Model Proficiency Evaluation Survey and NSQAP PT Program on T Cell Receptor Excision Circle (TREC) Assay for SCID

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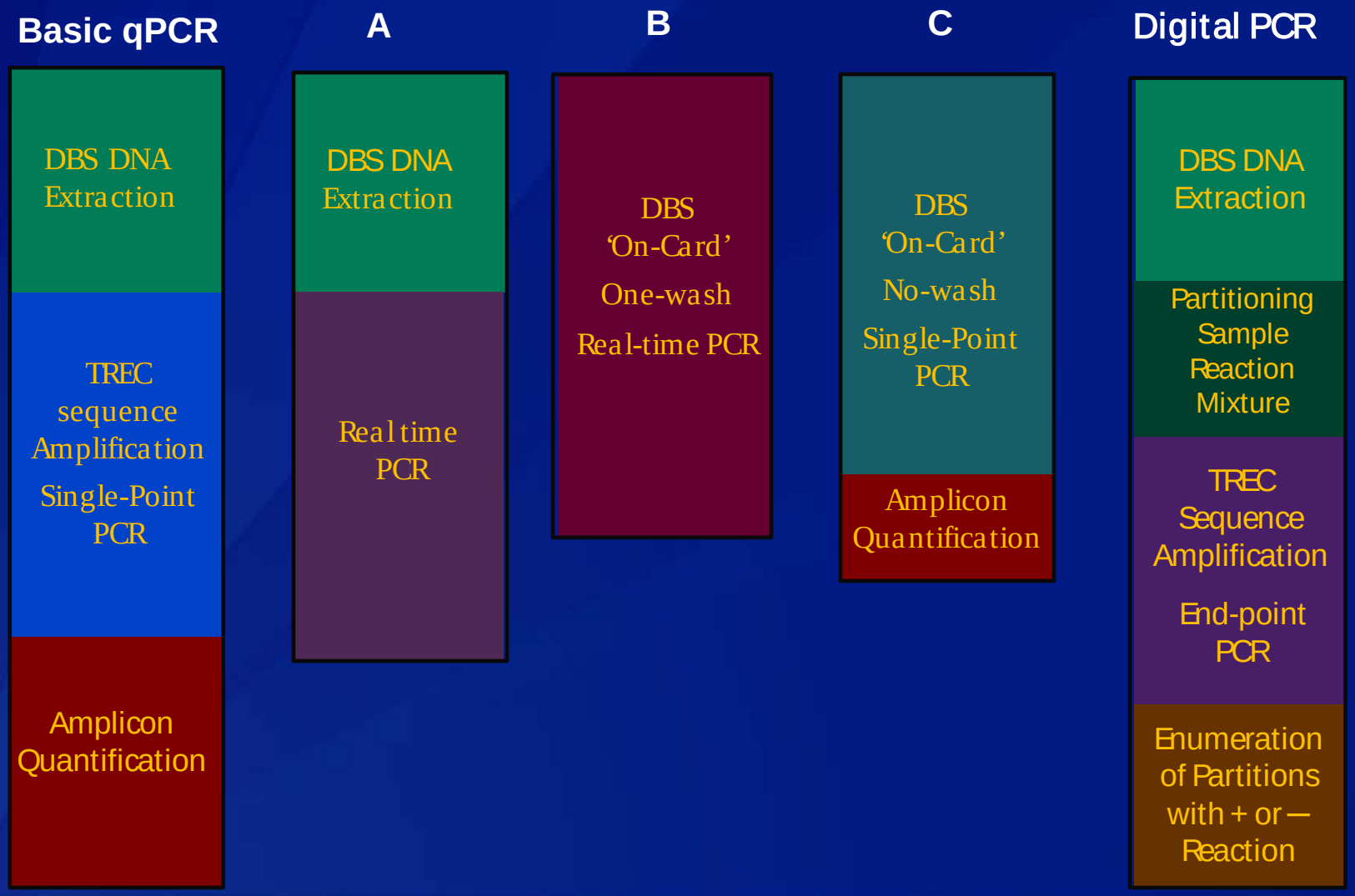
TREC Assay in Newborn Screening for SCID

- Laboratory-developed tests with limited standardization among labs
- Significant variations in major components of assay

TREC Quantitative PCR Assays



Other Variations in TREC Assay Protocols

- DNA Quantity

DNA Extract (from 3 mm punch)
Extraction Volume / Reaction Volume

DNA on DBS punch
2mm punch / 1.5 mm punch
(No wash/Wash 1x or 2x)

- Materials and Methods

Primers & Probes

Singleplex

Multiplex

96/384 well format

- Calibrators

Plasmids

Cell-based

Model Performance Evaluation Survey

- Started in February 2010 with three labs (WI, MA, CDC)
- 19 Laboratories currently participating
  - 10 PHL in routine population-based newborn screening for SCID
  - 9 labs in assay development or validation stages

TREC Model Performance Evaluation Survey Program (MPES)

Mission: To support state public health laboratories in

- Assay development and validation
- Accelerated proficiency testing
- Transition to NSQAP PT program
- Data harmonization

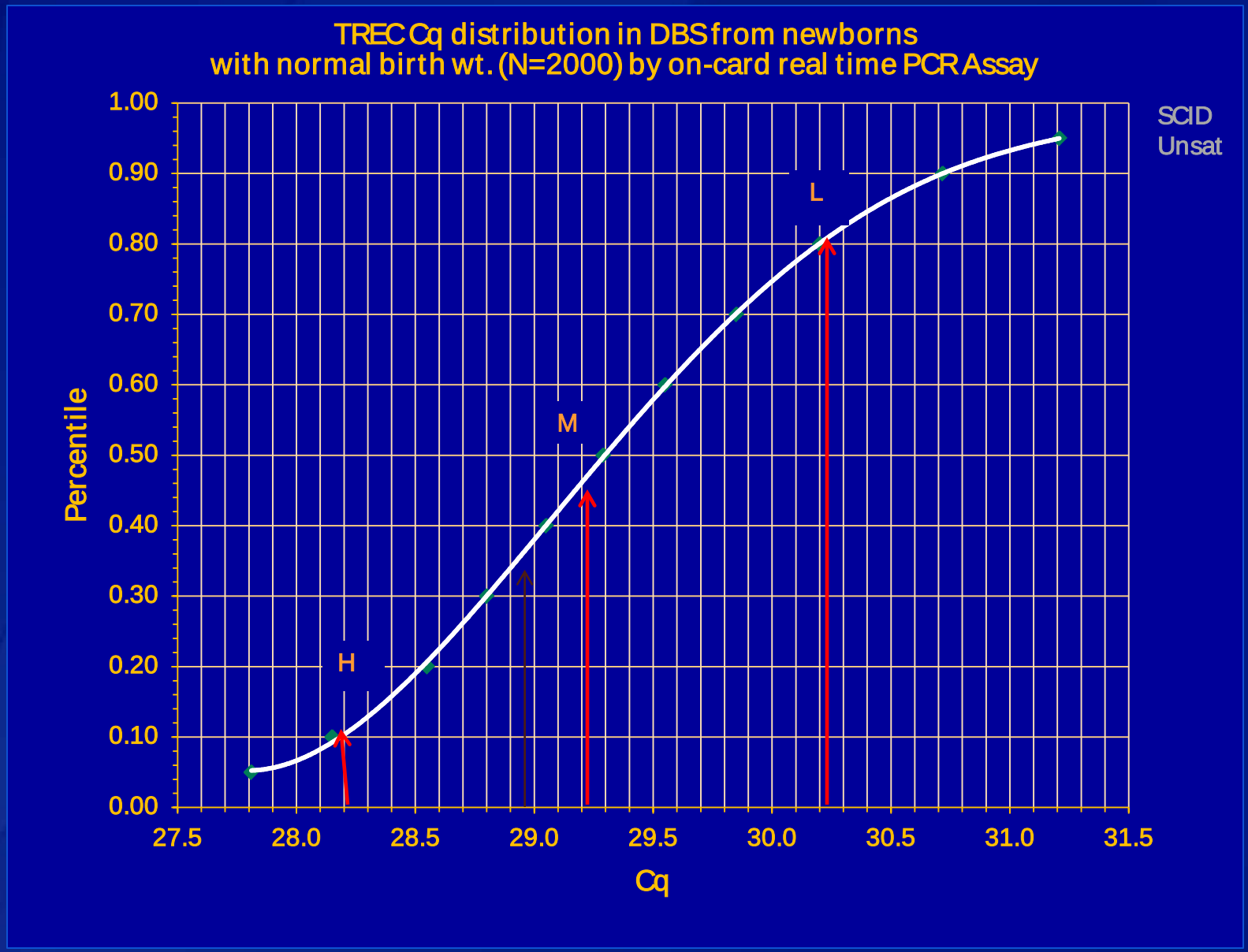
Supporting public health laboratories in

- Assay development and validation
  - Consultations on:
    - Physical laboratory layout and practices
    - TREC assay format selection
    - Instruments and reagents
    - Calibrators
    - Assay validation
  - Reference materials

CDC TREC Reference Materials

QC Materials

- SCID-like DBS: mononuclear cells – depleted blood (low/no TREC, normal reference gene level)
- "Unsat" DBS: leukocyte depleted blood (low/no TREC, low reference gene)
- Cord blood DBS: (TREC and reference genes in reference range)
  - High
  - Medium
  - Low


TREC Cq distribution in DBS from newborns
with normal birth wt. (N=2000) by on-card real time PCR Assay
SCID
Unsat
Percentile
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
27.5
28.0
28.5
29.0
29.5
30.0
30.5
31.0
31.5
Cq
H
M
L

Special Reference Materials for TREC Assay evaluation

Serial Dilutions of Cord Blood

- Begin at above median level of expected range for TREC
- Diluted into MNC-depleted blood

  (diluent *w* No detectable TREC; normal level of reference genes)
- 100%, 50%, 25%, 12.5%, 6.2%, 3.1%

Potential use: Assay developement; LOD/LOQ studies;

Calibration comparison; 'Cut-off' placement

Model Performance Evaluation Survey

an accelerated pilot PT program

- Panel sent out at 4-6 week interval
- Five well-characterized DBS with prior consensus categorization for proficiency assessment
- Additional ‘non-scoring’ DBS included for technical or harmonization studies
- All samples blinded
- Reports submitted by participants within 3 weeks

MPES Report Form

Lab # ___	TREC			Final Categorical Result				Reference Gene: ___			Comments
	Cq Value	Copy Number		No F/U	F/U action required			Cq Value	Copy Number		
Sample ID		per Rxn	per µL Bld	TREC NL	TREC ↓	Ref gene NL	Ref gene ↓		per Rxn	per µL Bld	
A											
B											
C											
D											
E											
F											
G											
		Cutoff			*If TREC↓ selected, indicate reference gene category*				Cutoff		

Sample Report from MPES Labs

Lab #300	TREC			Final Categorical Result				Reference Gene: RNase P			
	Cq Value	Copy Number		No F/U	F/U action required			Cq Value	Copy Number		Comments
Sample ID		per Rxn	per µL Bld	TREC NL	TREC ↓	Ref gene NL	Ref gene ↓		per Rxn	per µL Bld	
A	35.1	5	5		√	√		23.7			SCID-like
B	29.7	132	132	√		√		23.9			Normal
C	No Ct	0	0		√		√	30.5			Unsat
D	31.5	46	46	√		√		25.6			Normal
E	37.0	1	1		√	√		23.8			SCID-like
F	29.3	180	180	√		√		24.2			Normal
G	33.7	12	12		√	√		23.5			SCID-like
		Cutoff	*25*		*If TREC↓selected, indicate reference gene category*			27.5	Cutoff		

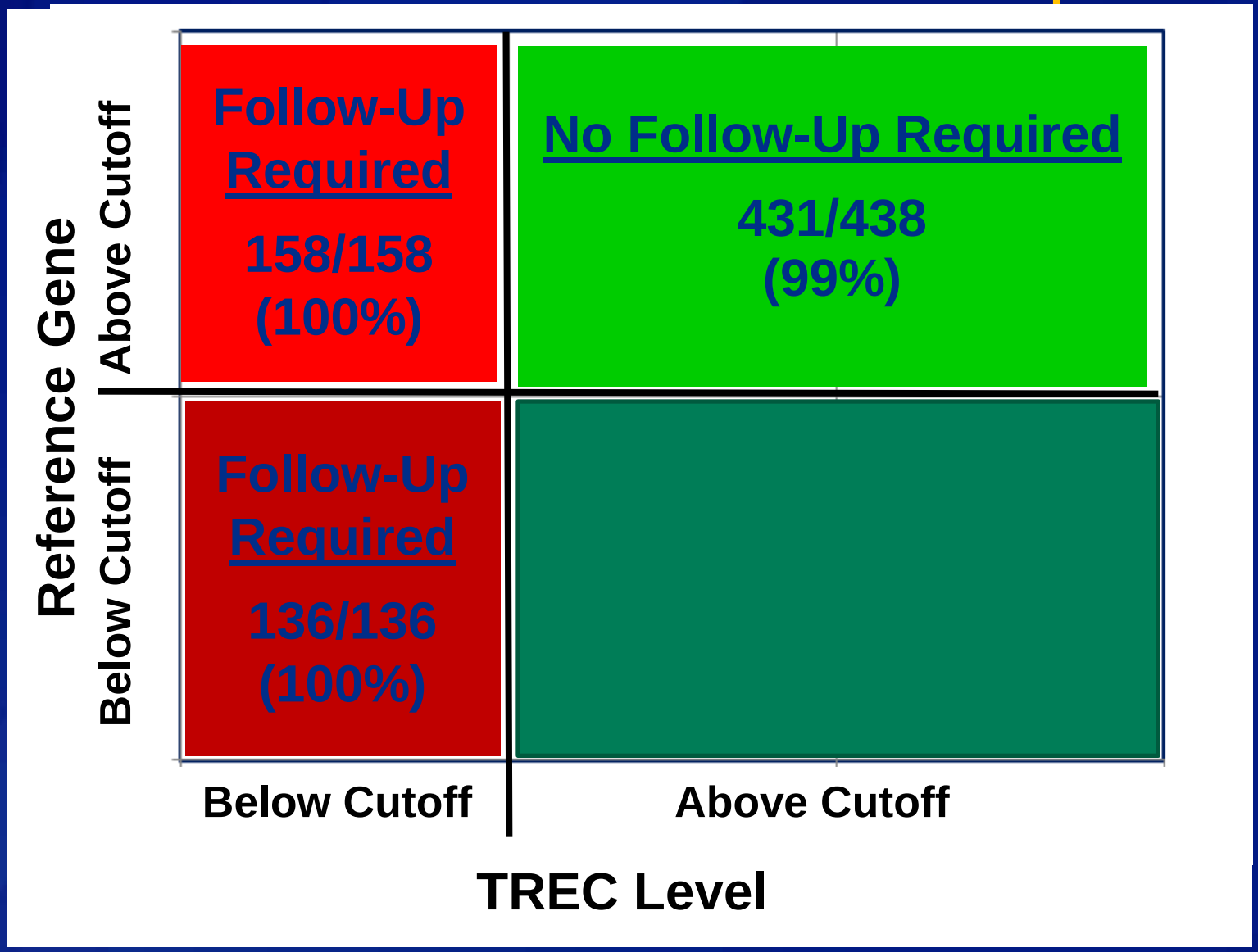
Lab #999	TREC			Final Categorical Result				Reference Gene: RNase P			
	Cq Value	Copy Number		No F/U	F/U action required			Cq Value	Copy Number		Comments
Sample ID		per Rxn	per µL Bld	TREC NL	TREC ↓	Ref gene NL	Ref gene ↓		per Rxn	per µL Bld	
A	Undeterm.	0	0		√	√		26.4	2144	13833	in report. range
B	32.7	146	943	√		√		26.7	3559	22960	in ref. range
C	Undeterm.	0	0		√		√	33.5	39	250	Unsat
D	34.6	46	296	√		√		27.7	2101	13554	in ref. range
E	Undeterm.	0	0		√	√		26.8	4095	26418	in report. range
F	32.3	195	1261	√		√		27.7	1754	11313	in ref. range
G	Undeterm.	0	0		√	√		26.8	3997	25785	in report. range
		Cutoff	*200*		*If TREC↓selected, indicate reference gene category*				Cutoff	*5000*	

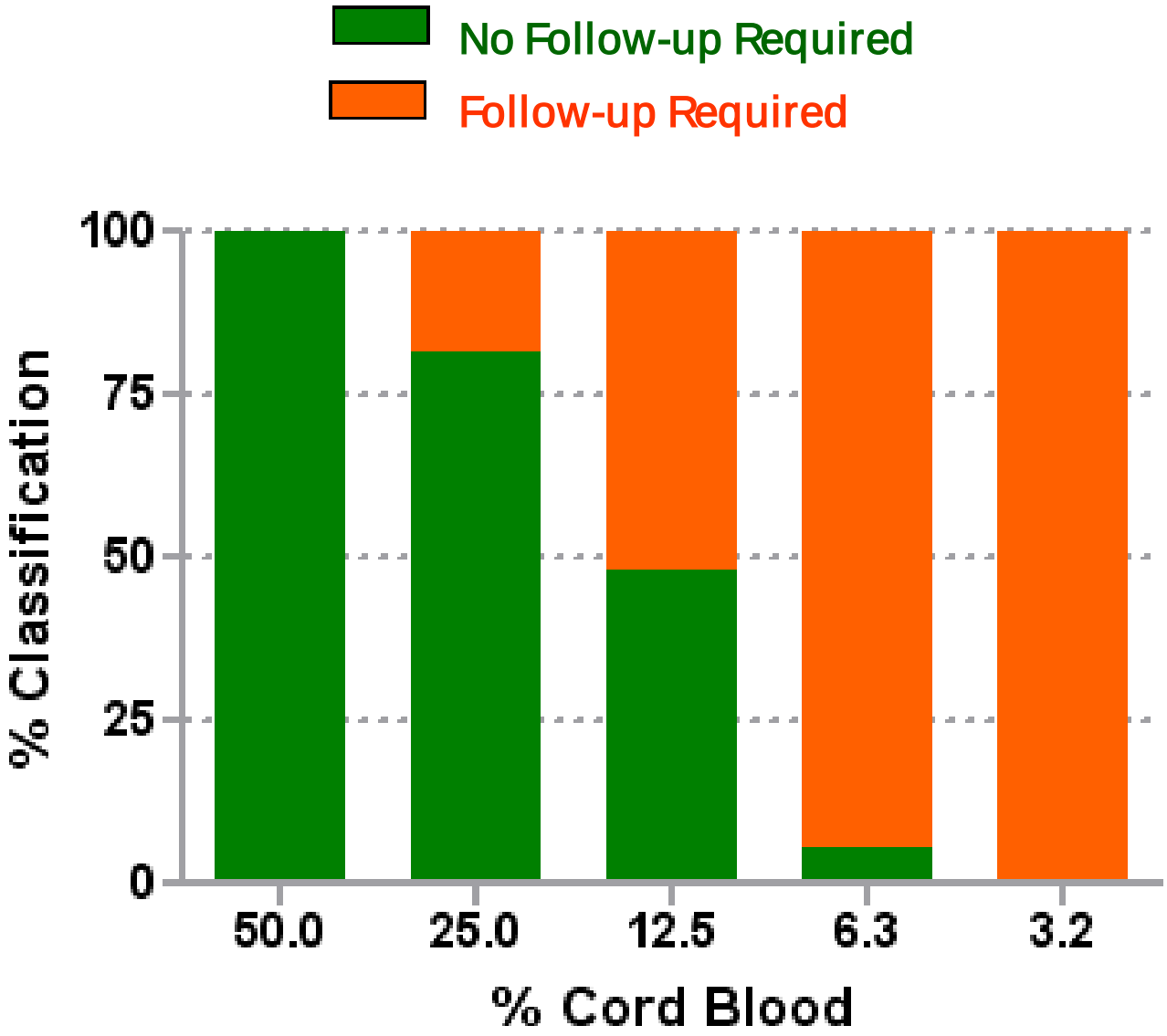
Sample CDC Report - Summary of Results

MPES#22

Sample ID	Sample Code	No F/U	F/U required		
		TREC NL	TREC ↓	Ref gene NL	Ref gene ↓
High Normal	E	9			
Low Normal	A	9			
SCID -like	F		9	9	
Leuko-depleted	B		9		9
CB-cal 5 (12.5%)	C	4	5	3	2
CB-cal 6 (6.3%)	H		9	5	4
CB-cal 7 (3.1%)	D		9	6	3

Cumulative PT Results from 17 MPES Sample Panels




No Follow-up Required
Follow-up Required
100
75
50
25
0
% Classification
50.0
25.0
12.5
6.3
3.2
% Cord Blood

NSQAP TREC Assay PT Program

- Currently restricts enrollment to domestic laboratories performing routine population-based SCID screening
- Quarterly panel of five DBS samples
- Report categorical results (f/u rquired or not required) only
- 10 labs currently enrolled

TREC Model Performance Evaluation Survey Program (MPES)

Data harmonization for result comparison

Development of consensus cell-based calibrators currently underway

Discussion

- Despite differences in assay format and reagents, all participating laboratories consistently identified samples with SCID-like phenotype correctly
- Results on the cord blood dilution series indicated good agreement on F/U requirement for samples across a full range of TREC levels, even as the absolute TREC copy numbers detected vary among laboratories.
- UCSF / MA NBS program has developed a TREC-transfected B-cell line currently under evaluation
- Consensus calibration for TREC in DBS will evolve quickly and may be achieved in the near future

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