Confirmatory Testing Following a Positive SCID Newborn Screen

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Following up an abnormal SCID newborn screen

- Assumptions:
  - The level of TRECs is below the laboratory-specific cutoff
  - Quality control indicates a valid result
- Secondary screen
  - Total T cell number
  - Proportion of naïve T cells

Secondary screen: T cell number

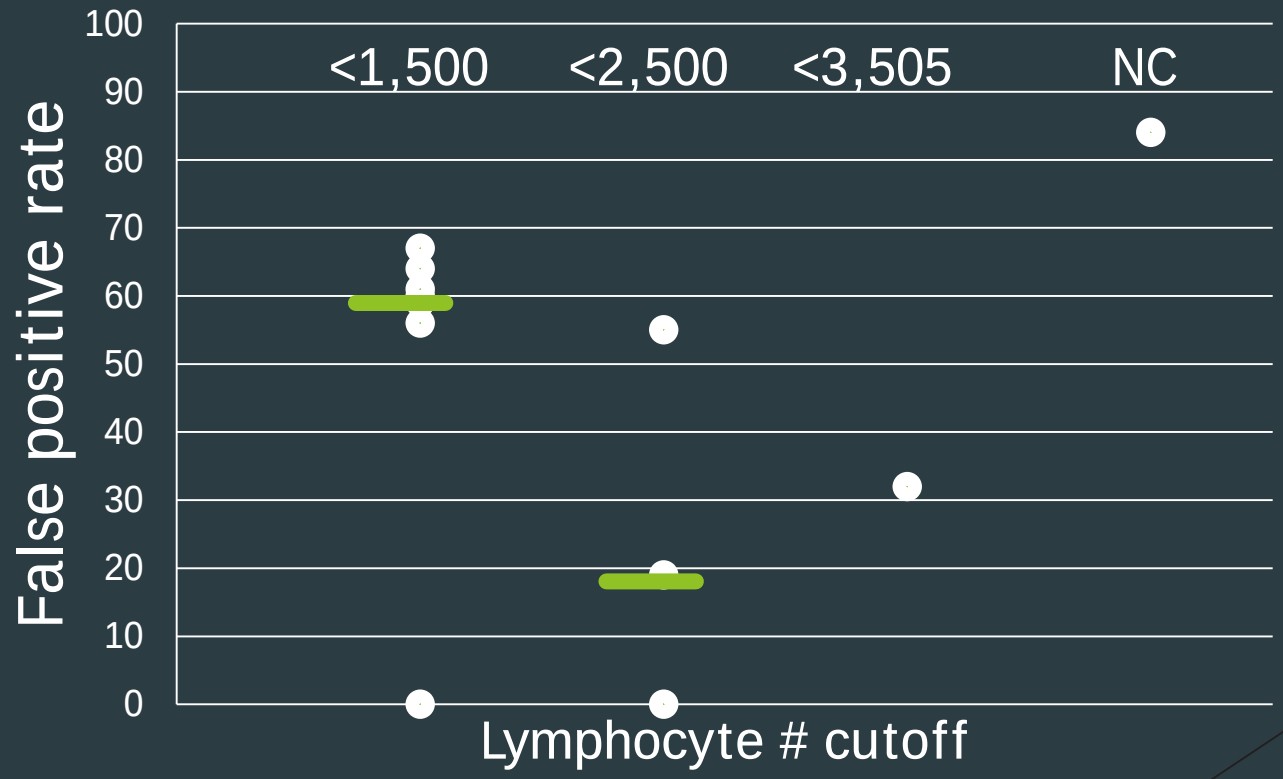
- No well-established normal ranges of absolute counts for first 1-7 days of life for term or premies

Shearer et al.	Comans-Bitter et al	
0-3 months, n = 700	Newborn, n = 20	2-5 months, n = 46
90th %ile - 5,500	95th %ile – 5,000	6,500
Median - 3,700	2,800	3,600
10th %ile - 2,500	5th %ile – 600	2,300

  - Shearer et al., J Allergy Clin Immunol 2003; 112:973
  - Comans-Bitter et al., J Pediatr 1997; 130:388

- State laboratory-specific lower limits vary between 1,500-3,505 cells/mm^3

Secondary screen: T cell number



Data from Kwan et al., JAMA 2014; 312:729

Secondary screen: Naïve T cells

- Spurious normal total T cell count due to:
  - Engraftment and expansion of maternal T cells
  - Autologous oligo/pauciclonal T cell expansion (Omenn's syndrome)
- In both cases, engrafted or expanded cells are not naïve
- Naïve cells $CD45RA^{+}$ or $CD45RO^{-}$
- Additional markers sometimes used but not essential here
- CD4 and CD8 populations may be measured separately, also not essential

Secondary screen: Naïve T cells

CD4/CD45RA	CD8/CD45RA
90th %ile - 95%	99%
Median - 90%	93%
10th %ile - 64%	80%

- Shearer et al., J Allergy Clin Immunol 2003; 112:973

- Convenient lower limit – 50%
- Not applied in all screening programs

Tertiary screen: T cell function

- Assumptions
  - T cell lymphopenia by laboratory-specific criteria
  - OR
  - Low proportion of naïve T cells, if applied

- Refer for testing of T cell function (preferably with clinical consultation)
- Gold standard: in vitro T cell proliferation to stimulation with phytohemagglutinin (PHA) by incorporation of ^{3}H
- Clinical consultation should be considered for any significant abnormality of lymphocyte populations, even if T cell lymphopenia is not present

SCID
PIDTC definitions

- Primary Immunodeficiency Treatment Consortium
- Typical SCID
  - Autologous T cells <300 cells/mm^3 AND
  - T cell proliferation <10% of a healthy control (or <10^{th} %ile of controls for the lab)
    - Maternal T cell engraftment
    - Known SCID-associated mutations
- "Leaky" SCID
  - Autologous T cells between 300-1,500 cells/mm^3, low proportion of naïve cells
  - T cell proliferation between 10-50% of normal
    - Incomplete/hypomorphic mutations in SCID genes

SCID
PIDTC definitions

- Omenn syndrome
  - T cell number low or normal, oligoclonal
  - T cell proliferation 10-50% of normal
    - Erythroderma,hepatosplenomegaly,eosinophilia, and elevated levels of serum IgE
- Generally occurs with SCID gene mutations but can be seen in other settings

Outcomes of SCID newborn screening

- T cells above cutoff, naïve proportion OK, no other lymphocyte abnormalities > no further evaluation
- T cells above cutoff, naïve proportion OK, yes other lymphocyte abnormalities
  - Follow clinically, consider further evaluation
- T cells below cutoff or naïve proportion not OK, function >50% normal
  - Follow clinically, consider further evaluation (DGS, T21)
- T cells below cutoff or naïve proportion not OK, function <50%
  - Evaluate for leaky SCID or other defect
- T cells <300 or naïve proportion not OK, function <10%
  - **SCID!**

Non-SCID T cell lymphopenia

- Syndromes with T-cell impairment 136
  - DiGeorge 78
  - Trisomy21 21
  - A-T 4
  - Trisomy18 4
  - CHARGE 3
  - Jacobsen 2
  - CLOVES 1
  - ECC 1
  - Fryns 1
  - Nijmegen 1
  - Noonan 1
  - Rac2 defect 1
  - Renpenning 1
  - TAR 1
  - Not specified 10
  - Cytogenetic 6
- Secondary 117
  - Cardiac anom. 30
  - Mult. Congen. 23
  - 3rd space 15
  - GI anom. 15
  - Neonatal leuk. 4
  - Not specified 30
- Preterm birth 29
- Variant SCID 12
- Unspecified T-cell lymphopenia 117

Data from Kwan et al., JAMA 2014; 312:729

Non-SCID T cell lymphopenia: Testing

- Karyotype
  - Major chromosomal abnormalities
  - Trisomy 21
  - Trisomy 18, etc.
- SNP or hybridization array
  - DiGeorge syndrome (focused analysis on 22q11)
  - Other microdeletion
  - Often useful in conjunction with targeted or whole exome/genome sequencing

Non-SCID T cell lymphopenia

- Ataxia-telangiectasia
  - Characteristic manifestations do not appear for months/years
  - Screening via alpha fetoprotein unreliable in first year of life
  - Consider for testing (chromosome fragility, Western blot or DNA analysis) in all possible cases, especially if there is also B cell lymphopenia
- CHARGE
  - *CCHD7*
- Nijmegen breakage syndrome
  - *NBS1*
- Rac2 deficiency
  - *RAC2*
- Other focused biochemical and/or genetic tests based on clinical features and laboratory phenotype

SCID/leaky SCID: Gene defects

T^-B^+ SCID	
IL-2R common gamma chain	IL2RG
Janus kinase 3	JAK3
IL-7R α chain	IL7RA
IL-2R alpha chain (CD25) deficiency	IL2RA
CD45 (protein tyrosine phosphatase, receptor type, C)	PTPRC
CD3 δ	CD3D
CD3 ε	CD3E
CD3 ζ	CD3Z
Coronin 1A	CORO1A

T^-B^- SCID	
Recombinase activating genes 1 and 2	RAG1 RAG2
DNA cross-link repair enzyme 1C (Artemis)	DCLRE1C
DNA-dependent protein kinase	PRKDC
Adenylate kinase 2 (reticular dysgenesis)	AK2
Adenosine deaminase	ADA
DNA ligase IV	LIG4
Non-homologous end-joining protein 1 (Cernunnos)	NHEJ1

SCID/leaky SCID: Genetic testing

- Next generation sequencing panels available from reference genetics laboratories
  - All SCID or segregate by T-B+, T-B-
  - Focused Sanger sequencing of a small number of candidate genes
- If all known genes and CGH/SNP array tests are unrevealing, consider whole exome or genome sequencing