Newborn Screening for Severe Combined Immunodeficiency (SCID)

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SCID Newborn Screening Implementation in the US

- **Y-axis**: No. of States, District & Territories
- **X-axis**: Year (2008 to Nov-16)

- **Graph Details**:
  - Green bars represent the number of newborn screening programs.
  - Red line represents the percentage of US newborns.
  - By Nov-16, 100% of US newborns were being screened.

Legend:
- # Newborn Screening Programs
- % US newborns
Screening for SCID (85% of all US newborns)

- At planning & procurement stage
- At assay validation stage
- Others

November, 2016 Newborn Screening Status for SCID – US States and Territories
What is SCID?

- A heterogeneous group of inherited disorders caused by single gene defects resulting in a combined immune deficiency
- Prevalence: ~ 1:50,000
- All have profound defects in T lymphocyte differentiation and function
- Some (not all) have defects in B cell and/or NK cell
- **End result**: patients can’t make antibodies, fight viral, bacterial, fungal or opportunistic infections

- Over 25 different genes have been identified: hundreds of mutation sites implicated
* No molecular defect in known SCID associated genes
SCID: Clinical presentation

- Infections start in first 3 - 6 months of life
- Opportunistic infections
  - *pneumocystis, cytomegalovirus*
- Persistent yeast - esophagitis
- Chronic diarrhea - rotavirus, giardiasis
- Red Scaly rash - erythroderma
- Failure to thrive/weight loss
- Sepsis - gram negatives
- Death usually before 1 year of age
SCID: Laboratory findings

- Nearly all forms have severe lymphopenia
  - Total lymphocyte counts $<<$ 2 SD below normal
- All forms have low CD3+ cells in FLOW cytometry
- *in vitro* tests of T cell function abnormal
- Serum Immunoglobulin A and IgM very low
  - IgG = maternal levels
- No thymic shadow on chest X-ray
- No specific antibodies/iso-hemagglutinins
SCID: Therapy

• Bone Marrow Transplantation:
  – Bone marrow or peripheral stem cells or cord blood
  – HLA identical siblings, matched unrelated donors
  – success for HLA-identical sibling donor > 90%

• Enzyme Replacement Therapy
  – PEG-ADA replacement therapy (ADA-SCID)

• Gene Therapy
  – modifying T cells with normal gene for ADA, IL-2RG, Jak3 and re-infusing into patients

**Early diagnosis is key**
Newborn Screening Test for SCID

Optimal test to screen for severe T cell lymphopenia -

• Must detect low/absent T cells
• Use existing NBS screening cards
• Sensitive and Specific
• Inexpensive

TREC Assay

measuring T cell receptor excision circles (TREC), using DNA from dried blood spots collected routinely on all newborns

* T cell receptors (TCR) are protein molecules on the surface of T cells, responsible for recognition of antigens
TREC
(T Cell Receptor Excision Circle)

Extrachromosomal DNA produced during rearrangement of V-D-J regions in TCR gene

– Gene rearrangement occurs when thymocytes → naïve T cells

– Any immune defect that affects T cell production or destruction will cause a decrease in TREC
Formation of $\delta$Rec-$\Psi$Ja TREC during *Delta segment* deletion in rearrangement of T cell receptor gene

Chromosomal 14 germline TCR $\alpha/\delta$ chain loci (all cells)

Chromosomal 14 TCR $\alpha/\delta$ chain loci (T cells)

Extrachromosomal DNA $\delta$Rec-$\Psi$Ja TREC

Chromosomal 14 TCR $\alpha$ chain locus

$V_\alpha - J_\alpha - C_\alpha$ rearrangement to form $\alpha$ chain exon
Why is TREC DNA sequence different from genomic DNA?

Orientation of δRec and ΨJα sequences in genomic DNA (chromosome 14)

Orientation of δRec and ΨJα sequences in TREC DNA

α chain V segments  δRec  Δ chain  ΨJα  α chain J segments

5’ GTGTCCTC 3’

Orientation of δRec and ΨJα sequences in TREC DNA

5’ CTCCTGTGCACGCTGA 3’

PCR Forward Primer Direction  →  PCR Reverse Primer Direction  ←
Real time PCR with extracted DNA

In-situ Real time PCR

PE EnLite Neonatal TREC Kit

TREC Quantitative PCR Assay Platforms Selected by US newborn screening laboratories
SEVERE COMBINED IMMUNODEFICIENCY

REALTIME PCR TREC ASSAY

1. Punch sample from the newborn dried blood spot
2. Dried blood spot
3. Add wash to solution
4. Shake
5. Remove solution, keep the washed punch
6. Add extraction solution
7. Heat
8. DNA in solution
9. DNA on punch
10. Add PCR reagent
11. Test the samples in a Real-time PCR machine
12. Amplification Plots
   - A specific piece of DNA present in normal babies is amplified, giving out increasing fluorescent signal.
   - DNA from babies with Severe Combined Immunodeficiency produces delayed signal or no signal at all.
PE EnLite Neonatal TREC Assay

1. PUNCHING
   - 1.5 mm DBS disc
   - Into PCR-plates directly

2. DISPENSING & SEALING
   - 10 µL of elution buffer
   - Sealing
   - Spin the plates
   - DNA elution 45 min at 98°C
   - 2 min at 4°C

3. DISPENSING & SEALING
   - Unsealing
   - 20 µL of PCR mix
   - Sealing

4. AMPLIFICATION & HYBRIDIZATION
   - Amplification and hybridization
   - In thermocycler
   - PCR 37 cycles: ~1 hr 40 min
   - 35°C x 1 hr
   - 24°C x 5 min

5. MEASUREMENT
   - TREC and β-actin
   - Two signals from each well

Time-resolve Fluorometer
All three assay platforms have been used successfully in newborn screening labs; all have some limitations.

Best choice? - depends on local conditions and local needs
TREC Assay

PCR
Real time PCR
TCRD TREC Sequence: 376 bp (out of 85 Kb) flanking the $\delta$Rec-$\Psi$J$\alpha$ signal joint

AAAGAGGGGCAGCCCTCTCTCAAGGCAAAATGGGGCTCCTGTGGGGAAAA
GAGGGGTGCCTCTGTCAACAAAGGTGATGCCACCATCCCTTCTACCCCAT
GCTGACACCTTTGGTTTTTTTTATGAAGGTCGCCACTCTCTGTG^CA
CACGTTG
ATGCATAGGGCACCCTCACCCTCGTGCTCTCTTTACTTTGCCTGTTTTATTGAGCTCAGTC
CTCCATGTCACACTCTGTGTTTTCCATCCTGGGGAGTGTTTCACAGCTATC
CCCAAGGCCACCAGCTGACGATCACGGCCGAAAACACACTCTCAGGCT
ATGCATAGGGCACCCTCACCCTCGTGCTCTCTTTACTTTGCCTGTTTTATTGAGCTCAGTC
CTCCATGTCACACTCTGTGTTTTCCATCCTGGGGAGTGTTTCACAGCTATC
CCCAAGGCCACCAGCTGACGATCACGGCCGAAAACACACTCTCAGGCT
ATGCATAGGGCACCCTCACCCTCGTGCTCTCTTTACTTTGCCTGTTTTATTGAGCTCAGTC

Color code: Blue – CDC forward primer, Green – CDC reverse primer binding site, Red – Taqman probe, ^ – signal joint position
Real-time PCR with TaqMan Probes

3’ F Primer | Polymerization | Fluorophore | Probe cleavage | Quencher | R Primer

5’
TREC Real-time PCR Amplification Profile

Correlation between Cq and Template Concentration
The number of PCR cycles to reach threshold depends on the initial concentration of target.

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Number of PCR cycles required to reach fluorescent threshold is inversely proportional to the template copies

\[ y = -3.335x + 37.454 \]

\[ R^2 = 0.998 \]

Efficiency = 99.5%
Test results on a dried blood spot sample obtained from a newborn infant. These amplification curves (● RNase P, ▲ TREC, □ CMV) indicated that this neonate had normal levels of TREC and RNase P, and was positive for cytomegalovirus (CMV).
Multiplex TREC/SMN1/RNaseP Assay

In Newborn Screening for SCID and Spinal Muscular Atrophy (SMA)
Format considerations:

• Real-time PCR vs Single-point PCR

• *In situ* vs Extracted DNA

• Singleplex vs Multiplex PCR
Instrumentation considerations

- Real time PCR format - 96 vs 384 wells

Automation

- Fully automatic robotics
- Semi-automatic pipetting stations
- Manual
Reagent considerations

- **Forward & Reverse Primers**
- **Probe** – conventional vs MGB
- **Real-time PCR pre-mix reagents**
  (new reagents have Taq polymerases that have been selected for relative resistant to PCR inhibitors in blood)
- **Reference gene** – **RNase P** (18 labs)
  **Beta-actin** (19 labs)
Assay Validation considerations

- Sample source
- Sample size
- Duration (pilot?)
Analysis considerations

• Quantitation units – Cq vs Copies

• Calibrators –
  • Plasmid
  • Human TREC DNA (ddPCR calibrated)
  • Cell-based DBS (transformed cell line)

• Standard curves – limitation of linear regression model

• Cutoff value determination
Problem of using simple linear regression model near limit of detection: significant variance difference along the range covered by curve → inconsistent slope and intercept
Summary

- SCID is a group of fatal inheritable immune disorders that affect about 1:50,000 babies, leading to recurring severe infections.

- Newborn screening (NBS) can identify affected patients, who may be treated by bone marrow transplantation, gene therapy or enzyme replacement therapy; best outcome is obtained if treated before infections occur.

- TREC assay is the preferred newborn screening test, which is based on quantitative PCR technologies to identify newborns with low level of T cell production, a common phenotypic marker for SCID.
Three available test platforms (2 LDT & 1 commercial kit) have been successfully applied in NBS programs;

All three approaches work, none perfectly; the best choice is the one that fits local needs

Newborns with confirmed low TREC levels should be referred promptly to a clinical immunologist for diagnosis and treatment.

Some primary T cell deficiencies not detectable by the TREC assay:
- Mutations that cause functional loss
  - e.g., MHC I, MHC II, CD40L
- Late onset ADA
Newborn Screening for SCID

available technical support and services

from CDC NSTRI and NSQAP programs
CDC Provides Technical and Scientific Support

Pre assay development consultation
- Laboratory set-up
- Assay platform options
- Equipment choices
- Reagents (primers, probes, qPCR pre-mix) and supplies

Post assay development consultation
- Cutoff determination
- Precision (CV%) improvement
- Assay validation

Reference materials
- For assay development and validation
Technical and Scientific Support (cont.)

Proficiency Testing: NSQAP TREC PT
- 3 times per year
- Panel of 5 samples
- To report categorical results (for follow-up decision and reference gene level)

Site visits
- SCID house call (urgent assistance)
- Molecular Assessment Program (MAP)
Individualized Training at CDC in TREC Assay for SCID

- Performing real-time PCR TREC Assay
  - Manual protocol
  - Automated protocol

- QA/QC materials preparation
Thank you for your attention!

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