excision circles (SCID)
 - RPP30 – Internal control
- Automated Extraction Tecan Evo 200
 - 2 step washing protocol
 - PBS/Tween 20 (room temperature)
 - Qiagen Solution 2 (room temperature)
 - Qiagen Solution 2 (70C) elution
 - 96 well to 384 well transition
- Real-Time PCR
 - Roche LightCycler 480 II
 - 384 well format

SMA/TREC Assay Results

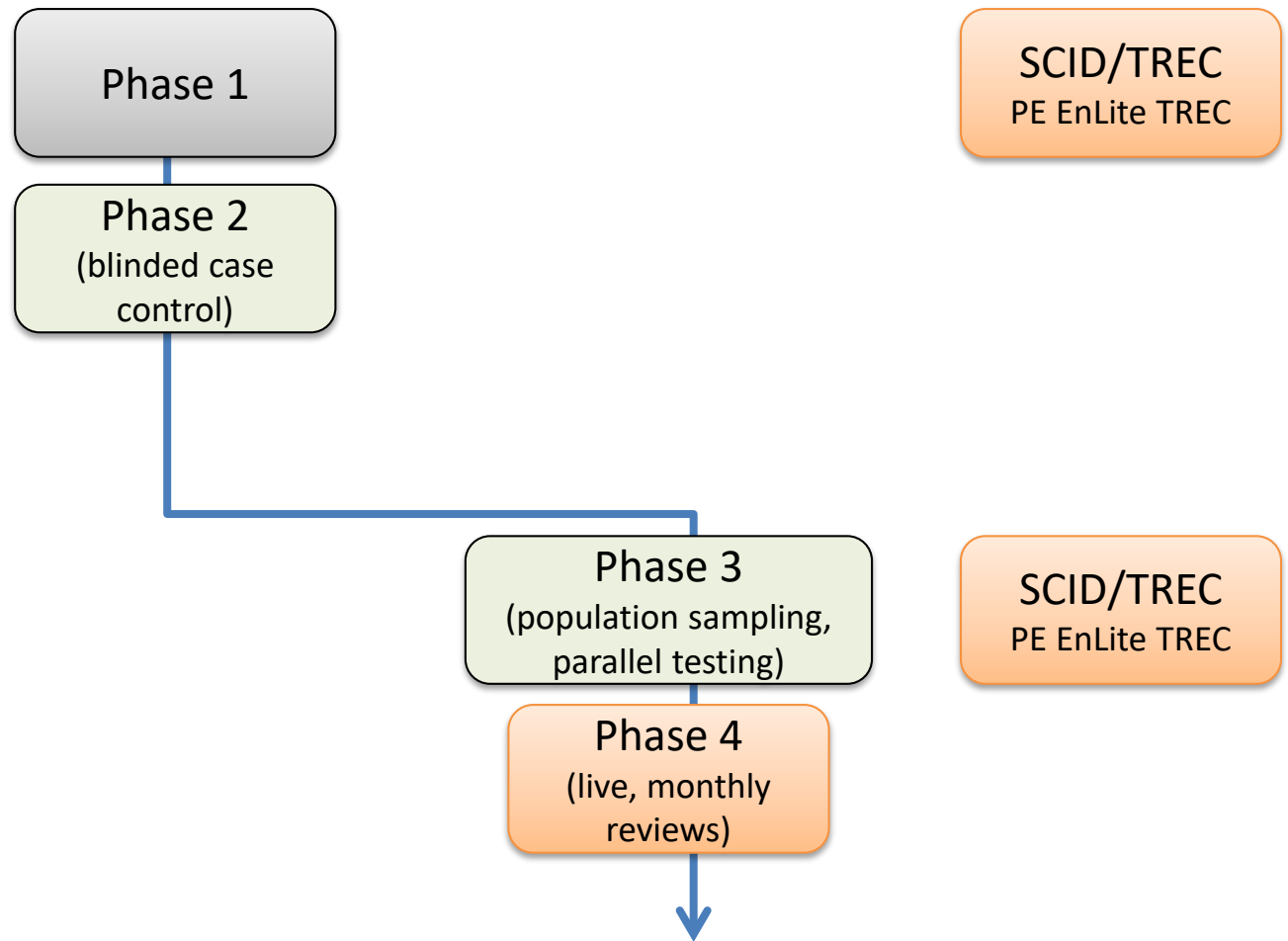
- Normal Control
 - Pooled known normal specimens
 - “Normal” control in DBS format
- Abnormal Controls
 - SMA like
 - SCID like

Validation Strategy

SMA



SCID/LDT



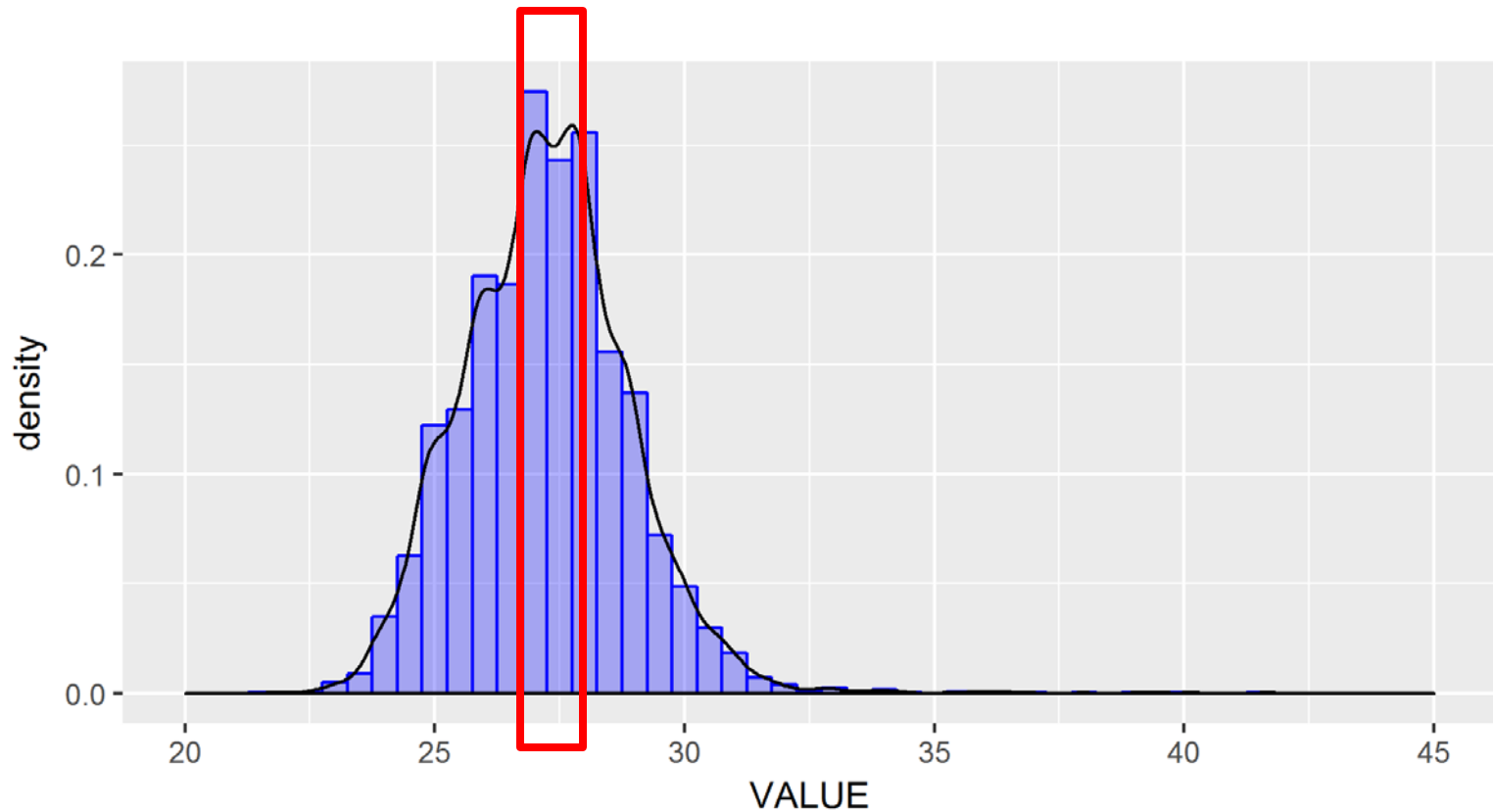
SMA: limited patient samples

Patient	Origin	SMN1 Cp	RNase P Cp	LDT Determination	Dx
1	Biogen	No Amp	27.64	Abnormal	SMA
2	Biogen	No Amp	26.41	Abnormal	SMA
3	Biogen	No Amp	27.61	Abnormal	SMA
4	Biogen	No Amp	28.91	Abnormal	SMA
5	Biogen	No Amp	28.45	Abnormal	SMA
6	Biogen	No Amp	28.67	Abnormal	SMA
7	Biogen	No Amp	29.82	Abnormal	SMA
8	Biogen	No Amp	29.67	Abnormal	SMA
9	Biogen	No Amp	27.91	Abnormal	SMA
10	Biogen	No Amp	28.85	Abnormal	SMA
11	Biogen	No Amp	29.55	Abnormal	SMA
12	Biogen	No Amp	28.12	Abnormal	SMA
13	Biogen	No Amp	29.92	Abnormal	SMA
14	Biogen	No Amp	28.89	Abnormal	SMA
15	Biogen	No Amp	27.28	Abnormal	SMA
16	CDC	No Amp	26.14	Abnormal	SMA
17	CDC	No Amp	27.85	Abnormal	SMA
18	Utah	No Amp	28.59	Abnormal	SMA
19	Utah	No Amp	29.08	Abnormal	SMA
20	Utah	No Amp	28.64	Abnormal	SMA
21	Utah	No Amp	28.55	Abnormal	SMA
22	Utah	No Amp	29.41	Abnormal	SMA
23	Utah	No Amp	29.82	Abnormal	SMA

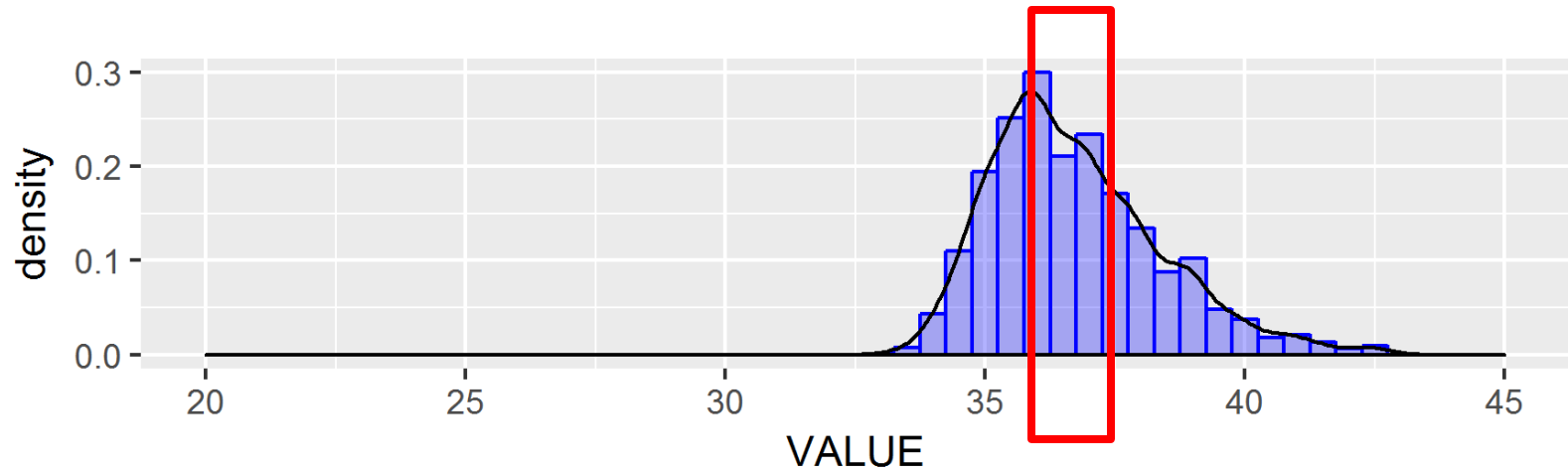
SCID Abnormals

Patient	TREC Cp	Z-Score	RNase P Cp	LDT Determination	Dx
1	No Amp	No Amp	28.97	Abnormal	Classic SCID
2	No Amp	No Amp	26.98	Abnormal	Classic SCID
3	No Amp	No Amp	30.34	Abnormal	SCID ADA
4	No Amp	No Amp	29.94	Abnormal	SCID ADA
5	No Amp	No Amp	29.94	Abnormal	DiGeorge Syndrome
6	No Amp	No Amp	30.21	Abnormal	DiGeorge Syndrome
7	No Amp	No Amp	33.13	Abnormal	Secondary T-cell Lymphopenia
8	No Amp	No Amp	31.37	Abnormal	Secondary T-cell Lymphopenia
9	No Amp	No Amp	28.86	Abnormal	Secondary T-cell Lymphopenia
10	No Amp	No Amp	26.54	Abnormal	Idiopathic T-cell lymphopenia asymptomatic
11	No Amp	No Amp	30.58	Abnormal	Variant T-cell lymphopenia
12	No Amp	No Amp	27.16	Abnormal	Microdeletion syndrome
13	40.8	2.30	29.35	Normal	Secondary T-cell Lymphopenia
14	41.39	2.66	31.61	Normal	Secondary T-cell Lymphopenia

SMN1 Population Analysis



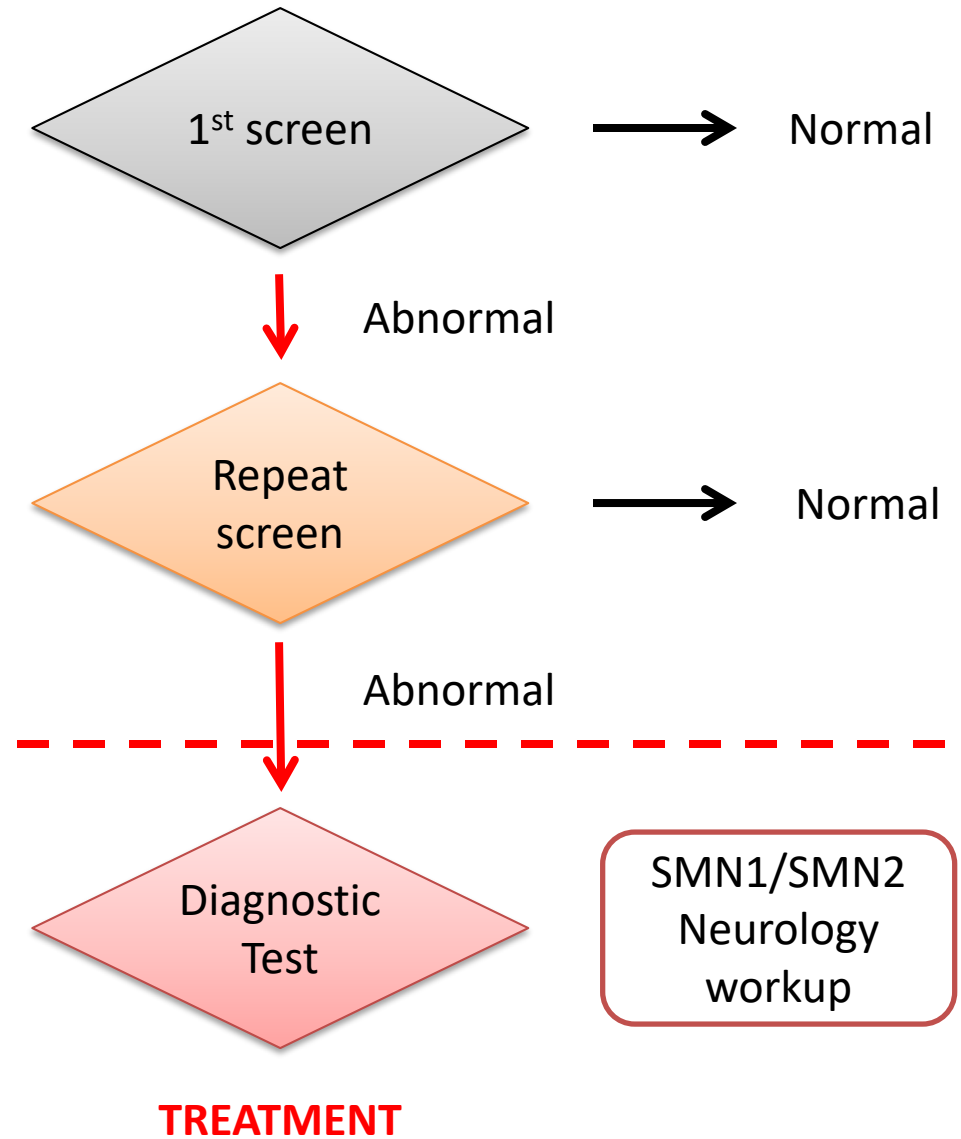
TREC Population Analysis



SMA/TREC Assay Cut-Offs

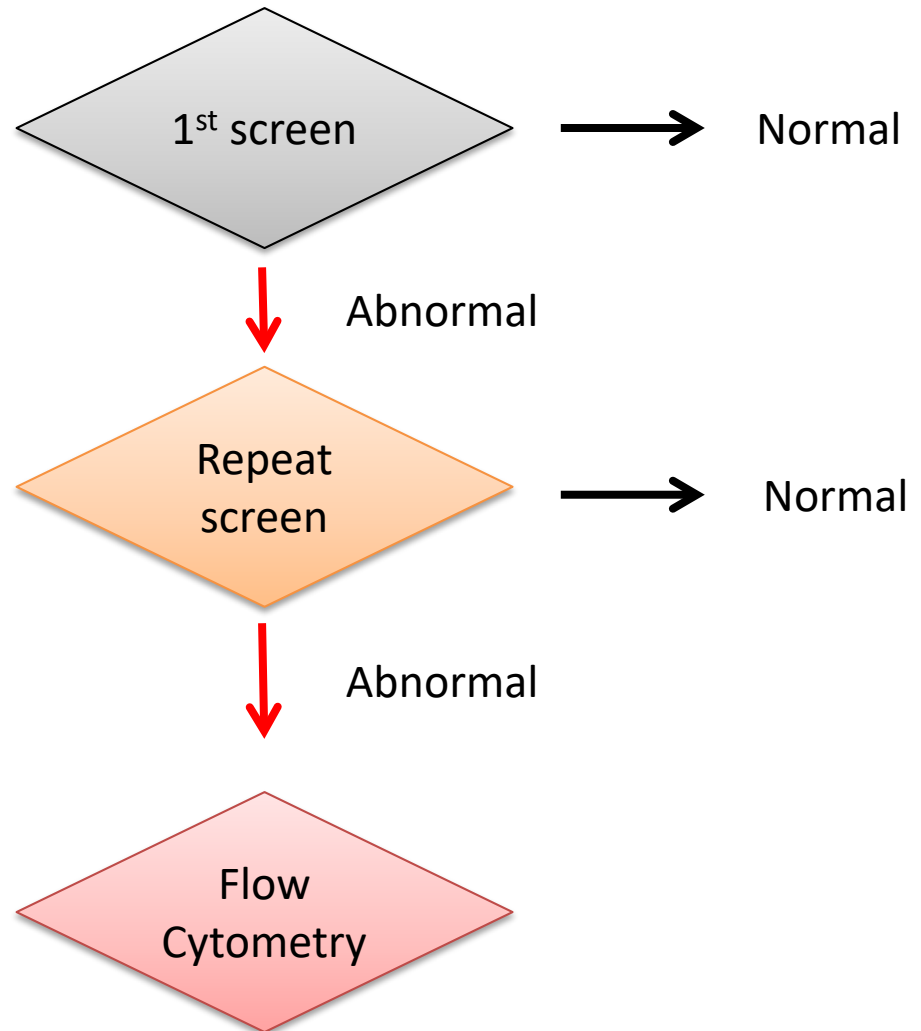
Analyte	Mean \pm SD	2 SD	99 th Percentile	3SD	99.5 th Percentile
SMN1	29.15 \pm 1.35	31.85	32.91	33.20	33.81
TREC	36.98 \pm 1.66	40.31	41.54	41.97	42.18
TREC cut-off	z = 2.8 (Cp equivalent 41.65)				
RPP30	29.71 \pm 1.39	32.49	32.99	33.88	34.14

SMA Screening and Diagnostic Workflow



SCID

Screening and Diagnostic Workflow





	Age at NBS report	Age at clinic evaluation	Age at confirmatory testing result	Confirmatory Result	Treatment type
Case 1	6 days	7 days	13 days	SMN1 = 0 SMN2 = 3	Gene therapy trial
Case 2	7 days	8 days	14 days	SMN1 = 0 SMN2 = 3	Gene therapy trial

Statistics

	n	Percent SMA
SMA	48,557	
repeat	466	0.96%

	n	Percent SCID	Percent SCIDC
SCID	22,525		
repeat	708	3.14%	

SMA: you have the deletion or you don't/binary

TREC as a SCID marker: continuous or quantitative trait phenotype



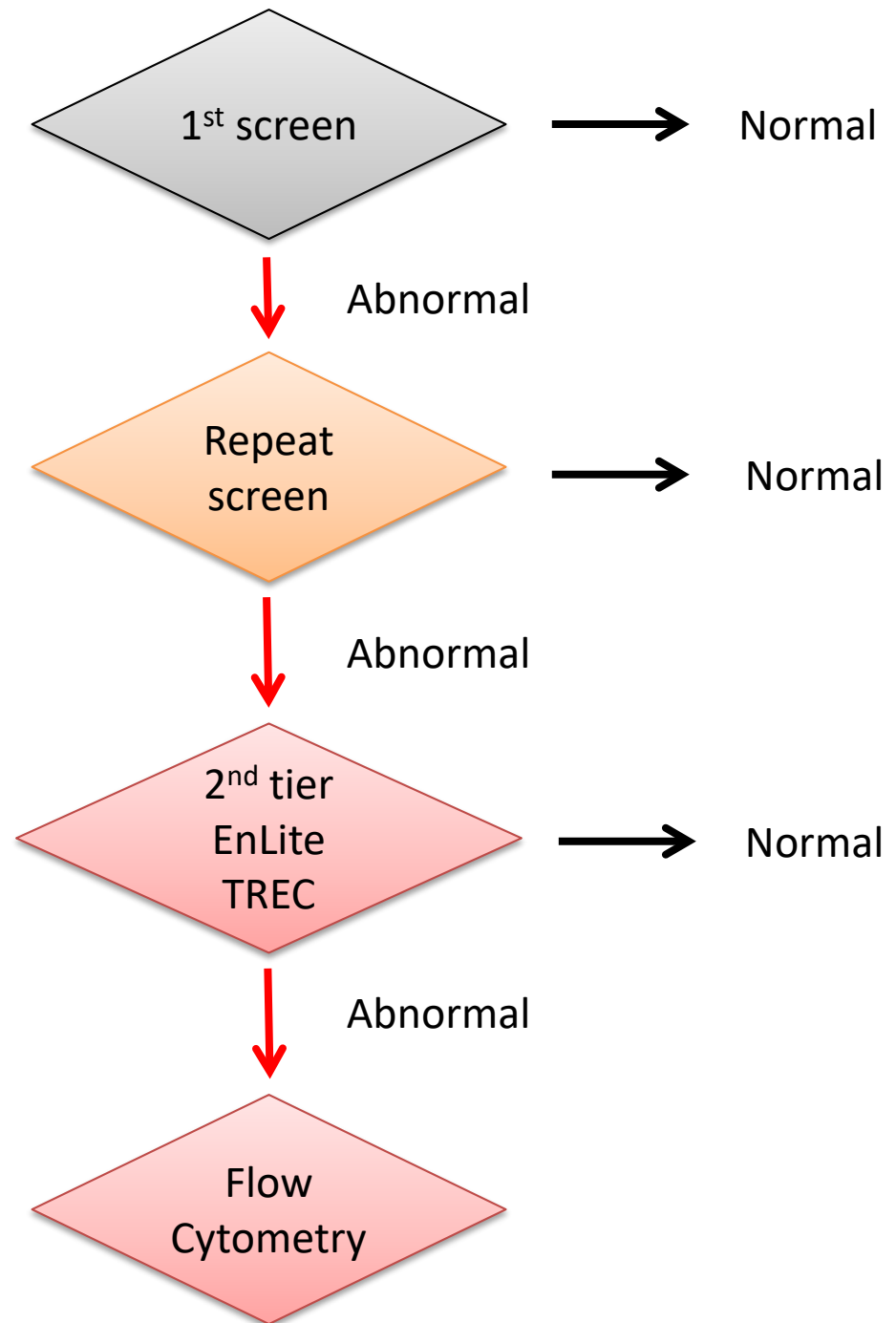
Problem: 2 false positive SCID cases

- *2 cases abnormal on 1st NBS; referred for flow cytometry; results normal/not consistent with SCID*
 - Retested SCID cases using EnLite TREC: Normal/low TREC levels
- Hypothesis: differential binding/elution kinetics TREC/gDNA

It is a Common Problem

Larger number of cases referred to
flow cytometry

SCID Screening and Diagnostic Workflow





Evie

Thank you

- Dr. Russ Butterfield and the Neurology team
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- cure SMA Foundation