

California Genetic Disease Screening Program – Lab Services

Pilot Testing for Severe Combined Immunodeficiency
Disease (SCID)

Ajit Bhandal, Ph.D., CLS, SC, Chief
Genetic Disease Screening Program Lab Services
Board Certified in Clinical Chemistry



Overview

Blood Spot Specimen

- Public Private Partnership
- Screening Model
- NBS Screening - SCID Pilot
- PNS Screening – Will not be discussed
- State of the Art Screening Information System (SIS) - Reports
- Bio-Specimen Bank – Research and Development



Public Private Partnership



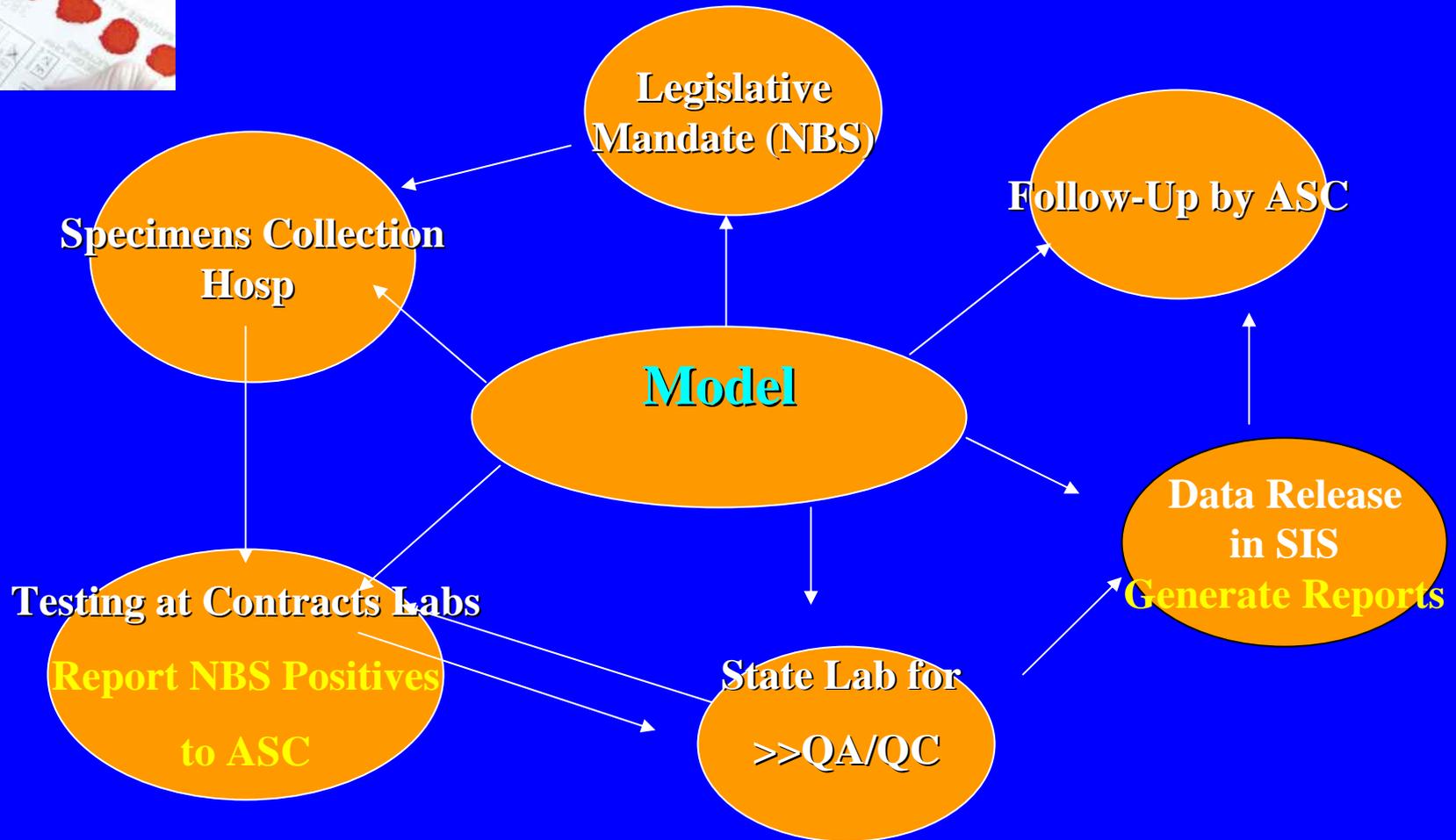


Public/Private Partnership

- Seven State contract laboratories in California for initial screening
- Contract labs use State approved protocols, lab equipment, and chemicals/reagents to ensure uniform testing of patients
- State lab serves as back-up of contract labs and maintains QA/QC
- Seven genetic area service centers for reporting and follow-up on positive NBS screening results
- Five confirmatory labs for confirmation of NBS screening results



Screening Model





Regulatory Compliance

- All contract labs including the central GDL State lab are certified by **CLIA** (federal) and by the **State of California**
- **CDC** for proficiency testing
- Follow **DEA**, **OSHA** and **HIPAA** regulations
- **DEA = Drug Enforcement Administration**
- **OSHA = Occupational Safety and Health Administration**
- **HIPAA = Health Insurance Portability and Accountability Act - 1996**



Annual Test Volume

NBS - ~ 520,000



Turn Around Time

- **NBS** – Positive results are reported over the phone within 24-48 hrs.



NBS Disorders

- **Currently** we test for **77 NBS** genetic disorders (including 30 core and 22 of 25 secondary targets recommended by ACMG)
- NBS disorders cause delays in development, neurological damage, dehydration, incorrect sex assignment, mental retardation, and death if remain untreated in early newborn age



1

NBS Disorders Untreated Patients

4

3

2



Fig. 21.3 Closer view illustrates M.G.'s eyes. Subluxed lenses had previously been removed bilaterally, after which he developed glaucoma in the left eye. He had fair skin and hair and a pronounced malar flush.



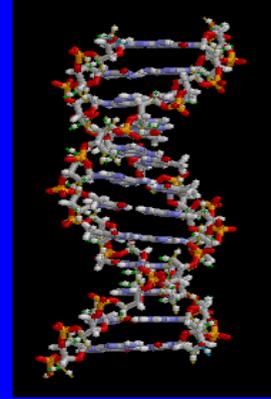
1. Normal
2. Two brothers with **PKU Disease**.
3. Biotinidase Disease
4. SCID Disease

SCID Pilot

Sponsored by

Vicki/Fred Modell – Jeffrey Modell Foundation

NIH



SCID Pilot- 2010/11

Severe Combined Immunodeficiency Disease

“Bubble Boy” Disease

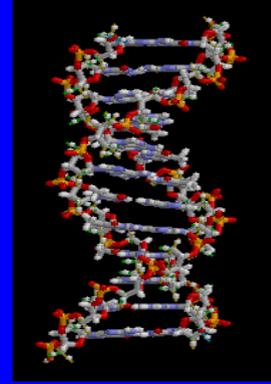
- **Phase 1**
- Start date – Aug 16, 2010.
- Method – Real-time PCR TREC assay- **DNA based**
- Expected to last ~7-8 months

- **Phase 2**
- Start date ~ Mar/April, 2011
- Method – End point assay (TREC assay – **DNA based**)
- Expected to last ~ 8-10 months
- Total specimens to be tested in phase 1 &2 ~ **one million**
- Positive results will be followed-up for diagnostic check

DNA Molecule



SCID Methodology



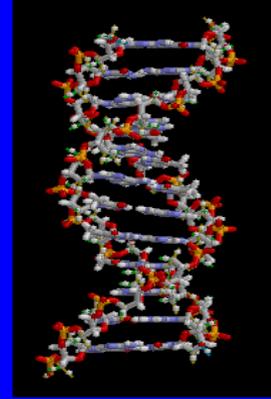
DNA Molecule

- **Background**

- SCID can be both x-linked and autosomal recessive disorder. Block T-cell development (low or no output of mature T-cells). Little or no immune system
- Can occur with no previous family history
- Prevalence - 1: 50,000 to 1: 100,000
- Babies are normal at birth
- Cause chronic and recurrent infections, death in first years of life if untreated
- **Treatment** – Hematopoietic stem cell transplantation (bone marrow trans).
 - Within 3.5 months of life = 95% long term survival rate
 - After 3.5 months of life = 60%-70% long term survival rate



SCID Methodology

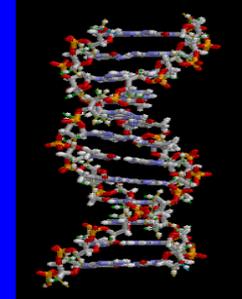


Real-Time PCR TREC Assay (PerkinElmer)

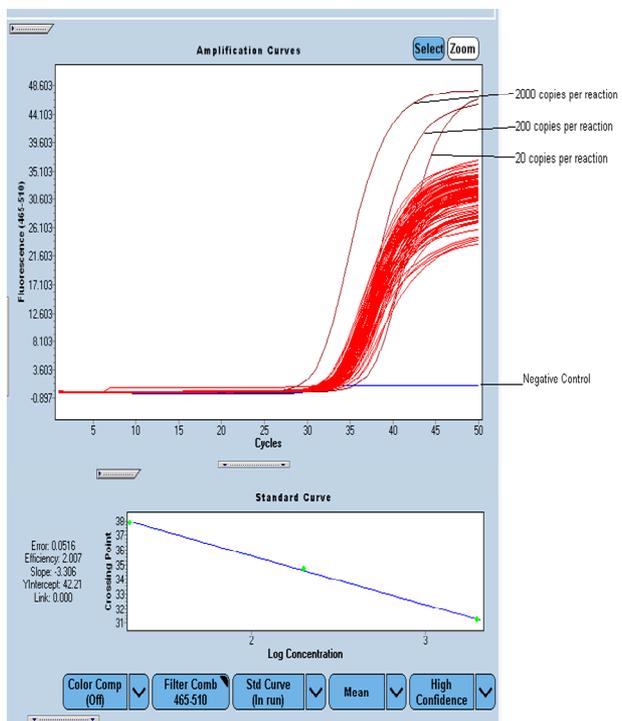
- TRECs (T-cell receptor excision circle) are stable circular DNA molecules generated during T- cell development
- TREC can be used as marker for naive T cell
 - TREC copies are measured from 3.2 mm dried-blood spot by polymerase chain reaction (PCR)
 - Low or absent of TRECs indicate genetic defect in newborn to generate T lymphocytes
 - TRECs below the cutoff value are considered as positive for T-cell lymphopenia including SCID
 - Positives are followed-up for further diagnostic testing and treatment.



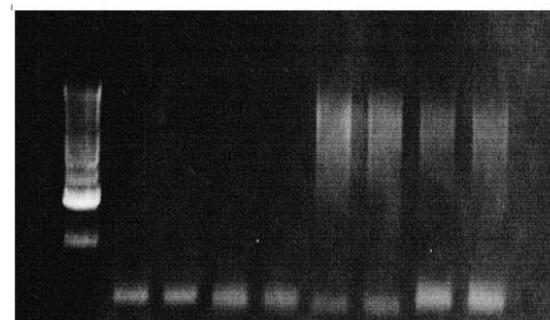
SCID Methodology - Results



Results of a plate full of regular DBS



Electrophoresis of Real Time PCR Products



M 1 2 3 4 5 6 7 8

M: Molecular Marker

1-2: PKI beta actin PCR products

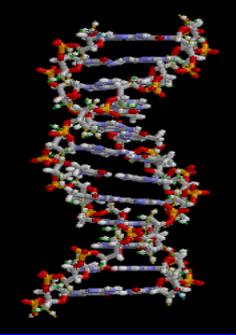
3-4: PKI TRFC PCR products

5-6: Wisconsin beta actin PCR products

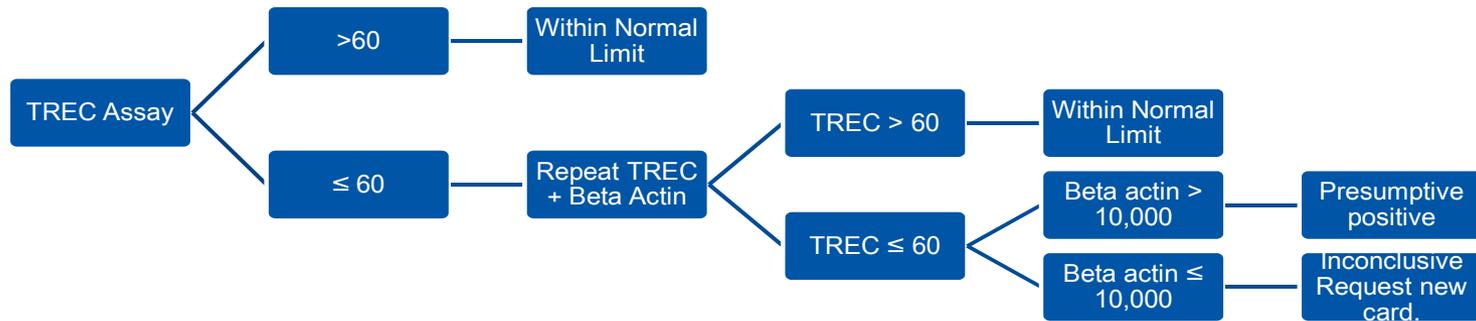
7-8: Wisconsin TRFC PCR products



SCID Methodology-Algorithm

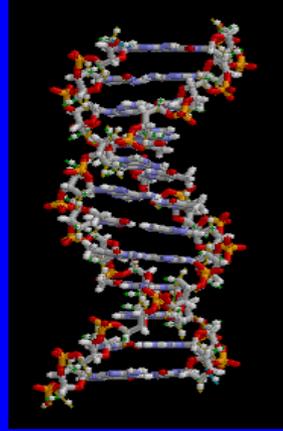


Reporting Algorithm





SCID Methodology



Why Did We Select PerkinElmer Method?

- Clean Assay
- Competitive with other assays (Dr. Puck, Wis, MA)
- Method Accuracy has been tested using CA samples
- Passed CDC proficiency test
- Short turn around time
- Result reporting - Same day
- Method is flexible to accommodate additional workload from other states if needed

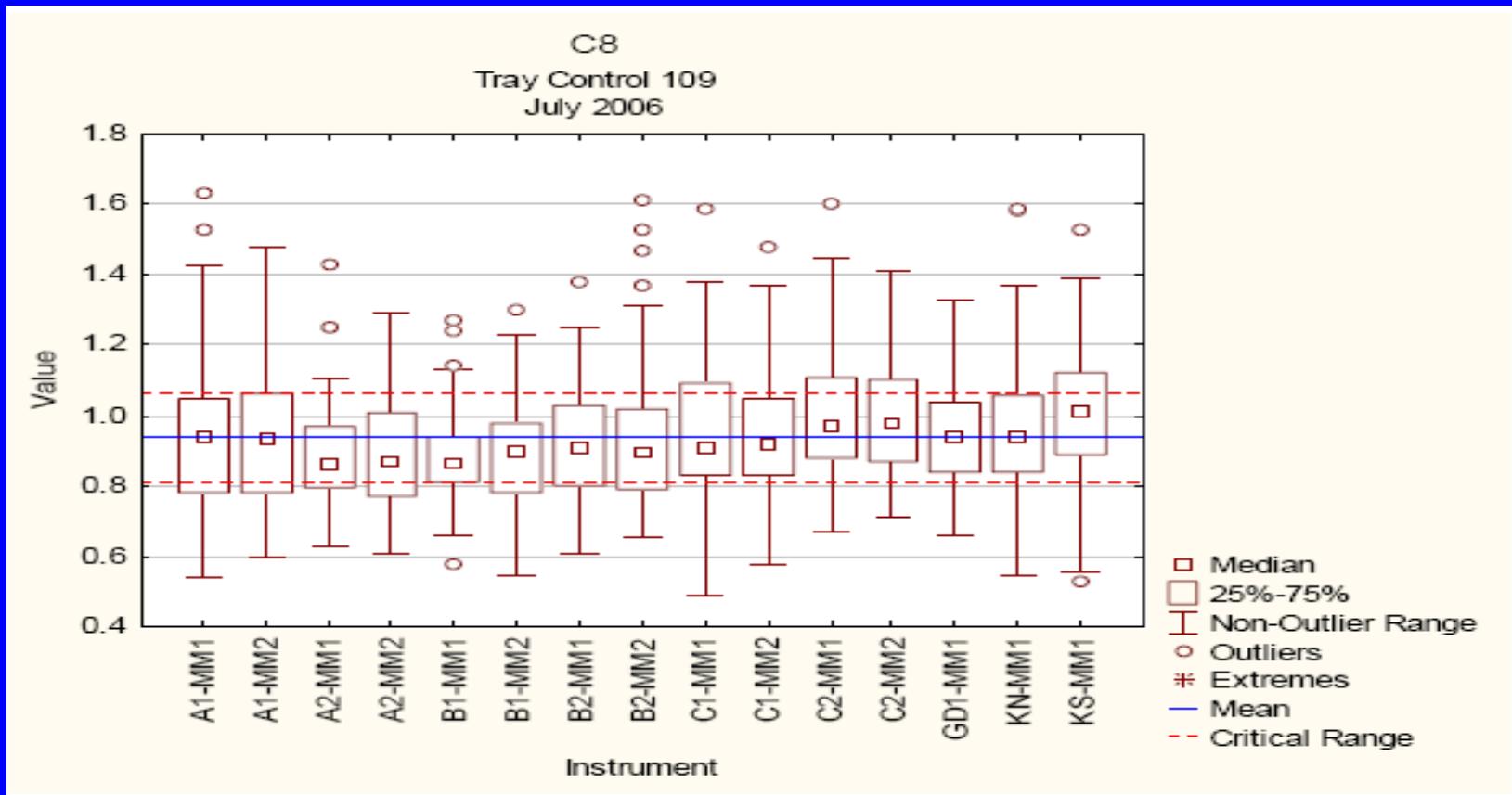


Laboratory Quality Assurance

- **Personnel** - Qualified and trained analytical staff
- **Equipment** – Well maintained machines equipped with diagnostic checks and automated turn key operation to reduce human errors
- **Reagents** – Use FDA approved reagents
- **Protocols** – State approved
- **Lab Reports** – Automated by SIS (LIMS) system
- **Inventory** – Turn Key Automated Inventory System
- **QC/QA Check** – Daily, weekly, monthly
- **CDC Proficiency Passing Rate (NBS)** – 100%



Quality Control/Quality Assurance Matching of Lab Instruments Monthly



Bio-Specimen Bank

- Blood spot Inventory ~15 million Specimens
 - Used for R&D of new bio-markers including cancer causing agents
- Redesign of Bio-specimen Bank - In progress

Acknowledgments

- **Jeffrey Modell Foundation**
- **NIH**
- **CFH/GDSP**
- **Dr. J. Puck**
- **PerkinElmer**