Newborn Screening and Diagnosis of Hemoglobin Traits and Diseases
Objectives

• Rationale for newborn screening (NBS) of hemoglobin disorders
• Methods of hemoglobin testing used by New England Newborn Screening Program (NENSP)
• Understanding process of NENSP for reporting abnormal results
• Interpretation and follow-up of abnormal NENSP results
Preventive Medicine

Primary Prevention
• Identifying persons at risk for condition to allow for education and follow-up (i.e. health promotion/protective measures)

Secondary Prevention
• Identification of patients with a disease prior to onset of symptoms or sequelae of the disease (i.e. screening)
Types of Newborn Screening

**Universal**
- Screening all newborns

**Targeted**
- Screening specific sub-groups of newborns based upon certain criteria (perceived race/ethnicity or other risk factors)
Newborn Screening Criteria

1. Life-threatening and/or catastrophic condition
2. Inexpensive and practical screening test
3. Effective treatment which prevents complications

Sickling disorders:
• Life-threatening
• Can be identified via screening hb electrophoresis
• Penicillin prophylaxis reduces mortality
1. Congenital Hypothyroidism
2. Congenital Toxoplasmosis
3. Phenylketonuria
4. Biotinidase Deficiency
5. Galactosemia
6. Maple Syrup Urine Disease (MSUD)
7. Homocystinuria
8. Congenital Adrenal Hyperplasia
9. Medium-chain acyl Co-A dehydrogenase deficiency (MCAD)
10. **Hemoglobin Disorders**
Newborn Screening for Hemoglobin Diseases

• Primary aim of NBS for hemoglobinopathy is to identify individuals with sickling disorders

• 1987 NIH recommends universal screening for sickling disorders be mandated by State Law

• Secondary outcome is the identification of a variety of other major and minor Hb disorders:
  - $\beta$ thalassemia major
  - $\alpha$ thalassemia minor
  - other variants
"We conclude that children should be screened in the neonatal period for sickle cell hemoglobinopathy and that those with sickle cell anemia should receive prophylactic therapy with oral penicillin by four months of age to decrease the morbidity and mortality associated with pneumococcal septicemia."
National NBS for Hemoglobin Diseases

- **Universal**
  - All but 7 states

- **Targeted/Optional**
  - Alaska, New Hampshire, North Dakota, South Dakota, West Virginia

- **Universal pilot**
  - Montana

- **None**
  - Idaho

As of December 2002
Hemoglobin and Hemoglobin Testing
Normal Hemoglobin (HbA)

- Heterotetramer
  2 α and 2 β chains (α₂β₂)

- Each globin chain is bound to a heme moiety
Globin Genes

α-like Genes


β-like Genes

ε:  γγ:  γγ:  ψβ:  δ:  β:  Chromosome 11

Kilobases

Hb Gower 1
(ζε:)

Hb Gower 2
(αε:)

Hb Portland
(ζγ:)

Hb F
(αγ:)

Hb A2
(αδ:)

Hb A
(αβ:)

Developmental Period

Embryonic

Fetal

Adult

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New England Pediatric Sickle Cell Consortium
Progression of Globin Synthesis

![Diagram showing the progression of globin synthesis over gestational and postnatal ages.](image-url)
Newborn Screening Process

• Mandated sample sent to State Lab on filter paper at about 48 hours of life

• Premature infants need testing before transfusion

• Results available within days of receipt
Methods of Newborn Screening

- **Hemoglobin electrophoresis**
  - Hemoglobins migrate and separate based on charge
  - Inexpensive, widely practiced, relatively slow.
  - Low sensitivity if Hb S <10% (i.e. preemies)
  - Difficulty with co-migration of some Hb variants

- **Methods of testing**
  - Isoelectric focusing*
  - Citrate agar*
  - Cellulose acetate
  - High performance liquid chromatography (HPLC)

* used in NENSC
Iso-electric Focusing (IEF)

Initial screening test performed by NENSP
Citrate Agar Electrophoresis

A2  S  F  A

Normal Adult
Normal Newborn
HPFH / β0 thalassemia
HPFH / A
β0 thalassemia / A
Hb SF
Control

Second test performed by NENSP

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Cellulose Acetate

Control
Normal adult
Hb AS
Control
Hb SS
Hb SC
Control

Adult samples

Alternative method of electrophoresis not used in NENSP
High-Performance Liquid Chromatography (HPLC)

- Alternative method of electrophoresis not used in NENSP
- Spectrophotometric analysis - allows for differentiation and quantification of hemoglobin variants
- Useful to verify or diagnose specific type of hemoglobin disease or trait
HPLC
Additional Methods of Testing Hemoglobins

• **Sickle solubility (Sickledex)**
  - Only identifies presence of HbS in sample
  - Cannot distinguish sickle cell disease from trait
  - Cannot identify Hb C trait
  - **NEVER** use as a screening tool

• **CBC**
  - Hb, RDW and MCV
  - Only useful as an adjunct to electrophoresis
NBS Hemoglobin Outcomes

• **Sickling disorders**
  - HbSS, HbS-β thalassemia, HbSC
  - HbS with other variant (i.e. HbSO_{Arab}, HbSD_{LosAngeles})

• **Non-sickling disorders and traits**
  - β thalassemia major, α thalassemia traits and disease.
  - Hb EE, Hb CC
  - HPFH
  - Multiple hemoglobin traits (HbAS, HbAC, HbAE)
Reporting Results: The New England Newborn Screening Program

- Hemoglobins are reported in order of relative abundance
  - FA → F > A
  - FAB → F > A > B
  - FAS* → F > A > S
  - FSA* → F > S > A

Important: FAS ≠ FSA
Reporting Sequence for Abnormal Results: Sickling Disorders

PCP Notification by NENSP

- Telephone notification to PCP
- Written report and fact sheet faxed to PCP
- Confirmation sample requested (filter paper) from PCP
- Confirmation results sent to PCP
Reporting Sequence for Abnormal Results: Sickling Disorders

By PCP
• Family notified and repeat specimen sent to NENSP

By NENSP
• Infant followed until family notified, penicillin prophylaxis initiated, and initial hematology visit
• After confirmatory test, family contacted by NENSP and offered home visit for teaching and family testing
Reporting Sequence for Abnormal Results by NENSP: Hemoglobin Traits and Non-Sickling Disorders

- Report and fact sheet mailed to PCP
- Initial letter mailed to family
- Second, more specific letter mailed to family with written information
- Home visit with family testing offered by NENSP
- Infant’s results confirmed only at family or PCP request
Limitations of NENSP for Hemoglobin Diseases

- Electrophoresis does not quantify hemoglobins other than relative abundance
- NENSP cannot distinguish between certain hemoglobins
  - HbE from HbO\textsubscript{Arab} OR HbD\textsubscript{LosAngeles} from HbG
- $\alpha$ thalassemia
  - Indicated by presence of Hb Bart’s
  - But cannot determine degree of $\alpha$ thalassemia in newborns and cannot identify in non-newborns
- $\beta$ thalassemia
  - Cannot identify $\beta$ thalassemia trait
How to Test Family Members for Hemoglobin Traits and Diseases

• CBC (including MCV)
• Hemoglobin electrophoresis
Interpretation of NENSP Results
What does this mean?

- HbF > HbA
- Normal

Is this a disease?

- No

What do you do?

- Nothing
FAS

What does this mean?
- Hb F > HbA > HbS
- Sickle cell trait

Is this a disease?
- No

What do you do?
- Family testing and counseling
What does this mean?
• A form of sickle cell disease
• Either HbSS or HbS-\(\beta^0\) thalassemia

Is this a disease?
• Yes

What do you do?
• Immediately start Penicillin VK 125 mg PO BID.
• See infant within 1-2 weeks.
• Send repeat NBS (filter paper) to state lab.
• Refer patient to a pediatric hematology program
If neither parent has a hemoglobin disease what are their hemoglobin types?

- Both parents are HbAS (sickle cell trait)

The infant has HbSS Disease
FS (continued)

OR

- One parent is HbAS (sickle cell trait) and the other is HbA-β₀ thal (β thalassemia trait)

The infant has HbS-β₀ thalassemia disease
What does this mean?
• A form of sickle cell disease
• HbS-ß+ thalassemia

Is this a disease?
• Yes

What do you do?
• Immediately start Penicillin VK 125 mg PO BID
• See infant within 1-2 weeks
• Send repeat NBS (filter paper) to state lab
• Refer patient to a pediatric hematology program
Remember !!!

- FAS ≠ FSA
- Why?
  - Hemoglobins are listed in order of relative abundance

- FAS is sickle cell trait
- FSA is HbS-β⁺ thalassemia
If neither parent has a hemoglobin disease what are their hemoglobin types?

- One parent is HbAS (sickle cell trait) and the other is HbA-β⁺ thalassemia (beta-thalassemia trait)

The infant has HbS-β⁺ thalassemia disease
FSC

What does this mean?
• A form of sickle cell disease
• HbSC

Is this a disease?
• Yes

What do you do?
• Immediately start Penicillin VK 125 mg PO BID
• See infant within 1-2 weeks
• Send repeat NBS (filter paper) to state lab
• Refer patient to a pediatric hematology program

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A Twist!

When You Speak to the Parents:

• Mother states her prenatal testing revealed she has sickle cell trait
• Father states that he has been “tested” and does not have sickle cell trait
• Father is upset and believes that this cannot be his baby
FSC (continued)

If neither parent has a hemoglobin disease what are their hemoglobin types?

- One parent is HbAS (sickle cell trait) and the other is HbAC (C trait)

The infant has HbSC Disease
FSC (continued)

The mother’s result is HbAS and the father’s is HbAC

How could this have happened?

• The father may have had a “sickledex” or “Sickle prep” screening test, not a hemoglobin electrophoresis
• The “sickledex” screening test identifies only Hemoglobin S and not Hemoglobin C
• On a “sickledex”, the father, with HbC trait would be “negative”
Conclusion

• Universal newborn screening in New England includes hemoglobin diseases
• Results available to any health care provider caring for child
• NENSP reports hemoglobins in order of relative abundance
• Every patient with a potential sickling disease needs to be followed by a pediatric hematology program
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