

Information		State				
			CA	NY	PR	LA
		Laboratory performing screen	CA	NY	MA	WI
Primary Screening Assay		TREC singleplex or TREC multiplex	Trec singleplex	TREC multiplex	TREC multiplex	TREC singleplex
Detection		real-time , endpoint or N/A (other)	real-time quantitative	real-time quantitative	real-time quantitative	real-time quantitative
TREC assay reference gene		actin , RNaseP or N/A	actin	RNaseP	RNaseP	actin
TREC FDA status		laboratory developed test; in vitro diagnostic device, research use only	RUO	LDT	LDT	LDT
INSTRUMENTATION for TREC ASSAY		AB 7900HT Fast Real-Time System, Lightcycler 400, Stratagene, other?	Roche LightCycler 480	ABI7900 FAST REAL TIME SYSTEM	ABI7900 FAST REAL TIME SYSTEM	ABI7900 FAST REAL TIME SYSTEM
Plate format for detection		384, 96	384	384	384	96
Automation_DNA extraction		Yes or No	yes	yes	yes	no
Automation_PCR set-up		Yes or No	Perkin-Elmer Janus	Biomek NX	MULTIDROP	epMotion5075

General TREC Assay information						
DBS punching size		3.2 mm, 1.5 mm	3.2	3.2	3.2	3.2
DNA template source		in situ= washed DBS, DNA extract	in situ/washed	DNA extract	DNA extract	DNA extract
reagents used for DNA extraction		lab-developed, generations, mix of both	Qiagen Generations	Lab developed	Lab developed	Qiagen Generations
DNA elution volume		24 uL, 30ul, 40 ul, 100 ul, N/A	30ul	100ul	100ul	24ul
volume of DNA per assay		2 ul, 5 ul, etc, N/A	10ul	2ul	5ul	8ul
total PCR reaction volume		10 ul, 20 ul	20ul	10ul	20ul	20ul
TREC primer sequences		citation or specify sequence	F:5' ACACCTCTGGTTT TTGTA A3' R:5' CAGCTGCAGGGT TTAG3'	F:5'TGACACCTCT GGT TTTTGTAAA GG3' R:5'TGCAGGTGC CTATGCATCA3'	F:5'TGCTGACAC CTCTGGTTTTTGT AA3' R:5'GTGCCAGCT GCAGGGTTTAG3 '	F:5'CACATCCCTTTC AACCAGCT3' R:5'GCCAGCTGCAG GGT TTAGG3'
TREC probe sequences		citation or specify sequence	5' TAGGCACCTGCAC CCC 3'	5'-FAM- CCCACTCCTGTG CACG-NFQ3'	5'-FAM- ATGCATAGGCAC CTGC-MGB3'	5'-FAM- ACACCTCTGGTTTTT GTAAAGGTGCCAC T3'-TAMRA
reference gene sequence primers/probe		citation or specify sequence	F: 5' ATGGTGTATCTCT GCCTT 3' R:5' GTCACCGGAGTCC ATC 3' 5' TACGTTGCTATCC AGGCTG3'	TaqMan RNaseP Vic Control Reagent (AB4316844)	TaqMan RNaseP Vic Control Reagent (AB4316844)	F:5'-ATTTCCCTCTC A GGCATGGA-3' R:5'-CGTCACACT TCATGATGGAGTTG 3' 5'-VIC- GTGGCA TCC ACGAAA CTA3TAMRA

Assay Calibrators_TREC		TREC_plasmids, TREC_Hela cells, etc	TREC_plasmids	TREC_plasmids, Dr. Douek	TREC_plasmids, Dr. Douek	TREC_plasmids, Dr. Douek
Assay Calibrators_reference gene		Actin_plasmids, etc.	Actin_plasmids	RNaseP_human DNA	RNaseP_human DNA	Actin_plasmid
TREC Assay Controls						
Number of Normal controls per 96 wells			2	3	0 due to internal controls	1
Type of Controls	Infant DBS, spiked adult blood, etc.)		pooled cord blood	Infant DBS, spiked adult blood	sample failure rate monitored	cord blood from infants
Number of SCID patient or SCID-like controls per 96 wells			2	3	1 [2*]	1 [1]
Type of SCID patient or SCID-like controls	Adult, lymphocyte depleted, RBC only, other?		Adult, lymphocyte depleted	Lymphocyte depleted and/or confirmed SCID specimens	1 blank filter paper punch; *2 per 384-well plate pre-extracted DNA from SCID infant or lymphocyte-depleted blood	1 SCID-like sample from elder adults AND [1 SCID infant per week]
Number of no target controls per 96 wells			2	2	1 per amplification	1

Type of "no target" controls	Blank DBS? No DBS? Water? Buffer?, other?		punched filter paper with DNA extraction and no DBS (water)	punched filter paper with DNA extraction and no DBS (water)	no DNA e.g. water	punched filter paper with DNA extraction
PCR Cycle Detail			1 cycle at 95 °C for 10 min, 50 cycles at 95 °C for 10 sec and 56 °C for 1 min	1 cycle of 10 mins at 95 degree C, and 50 cycles of 15 secs at 95 degree C and 60 secs at 60 degree C.	1 cycle of 10 mins at 95 degree C, and 40 cycles of 15 secs at 95 degree C and 30 secs at 60 degree C.	1 cycle of 2 mins at 50 degree C, 1 cycle of 5 mins at 95 degree C, and 40 cycles of 30 secs at 95 degree C and 60 secs at 60 degree C.
REPORTING						
TREC cutoff: Full-term infants	Value below which an out of range result is reported; copies/μL whole blood		25 copies/ul blood	150 copies/ul blood repeat request; 200 copies/ul blood on second specimen	252 copies/ul blood	25 copies/ul blood screen positive; 25-39 copies/ul blood possible screen positive--repeat request

TREC cutoff: Pre-term infants	Value below which an out of range result is reported; copies/μL whole blood		25 copies/ul blood	200 copies/ul blood	252 copies/ul blood	25 copies/ul blood
reference sequence cutoff	Value below which an out of range result is reported; copies/μL whole blood or Cq		10,000 copies/ul blood	>35 Cq	4,062 copies/ul blood	10,000 copies/ul blood
Laboratory screening algorithm						

