Quick Reference Guide to Results from Virginia Newborn Screening for Hemoglobinopathies

A hemoglobinopathy is a condition (disease or trait) caused by a defect in the genetic code for hemoglobin synthesis. There are over 600 known hemoglobin variants. These variants are characterized as either qualitative or quantitative. The vast majority of abnormal hemoglobin result from the mutation of a single polypeptide chain. The anomalies are transmissible, hereditary, autosomal traits. In the heterozygous subject (trait carrier), an abnormal gene is inherited from one parent and it directs the formation of abnormal hemoglobin. Theoretically, one part of the hemoglobin is abnormal and the other is normal, such as in sickle cell trait (A/S). In the homozygous subject, identical abnormal genes are inherited; one from each parent and the majority of the hemoglobin is abnormal, such as in sickle cell anemia (S/S).

1) Hemoglobin Structure:
   a) Normal Adult Hemoglobin
      Normal adult hemoglobin consists primarily of hemoglobin A. Hemoglobin A consists of two alpha chains and two beta chains. Beta chain synthesis begins early in fetal development. At the sixth week of gestation, hemoglobin A composes about 7% of the total hemoglobin; the percentages slowly increase throughout the pregnancy. At the thirtieth week, there is a switch from gamma chain to beta chain production.

   b) Fetal Hemoglobin
      At birth babies have mostly fetal or F hemoglobin. Fetal hemoglobin falls to the normal level of less than 3 to 5% by the time the infant is 5-6 months of age. Most adults have less than 2% fetal hemoglobin. Fetal hemoglobin consists of two alpha and two gamma chains.

   c) Hemoglobin A2
      Besides hemoglobin A and F, human red blood cells normally contain a third hemoglobin component, hemoglobin A2. Two alpha and two delta chains make up hemoglobin A2, which constitutes less than 3.5% of hemoglobin in a normal individual. A2 is usually elevated in individuals with Beta Thalassemia trait.

2) QUALITATIVE DEFECTS
   Qualitative defects refer to structural variations that result in a change of the type of hemoglobin produced. Ninety-five percent of the structural variants are caused by a single amino acid replacement. The amino acid replacement or substitution changes the quality, or characteristic of the hemoglobin. For example, hemoglobin S, C, E, D, G, and O, all contain a substitution of a different amino acid into the normal amino acid sequence of the beta globin chain. Each substitution changes the function of the hemoglobin molecule in a particular way. Thus, each hemoglobin disease or trait has a different characteristic clinical picture.

3) QUANTITATIVE DEFECTS
   Quantitative defects are characterized by a reduction or absence in the amount of normal alpha and/or beta globin chains produced. An example of a quantitative
defect is beta thalassemia. When an individual has beta thalassemia trait, beta chains are being produced, but in a lesser quantity. Because individuals with quantitative defects may still have hemoglobin A, hemoglobin electrophoresis alone cannot diagnose them alone.

a) Beta Thalassemia
Beta thalassemia syndromes are caused by genetic deletions of the beta genes. Progressive decrease in beta chain synthesis results in more severe anemia and symptoms as more beta genes are deleted. There are three classifications of beta thalassemia: thalassemia minor or trait, thalassemia intermedia and thalassemia major (Cooley's Anemia). Beta Thalassemia trait cannot be detected on cord blood electrophoresis or high pressure liquid chromatography. It cannot be diagnosed in the newborn because it is impossible to quantitate the percent of Hemoglobin A2, which is necessary to make the diagnosis.

b) Alpha Thalassemia
Alpha thalassemia syndromes are caused by genetic deletions of the alpha genes. Progressive decrease in alpha chain synthesis results in more severe anemia and symptoms as more alpha genes are deleted. There are four classifications of alpha thalassemia, the type an individual has depends upon their inheritance pattern for alpha globin chain production. In the newborn, alpha thalassemia is detected through the presence of Hemoglobin Bart's on the newborn screen. The percentage of Hemoglobin Bart's in the cord blood sample may indicate the number of alpha genes that have been lost. See Table 6 for more explanation.

4. Virginia Hemoglobin Methodology
All hemoglobin newborn screening samples sent to the Division of Consolidated Laboratory Services (DCLS) are tested using isoelectric focusing (IEF) and high performance liquid chromatography (HPLC). Screening will identify sickle cell disease, other hemoglobinopathies, hemoglobinopathy carriers and alpha thalassemia syndromes. The methodology used by DCLS, NBS Laboratory specifically detects hemoglobins A, S, C, D, E, F and a FAST region that includes Bart's hemoglobin. If hemoglobins other than those specified are detected, they are reported as "V". These unidentified hemoglobins invariably have no or minimal clinical or genetic significance.

<table>
<thead>
<tr>
<th>RESULT CODE*</th>
<th>DIAGNOSTIC POSSIBILITIES **</th>
<th>ACTION REQUIRED***</th>
</tr>
</thead>
</table>

Table 1: Most Commonly Reported FS Hemoglobin Results

Quick Reference Guide to Results from Virginia Newborn Screening for Hemoglobinopathies
Information adapted from the Michigan Department of Health and Human Services' Interpretation of Newborn Hemoglobin Screening Results
### Hemoglobins

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS</td>
<td>Fetal and sickle hemoglobin</td>
</tr>
<tr>
<td>FSA</td>
<td>Fetal hemoglobin, sickle hemoglobin and small amount of adult hemoglobin</td>
</tr>
<tr>
<td>FSC</td>
<td>Fetal hemoglobin, sickle hemoglobin and hemoglobin C</td>
</tr>
<tr>
<td>FSD</td>
<td>Fetal hemoglobin, sickle hemoglobin and hemoglobin D</td>
</tr>
<tr>
<td>FSV</td>
<td>Fetal hemoglobin, sickle hemoglobin and an unidentified variant</td>
</tr>
<tr>
<td>FSE</td>
<td>Fetal hemoglobin, sickle hemoglobin and hemoglobin E</td>
</tr>
</tbody>
</table>

For all cases:
- Confirmatory testing
- Disease education
- Referral to Comprehensive Pediatric Sickle Cell Center

FS
- Sickle cell anemia
- Sickle cell-β thalassemia zero or plus
- Sickle cell – hereditary persistence of fetal hemoglobin, a benign condition

FSA
- Sickle β-thalassemia plus
- Sickle cell trait

FSC
- Hemoglobin SC disease; generally a milder form of sickle cell disease sometimes confused with sickle cell trait

FSD
- Hemoglobin SD disease – moderate sickling disorder

FSV
- Sickle cell anemia
- Sickle cell-β thalassemia
- Sickle cell – hereditary persistence of fetal hemoglobin (benign)
- Hemoglobin S C Harlem - moderate sickling disorder
- Hemoglobin S O Arab – moderate sickling disorder
- **Conditions phenotypically identical to sickle cell trait**

FSE
- Hemoglobin SE Disease
- A mild form of sickle cell disease similar to sickle beta thalassemia plus

### Conditions

- FS
  - Fetal and sickle hemoglobin
- FSA
  - Fetal hemoglobin, sickle hemoglobin and small amount of adult hemoglobin
- FSC
  - Fetal hemoglobin, sickle hemoglobin and hemoglobin C
- FSD
  - Fetal hemoglobin, sickle hemoglobin and hemoglobin D
- FSV
  - Fetal hemoglobin, sickle hemoglobin and an unidentified variant
- FSE
  - Fetal hemoglobin, sickle hemoglobin and hemoglobin E

*Hemoglobins are reported in decreasing order of concentration.

** In the newborn period, only a presumptive diagnosis of sickle cell anemia can be made. The HPLC pattern typical of sickle cell anemia (FS) is also found in sickle cell beta thalassemia zero (Sβ°), sickle cell beta thalassemia plus (Sβ+) and sickle cell-hereditary persistence of fetal hemoglobin.

*** A sickle cell screen (hemoglobin solubility or sickle dex) is not recommended as an option for confirmatory testing. Recommended options are repeat filter paper screen or hemoglobin electrophoresis.
Table 2: Most Commonly Reported FC/FD Hemoglobin Results

<table>
<thead>
<tr>
<th>RESULT CODE</th>
<th>DIAGNOSTIC POSSIBILITIES</th>
<th>ACTION REQUIRED</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>• Hemoglobin C disease; usually a mild form of hemolytic anemia with no need for intervention in the newborn period • Hemoglobin C thalassemia zero or plus</td>
<td>For all cases: • Confirmatory Testing • Genetic Counseling • Disease Education • Offer family testing • Referral to Pediatric Hematology/Oncology</td>
</tr>
<tr>
<td>FCE</td>
<td>• Hemoglobin CE disease; a benign, but genetically significant, condition</td>
<td></td>
</tr>
<tr>
<td>FCA</td>
<td>• Hemoglobin C thalassemia plus; usually a mild form of hemolytic anemia with no need for intervention in the newborn period • Hemoglobin C trait</td>
<td></td>
</tr>
<tr>
<td>FCV</td>
<td>• Hemoglobin C Disease • Hemoglobin C Thalassemia • Conditions phenotypically identical to hemoglobin C trait</td>
<td></td>
</tr>
<tr>
<td>FDA</td>
<td>• Hemoglobin D thalassemia; a benign condition • Hemoglobin D trait</td>
<td></td>
</tr>
<tr>
<td>FDV</td>
<td>• Hemoglobin D Disease; a benign condition • Hemoglobin D thalassemia • Conditions phenotypically identical to hemoglobin D trait</td>
<td></td>
</tr>
<tr>
<td>FD</td>
<td>• Homozygous hemoglobin D; a benign condition • Hemoglobin D thalassemia</td>
<td></td>
</tr>
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</table>
### Table 3: Most Commonly Reported FE Hemoglobin Results

<table>
<thead>
<tr>
<th>RESULT CODE</th>
<th>DIAGNOSTIC POSSIBILITIES</th>
<th>ACTION REQUIRED</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE Fetal hemoglobin and hemoglobin E</td>
<td>• Hemoglobin E disease; a mild form of hemolytic anemia.  • Hemoglobin E thalassemia; a more severe form of hemolytic anemia causing transfusion dependence</td>
<td>• Confirmatory testing  • Genetic counseling  • Referral to Pediatric Hematology/Oncology if appropriate</td>
</tr>
<tr>
<td>FEA Fetal hemoglobin, hemoglobin E and a small amount of adult hemoglobin</td>
<td>• Hemoglobin E thalassemia; a more severe form of hemolytic anemia causing transfusion dependence  • Hemoglobin E trait</td>
<td></td>
</tr>
<tr>
<td>FEV Fetal hemoglobin, hemoglobin E and unidentified hemoglobin variant</td>
<td>• Hemoglobin E disease  • Hemoglobin E thalassemia  • Conditions phenotypically identical to hemoglobin E trait</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Other Commonly Reported Hemoglobin Results

<table>
<thead>
<tr>
<th>RESULT CODE</th>
<th>DIAGNOSTIC POSSIBILITIES</th>
<th>ACTION REQUIRED</th>
</tr>
</thead>
<tbody>
<tr>
<td>F with Low A Possible beta thalassemia major</td>
<td></td>
<td>• Confirmatory testing  • Genetic counseling  • Referral to Pediatric Hematology/Oncology if appropriate</td>
</tr>
<tr>
<td>FV Fetal hemoglobin and unidentified hemoglobin variant</td>
<td>Unknown variant hemoglobin disease</td>
<td></td>
</tr>
<tr>
<td>FAB ≥ 30%</td>
<td>Possible Hemoglobin H disease</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5: Commonly Reported Traits
Quick Reference Guide to Results from Virginia Newborn Screening for Hemoglobinopathies
Information adapted from the Michigan Department of Health and Human Services’ Interpretation of Newborn Hemoglobin Screening Results

<table>
<thead>
<tr>
<th>Result</th>
<th>Diagnostic Possibilities</th>
<th>Action Required</th>
</tr>
</thead>
</table>
| FAS    | Fetal hemoglobin, normal adult and sickle hemoglobin | • Sickle cell trait; which is clinically benign but genetically significant | • No confirmatory testing required.  
• Genetic counseling |
| FAC    | Fetal hemoglobin, normal adult and hemoglobin C | • Hemoglobin C trait; which is clinically benign but genetically significant | |
| FAD    | Fetal hemoglobin, normal adult and hemoglobin D | • Hemoglobin D trait; which is clinically benign but genetically significant | |
| FAE    | Fetal hemoglobin, normal adult and hemoglobin E | • Hemoglobin E trait; which is clinically benign but genetically significant | |
| FAV    | Fetal hemoglobin, normal adult and an unidentified hemoglobin variant | • Most likely clinically insignificant hemoglobin variant | • Physician of record responsible for reassuring the parent that this is clinically insignificant.  
• No confirmatory testing required. |
| FAB <30% | Fetal Hemoglobin, Hemoglobin A and Bart’s Hemoglobin | • Alpha Thalassemia Trait | • No confirmatory testing required  
• Order CBC between 9 and 12 months |

**Table 6: Classification of Alpha Syndromes**

<table>
<thead>
<tr>
<th>Gene Deletion</th>
<th>Classification Complications</th>
<th>Notes?</th>
</tr>
</thead>
</table>

Quick Reference Guide to Results from Virginia Newborn Screening for Hemoglobinopathies  
Information adapted from the Michigan Department of Health and Human Services’ Interpretation of Newborn Hemoglobin Screening Results
| Single gene deletion: Remaining three genes compensate almost completely. | • Silent Carrier  
• Clinically and hematologically normal | Traces of Hb Barts at birth that disappear. Diagnosed by enumeration of the alpha genes by recombinant DNA technology. This is both technically difficult and expensive. |
|---|---|---|
| Deletion of two alpha genes | • Alpha Thalassemia Trait  
• Mild anemia with small red cells.  
• No evidence of iron deficiency  
A2 levels are normal | Traces of Hb Barts at birth that disappear at 3-4 months of age |
| Deletion of three alpha genes | • Hemoglobin H Disease  
• Moderately severe microcytic hemolytic anemia resembling mild Cooley’s anemia.  
(See section on Hemoglobin H Disease) | |
| Deletion of four alpha genes | • Fetal Hydrops Syndrome  
Severe hemolytic anemia beginning in utero. Affected infants develop heart failure, often stillborn between 34 and 40 weeks or dies within the first hours of birth.  
• Pregnant women carrying an infant with Fetal Hydrops Syndrome have a high rate of severe toxemia of pregnancy and postpartum bleeding.  
• Hemoglobin Barts with small amounts of Hemoglobin H and Portland.  
No hemoglobin A or F  
The parents have a thalassemic blood picture with low MCV and MCH and normal hemoglobin electrophoresis. | |