

# **Georgia Newborn Screening Manual**

**for**

## **Metabolic Diseases & Hemoglobinopathies**

*A Practitioner's Guide*

**Georgia Department of Human Resources**  
**Division of Public Health**  
**Family Health Branch**



# **GEORGIA NEWBORN SCREENING MANUAL**

## **CONTENTS**

<b><u>Introduction</u></b> .....	<b>1</b>
<u>The Collection of Newborn Screening Specimens</u> .....	<b>3</b>
<u>Metabolic and Hemoglobin Screening Notice</u> .....	<b>7</b>
<u>Screening The Critically Ill and Premature Infant</u> .....	<b>8</b>
<u>Newborn Screening Form 3491</u> .....	<b>9</b>
<u>Newborn Screening Instructions</u> .....	<b>10</b>
<b><u>What Do I Do If?</u></b> .....	<b>14</b>
<b><u>Understanding Metabolic Disorders</u></b>	
<u>Summary Table Metabolic Disorders</u> .....	<b>20-21</b>
<u>Phenylketonuria (PKU)</u> .....	<b>22</b>
<u>Tyrosinemia</u> .....	<b>25</b>
<u>Homocystinuria</u> .....	<b>27</b>
<u>Maple Syrup Urine Disease (MSUD)</u> .....	<b>28</b>
<u>Galactosemia</u> .....	<b>29</b>
<u>Congenital Hypothyroidism</u> .....	<b>32</b>
<u>Congenital Adrenal Hyperplasia (CAH)</u> .....	<b>34</b>
<b><u>Understanding Abnormal Hemoglobins</u></b>	
<u>Hemoglobinopathies</u> .....	<b>38</b>
<u>S/Beta Thalassemia</u> .....	<b>40</b>
<u>Bart's Hemoglobin And Alpha Thalassemia</u> .....	<b>42</b>
<u>Beta Thalassemia Minor, Intermedia and Major</u> .....	<b>44</b>
<u>Conclusion</u> .....	<b>47</b>
<u>Summary Table Hemoglobin Results</u> .....	<b>48</b>
<u>Sickle Hemoglobin</u> .....	<b>49</b>
<u>Hemoglobin C</u> .....	<b>51</b>
<u>Bart's Hemoglobin And Alpha Thalassemia</u> .....	<b>52</b>
<u>Beta Thalassemias</u> .....	<b>54</b>
<u>Hemoglobin F</u> .....	<b>56</b>
<u>Hemoglobin E</u> .....	<b>57</b>
<u>Hemoglobin "D"</u> .....	<b>58</b>
<u>Hemoglobin "J"</u> .....	<b>59</b>
<u>Hemoglobin G - Philadelphia</u> .....	<b>60</b>
<u>Hemoglobin Constant Spring</u> .....	<b>61</b>
<u>Hemoglobins "N"</u> .....	<b>62</b>
<u>Hemoglobin O - Arab</u> .....	<b>63</b>
<b><u>Georgia Law</u></b> .....	<b>65</b>
<b><u>DHR Rules and Regulations</u></b> .....	<b>69</b>
<b><u>Resources</u></b> .....	<b>74</b>

## INTRODUCTION

The Georgia Newborn Screening System (NBS) has five components of disease prevention including:

1. **Screening:** universal testing of all newborns
2. **Follow-up:** rapid retrieval and referral of the screen-positive newborn
3. **Medical Diagnosis:** confirmation of a normal or abnormal screening test result by a private physician or tertiary treatment center
4. **Management:** rapid implementation and long-term planning of therapy
5. **Evaluation:** validation of testing procedures, efficiency of follow-up and intervention, and benefit to the patient, family and society. Include consideration of adding other tests to the system as indicated by appropriate research and scientific evidence.

Newborn screening is an essential, preventive public health function to identify at risk infants in the first few days of life so that early intervention can be implemented to prevent severe mental retardation, chronic disability or death. The cost of these disorders when left untreated is enormous, both in human suffering and in economic terms. Georgia law directs that a statewide network for genetics services be developed as a cooperative effort between public health, appropriate medical centers and private practitioners.

The goals of the Georgia Newborn Screening System are to ensure that:

1. Every newborn in Georgia has a specimen collected for newborn screening tests prior to discharge from the hospital **regardless of the age of the baby.**
2. **If the baby is discharged prior to 48 hours after birth the hospital administrator or his/her designated representative must give the parent(s) written and verbal instructions to have the baby tested again prior to one week of age.**
3. All infants whose test results are outside the normal limits for a newborn screening disorder receive prompt and appropriate confirmatory testing.
4. All newborns diagnosed with a metabolic disease or hemoglobin abnormality are entered into and maintained on appropriate medical therapy.

Achieving these goals is dependent upon coordinated, systematic efforts from these groups:

1. **Hospitals** are responsible for the collection, labeling and mailing of the first screening specimens, and for informing the parents or guardian both verbally and in writing when a second specimen should be collected prior to one week of age.
2. **The Practitioners** are responsible for prompt collection and submission of repeat specimens if indicated, by screening results or timing of first specimen; medical care; provision of parent education, support and when needed, referral to specialty care.
3. **The Georgia Public Health Laboratory** is responsible for specimen analysis, record keeping (as per CLIA (88) requirements), quality control of laboratory methods and notification of results to hospitals, practitioners and follow-up programs.
4. **The Follow-up Programs** are responsible for tracking abnormal screening results, diagnosed cases, linking confirmed cases to appropriate medical care and serving as a source of information about the newborn screening disorders for practitioners, parents and consumers. The Metabolic Follow-up Programs will be coordinated by the Division of Medical Genetics at Emory University. The Sickle Cell Disease Follow-up Programs will be coordinated by the Sickle Cell Center at Grady Hospital and Sickle Cell Center at Medical College of Georgia.
5. **The Genetics Program** is responsible for (1) monitoring and evaluating newborn screening practices (2) developing a quality control program (3) electronic data surveillance and tracking system including maintenance of long term results (4) facilitating communication between practitioners, the laboratory personnel and the follow-up team (5) providing ongoing education for practitioners, and (6) reporting results to state and federal officials and to the public.

This manual describes the operational requirements of the newborn screening system, the disorders currently screened for by the program, standards for follow-up of abnormal screening test results and appropriate medical management of diagnosed cases.

# THE COLLECTION OF NEWBORN SCREENING SPECIMENS

**Responsibility for performing newborn screening** is assigned by law as follows: "When a live birth occurs in a hospital the physician shall have a specimen of the infant's blood taken prior to the infant's discharge from the hospital", and, "When a live birth occurs in a facility other than a licensed hospital, it shall be the responsibility of the person in charge of the facility or the person in attendance, to give written notice to the parents, guardian or other legally responsible person of the legal requirements for the newborn to be tested and to advise where testing can be obtained."

**Timing of screening:** State rules require that a blood specimen be collected between forty-eight (48) hours after birth and no later than when the infant is one week old. It is **preferable** to obtain the first specimen 24 hours after the first protein feeding in order to detect Phenylketonuria (PKU). **However, the State rules require that if the infant is discharged before 48 hours after birth, a blood specimen must be collected prior to discharge.** In addition, the hospital administrator or a designated representative must provide written notice to the parents, guardian or legally responsible person that the infant must be tested again prior to one week of age. Discharging a newborn without collecting a specimen, even with the intent to collect it later, greatly increases the risk of missing an infant affected with one of the screened disorders.

**Transferred infants:** Since the responsible party is the one who attended the delivery of the newborn, in the event of transfer to another facility shortly after birth or before screening has been accomplished, the transferring facility must ensure that the next facility is aware of the need for screening and should document this in their records.

**Transfusion:** If a newborn is to receive a transfusion, collect a specimen prior to the transfusion since even small transfusions may invalidate galactosemia and hemoglobin screening test results. If the infant is less than 48 hours old or has not been on a protein diet for 24 hours, collect a second sample for newborn screening one week after the last transfusion and a third sample two months post transfusion.

**Premature infants** may have persistent abnormalities in newborn screening test results without having an abnormal condition. Prematurity may be associated with physiological elevation of 17-hydroxyprogesterone (17-OHP) and reduction of thyroxine. Testing for PKU and other disorders can be affected by Total Parenteral Nutrition (TPN) and antibiotics. Galactosemia results can be affected by antibiotics. A premature infant should receive the first screen before seven days of age.

A premature infant with abnormal newborn screening results should be rescreened at one month of age, at the time of discharge (whichever comes first) or when requested by the NBS system. Physical or metabolic signs suggestive of the presence of a screened condition should prompt immediate and appropriate diagnostic testing for the suspected disorder. (See *Screening the Critically Ill and Premature Infant*, page 8)

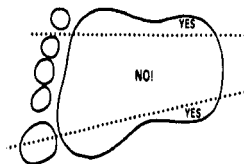
**Reports** of screening results and notice of unsatisfactory specimens are mailed to the **physician** usually within 7 days of receiving the specimen. Abnormal results are communicated immediately to follow-up programs to assure appropriate retrieval and follow-up. A Voice Response System (VRS) to allow 24 hour access to laboratory testing results is being installed, and is expected to be operational by October 1, 1998. Call Muthukrishnan Ramachandran, Ph.D. at (404) 327-6800 or (404) 327-7937 for more VRS enrollment information.

**Blood Collection:** Gloves should be worn for personal safety. Care should be taken to avoid contamination of blood collection circles with antiseptic solutions, powders, lotions or other materials which may adversely affect the testing process. A videotape describing proper collection procedures and posters illustrating proper blood collection and inadequate specimen are available from the Genetics Program, (404) 679-0547.

**Procedure:**

1. Position the infant with feet lowered below the heart to help to increase the blood flow.
2. Warm the heel to increase the blood flow to the area by covering the puncture site for three to five minutes with a warm, moist towel which has been run under tap water at a temperature of not more than 42 degrees centigrade or 107.6 degrees F.
3. Clean the puncture site with a sterile alcohol pad. Wipe dry with sterile gauze. Excess alcohol may cause hemolysis and denature some of the enzymes tested.
4. Use a sterile disposable lancet with a 2.45 mm tip or an automatic lancet e.g., Tenderfoot™ device, to perform a swift clean puncture in the areas indicated on the diagram. Wipe away the first drop of blood with dry sterile gauze.

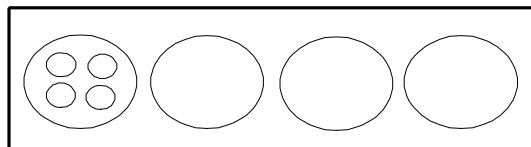
Recommendation for Heel Puncture Site in Newborns



Perform punctures on the most lateral portions of the plantar surface (in the colored portion of the foot).

5. Allow a large drop of blood to form. To enhance blood flow during collection, very gentle intermittent pressure may be applied to the area surrounding the puncture site. Excessive "milking" causes an admixture of tissue fluids with the blood specimen, invalidating the specimen.
6. **Do not use a capillary tube.** Lightly touch the filter paper against a large drop of blood and allow a sufficient quantity of blood to soak through to completely fill the circle. Apply blood to one side of the filter paper only, allowing full saturation of each circle area. Either side may be chosen for this procedure. Fill all circle areas. Do not layer successive small drops of blood to the same circle. Avoid touching or smearing the blood spots.
7. If blood flow is diminished, repeat steps three through six with sterile equipment.
8. Allow the blood specimens to air-dry **for at least 3 hours, preferably 4 hours**, on a flat, nonabsorbent surface protected from heat or direct sunlight. **Do not refrigerate the samples.**
9. **Mail collection forms to the Georgia Public Health Laboratory within 24 hours of collection. Do not accumulate specimen before mailing since this may result in specimen too old to test. When placing more than one specimen in an envelope, alternate orientation of collection forms so that blood spots on adjacent forms are not in contact. Delayed submission to laboratory may result in significant delay in identification of an infant with metabolic or hemoglobin disorder.**

**Unsatisfactory Specimens:** The State Public Health Laboratory receives many blood spot specimens in a condition unacceptable for testing. Certain types of specimens are known to give invalid results. These include old specimens or those with incompletely filled, abraded, discolored, diluted or clotted spots and those showing serum "rings". The newborn screening report will state **"UNSATISFACTORY - PLEASE RESUBMIT"**. Submitting invalid specimens results in the inconvenience of retesting and delays the screening of the newborn, placing the newborn at risk for delayed diagnosis of a screened condition. **THE INFANT MUST BE RE-SCREENED AS SOON AS POSSIBLE.**



The Laboratory makes four 1/8" punches from each "circle". It is necessary that blood fills up the entire circle and soaks through the filter paper but does not alter the homogeneity of the filter paper surface. (Excess application must be avoided)

**Parents Refusal to have the baby tested:** Religious grounds are the only

valid reason for refusal of newborn screening. If a parent objects to testing based on religious grounds, a hospital official is to inform the parent of the consequences of refusal (possible infant death or retardation) and require the parent to complete a statement indicating their declination of newborn screening for religious reasons. This signed refusal should be retained in the record of the physician, midwife or person attending the delivery.

**Recording Newborn Screening Results:** Each hospital, doctor or midwife caring for an infant should document that newborn screening has been done, the results, when it was done and by whom.

**Responding to requests for rescreening:** The hospitals, public health clinics and genetics centers may request, by phone and letter, that an infant be retested.

1. When submitting a specimen for repeat testing, note if the last name has changed from that given at birth. Give the hospital facility code number (not that of the hospital of birth) where you want the hard copy of the results to be mailed.
2. Give the complete information requested on form #3491. Make certain all copies are legible. Give the name of the physician who is to provide follow-up on the infant.
3. Use the DHR kits only. The filter paper collection kits are to be used on or before the expiration date printed on the filter paper margin. Destroy all outdated kits immediately and request a new supply.
4. When collecting a repeat specimen, please check the appropriate boxes on form #3491.

Examples:

- (a) The first test was unsatisfactory. "Check ROUTINE RETEST".
- (b) The infant was discharged before 48 hours old, "Check ROUTINE RETEST".
- (c) The infant is premature or low birth weight. "Check ROUTINE RETEST".
- (d) The infant had a previous abnormal test result. "Check PRIOR ABNORMAL" requested by the State Laboratory.
- (e) The physician routinely retests for the metabolic disorders. "Check ROUTINE RETEST".
- (f) The physician retests a previously diagnosed case - "Check DIAGNOSED CASE".



# METABOLIC AND HEMOGLOBIN SCREENING NOTICE

I, the parent of Baby \_\_\_\_\_, understand that Georgia law requires that all infants born in Georgia have the Newborn Screening test performed, unless the parent's object to such testing for religious reasons.

The Newborn Screening Test, which tests for a number of inherited disorders, involves the collection of blood obtained by pricking the heel of the baby's foot. Temporary pain might be experienced during the collection procedure. The inherited disorders are galactosemia, maple syrup urine disease, homocystinuria, phenylketonuria, tyrosinemia, hypothyroidism, congenital adrenal hyperplasia and hemoglobin disorders as of October 1, 1998.

I understand that if the Newborn Screening Test detects one of these inherited disorders, appropriate treatment may be provided and irreparable injury or death may be prevented. If one or more of these disorders exists but is not detected, mental retardation, physical handicaps or death may result. I understand that this test does not check for all genetic disorders.

I understand that the Newborn Screening Test described above must be performed when the infant is at least 48 hours old and has been taking protein feeding (formula or breast milk) for at least 24 hours. If an infant is discharged prior to that time, Georgia law requires that the infant be tested on discharge and that the test be repeated before one week of age. If the infant is taking an antibiotic, mark yes on the form and write in the name of the antibiotic.

**IF MY CHILD IS DISCHARGED FROM THE HOSPITAL BEFORE HE OR SHE IS 48 HOURS OLD OR HAS HAD PROTEIN FEEDING FOR 24 HOURS, I UNDERSTAND THAT I AM RESPONSIBLE FOR ARRANGING FOR THE FOLLOW-UP TEST PRIOR TO ONE WEEK OF AGE. I UNDERSTAND THAT THIS TEST CAN BE PERFORMED AT MY CHILD'S DOCTOR'S OFFICE, A HEALTH DEPARTMENT CLINIC, OR AT THE HOSPITAL LABORATORY.**

I acknowledge that I have received and understand the above information about the Newborn Screening Test and that I have been given an opportunity to ask questions and receive additional information and an opportunity to refuse the test, if I object for religious reasons.

\_\_\_\_\_  
Parent(s)

Date \_\_\_\_\_ Time \_\_\_\_\_

\_\_\_\_\_  
Witness

Date \_\_\_\_\_ Time \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## **SCREENING THE CRITICALLY ILL AND PREMATURE INFANT**

Infants in the neonatal intensive care unit (NICU) have so many critical needs that their Newborn Screening test may be overlooked. It is advisable to establish a protocol to be sure that this screening is done.

- \* Hospitals transferring a sick neonate to a NICU should document in the record whether the first newborn screen has been done.
- \* The receiving NICU should note whether newborn screening has been done. If not, the neonate should have newborn screening done within the first 7 days of life and this should be documented in the record and reported to the transferring hospital.
- \* The newborn screening test should be done before a transfusion is given. Unscreened infants transfused before admission to the NICU should be screened regardless, but will need rescreening 3 months post transfusion.
- \* Screening for PKU and galactosemia is most accurate 48 hours or more after the infant has received enteral feedings. Adequate enteral feeding is considered to be at least 75 calories/kg/day. If a neonate is screened before having or retaining enough milk feedings to provide accurate results for amino acid tests and galactosemia screening, "insufficient milk intake" should be noted on a normal report as a reminder that repeat screening is indicated. A normal screen in an infant who has insufficient enteral intake does not rule out metabolic disease. Total parenteral nutrition may cause a false positive test for PKU and several other disorders. Feeding soy formula may result in a false negative galactosemia test because galactose accumulation depends on lactose ingestion. Infants with classic galactosemia are treated from birth with galactose-free diets are spared the acute complications of galactosemia.
- \* An abnormal screening test result should be noted in the chart and if the premature/ill infant shows clinical signs compatible with the disorder, confirmatory testing should be immediately done. If the child shows no signs of the disorder, repeat screening should be done by age 4 weeks or at discharge, whichever occurs first. A tickler system is advised to assure appropriate follow-up.





**Right side of form:**

Infant's Name: Print legibly, one letter in each block, the infant's last name and first name or Baby Boy, Baby Girl

Birth Date: Print the month, day, year of birth

ID#: Print in your medical record ID#

Hospital of Birth: Print in the 3 digit code of your hospital and the name or abbreviated name of your hospital. If you are collecting a blood specimen for a retest, please provide the name of the hospital/facility where you want the hard copy of the results mailed.

Date Collected: Print in the month, day, year that the sample was collected

Sex: Mark Male or Female

Birth Weight: Record the baby's birth weight in pounds and ounces

Premature: Mark Yes or No

Time of Birth: Record the time of the baby's birth, be sure to include A.M. or P.M.

Time of Collection: Record the time that the specimen is collected, be sure to include A.M. or P.M.

Mother's Name: Print the mother's last name, then first name

Mother's Address: Print the mother's street address

City/State: Print the mother's city and state of residence

Zip Code: Print the mother's zip code

Mother's Age: Record the mother's age at date of delivery

Phone: Print the mother's area code and phone number

County of Residence: Print the mother's county of residence

Clinician's Name: Print the name of the primary care provider who will be providing care to the baby after discharge from the hospital

Address: Print the address of the above primary care provider

Zip Code: Print the clinician's zip code

Clinician's Phone#: Record the area code and phone number of the above physician

**Left side of form:**

1. 1st test; Routine Test; Diagnosed Case; (Prior Abnormal) Requested by State Lab: Put an (x) in one of the boxes
2. Antibiotics: Mark Yes or No. If yes, write in the name of the antibiotic above the yes. If the antibiotic affects the bioassay, the laboratory will request a repeat test.
3. Transfusion: Mark Yes or No. If yes, record the date of last transfusion
4. Race/Ethnicity: Mark one box
5. Protein Feeding: Mark Breast, Formula or Both. If formula, print the trade name of the formula.
6. When collecting blood, fold back the cover sheet to expose filter paper. Do not touch or handle filter paper before or after applying blood.
7. Clean puncture site with 70% alcohol; allow to dry. Make puncture using sterile lancet with 2.45mm point. Wipe away first drop blood. When second large drop appears, touch filter paper to drop. Allow blood to saturate through the filter paper to fill the circle both in the front and back but do not layer drops one upon another. Fill all six circles with blood. For a copy of the Video on blood collection, call 404-679-0527.
8. Before re-folding back cover sheet, allow blood to dry. Place form at edge of table so blood does not touch anything during drying period. Form must be in horizontal position.
9. CAUTION: Do not collect blood in capillary tubes and apply to filter paper. **Testing must begin within 6 days after collection.** A delay in testing results in deterioration of the specimen. Antibiotics which interfere with test; Penicillin G, Kanamycin, Methicillin, Chloramphenicol, Ampicillin and Oxytetracycline.
10. The State Laboratory assumes responsibility for testing only; whoever submits specimens must assume liability for proper identification, collection and prompt delivery of specimens to the State Lab.
11. Test results will be computer generated.
12. Store kits in cool, dry place.
13. Order no more kits than can be used in 6 months.
14. Mail specimen no later than 12 hours after collection to:

**Georgia Public Health Laboratory  
1749 Clairmont Road  
Decatur, GA 30033-4053**

**WHAT DO I DO IF?**

# WHAT DO I DO IF?

## NEWBORN SCREENING

1. **I WANT A COPY OF THE INFANT'S NEWBORN SCREENING RESULTS?**  
Call the Voice Response System at (404) 321-2293 or 2294 or 2297.
  
2. **I WANT TO HAVE AN INFANT TESTED FOR THE FIRST TIME?**  
Use the Newborn Screening Metabolic and Sickle Cell Disease Test Kits, Form 3491, to obtain the blood sample from the infant. Test Kit can be obtained by calling the State Public Health Laboratory at (404) 327-7920. Follow the instructions sheet for collecting blood. Submit the collected blood sample to the Georgia Public Health Laboratory.
  
3. **I AM ASKED TO COLLECT A SPECIMEN FOR REPEAT TESTING?**  
Follow the instructions you received in the letter requesting you to obtain the blood sample. Make certain that you make a **notation on the form in red ink that the child had a prior abnormal test result.** (See page 6)
  
4. **I AM ASKED TO TEST A PREMATURE INFANT, A SICK INFANT OR AN INFANT WHO HAS HAD AN EXCHANGE TRANSFUSION?** (See page 8)
  
5. **I NEED TO TALK WITH SOMEONE AT ONE AT THE TERTIARY CENTERS?**  

Call for metabolic: Emory University Division of Medical Genetics (404) 727-0486 during business hours After hours/holidays (404) 778-5000, ask for Geneticist on call  or Medical College of Georgia Pediatric Genetics (706) 721-2809	Call for sickle cell: Medical College of Georgia Pediatric Sickle Cell Center HF 1107 Augusta, GA 30912-3730 (706) 721-0174 or Georgia Sickle Cell Center Grady Memorial Hospital (404) 616-3572
--	---
  
6. **THE BABY IS BEING BREAST FED AND NEEDS A METABOLIC SCREEN?**  
Breast milk is an adequate protein challenge. Collect the blood when the baby has been on protein feeding at least 24 hours and the infant is 48 hours old.
  
7. **IF THE CHILD HAS MOVED HERE FROM OUT OF STATE?**  
Retest the child since not all states screen for the same disorders.



8. **A CHILD WHO APPEARS NORMAL HAS A POSITIVE GALACTOSEMIA SCREENING TEST?**  
This is a potential **Medical Emergency!!** Locate the baby immediately. Change the infant's formula to a soy formula such as Isomil, Prosobee, etc. Repeat the galactosemia screening test. Contact the tertiary genetics center for further instructions.
9. **A PREMATURE INFANT CONTINUES TO HAVE LOW T<sub>4</sub> TEST RESULTS AFTER SEVERAL REPEAT TESTS?**  
Contact Karen Grinzaid, M.S. or Sharel Breen, R.N. at Emory University (404) 727-0486.
10. **THE INFANT IS RECEIVING ANTIBIOTICS?**  
Submit the blood specimen within the proper time frame. Indicate which antibiotic the infant is taking. If the antibiotic affects the bioassay, the laboratory will request a repeat test.
11. **IF THE T<sub>4</sub> CONCENTRATION IS ELEVATED?**  
Our screening method detects low concentration of T<sub>4</sub> and, therefore, it is not sensitive for very high concentrations. Our screening method is not intended to detect hyperthyroidism.
12. **A TEST COMES BACK ABNORMAL, DO I HAVE TO FILL ALL THE CIRCLES ON THE REPEAT TEST SPECIMEN WITH BLOOD?**  
No. Three or four properly filled circles of blood are sufficient. Be sure, however, to indicate that you are submitting for repeat testing and not an initial test request. For example, if the report from the initial test is positive for galactosemia, collect the new sample of blood on the filter paper outfit, 3491. Fill in the requested information, check repeat box at the top of the form and write next to the box "REPEAT GAL".
13. **I AM HAVING TROUBLE COLLECTING BLOOD, SHOULD I TRY TO GET SOME BLOOD INSIDE EACH CIRCLE?**  
No. It is better to have 3 or 4 fully filled circles than 6 partially filled ones.

## **HEMOGLOBIN TESTING**

1. **I NEED INFORMATION ON A HEMOGLOBIN I AM NOT FAMILIAR WITH?**  
Contact the Augusta Comprehensive Sickle Cell Center (706)721-0174 or the Georgia Sickle Cell Center at Grady Memorial Hospital (404)616-3994.
2. **I SUBMITTED A SPECIMEN FOR TESTING FOR SICKLE HEMOGLOBIN AND THE TEST RESULTS CAME BACK WITH ANOTHER TYPE OF HEMOGLOBIN.**  
The testing procedure is Isoelectric Focusing which detects many hemoglobin variants - most of which do not cause disease. Because they are genetic in nature, the family should be made aware of this finding.
3. **I HAVE A PATIENT WHO NEEDS TREATMENT FOR A SICKLE CELL CRISIS?**  
Contact either the Grady Hospital Sickle Cell Clinic (404)616-3572 or the Augusta Sickle Cell Center (706)721-0174 for pediatrics or (706) 721-2171 for adults. These centers will help you find appropriate local resources and/or provide you with clinical information.
4. **I NEED LITERATURE TO GIVE MY PATIENTS IN COUNSELING SESSIONS?**  
Contact the Georgia Division of Public Health, Genetics Program Office at (404) 679-0529 or 0530.
5. **SHOULD PATIENTS (CHILDREN OR ADULTS) WITH HEMOGLOBINOPATHIES BE TREATED WITH IRON?**  
Patients with clinically significant hemoglobinopathies should not receive long term iron therapy without appropriate iron binding studies or other diagnostic tests. Formulas do not contain enough iron to be harmful.
6. **I NEED TO SUBMIT A SECOND LIQUID BLOOD SAMPLE TO CONFIRM A SICKLE CELL DISEASE?**  
Submit a 2-4 ml sample of EDTA blood to:

Medical College of Georgia  
Hemoglobin Laboratory  
1120 15th Street  
AC 1004  
Augusta, Georgia 30912

For further information, call Leslie Holley at (706) 721-9640 or Abdullah Kutlar, M.D. at(706)721-2171.

## GENERAL QUESTIONS

1. **WHY IS IT NECESSARY TO RETEST SOME BABIES SEVERAL TIMES?**  
Premature babies may have immature enzyme systems or thyroid functioning. It may be necessary to monitor their progress to be certain they reach normal levels. Collecting blood specimens 48 hours after birth, if possible, and using correct specimen collection procedures will avoid several repeat tests. Care must be taken to see that the collected specimens reach the State Public Health Laboratory within 7 days of collection. Beyond 7 days will require a retest.
2. **WHY ARE SO MANY SPECIMENS MARKED UNSATISFACTORY WHEN I CAN SEE PLENTY OF BLOOD IN THE CIRCLES?**  
There must be an even penetration of blood for the test to be accurate. This means soaking through the filter paper with one application and filling the whole circle so the blood is evenly distributed on both sides of the filter paper.
3. **WHY DO I HAVE TO FILL OUT ALL THE INFORMATION WHEN I KNOW THE BABY?**  
It may be necessary to reach the family in a hurry when you are not available. Also, the State Laboratory receives so many specimens that often there are several babies with the same name and the same birthdate.
4. **WHY ARE THERE SO MANY "FALSE POSITIVES" FOR METABOLIC TESTS?**  
Since this is a screening test, we must be certain that there is no chance of missing a child with a metabolic disorder. Only about 3% of all specimens are actually recalled.
5. **IF A TEST IS NOT DONE FOR SOME REASON IN THE FIRST THREE WEEKS OF LIFE, ISN'T IT USELESS TO RETEST LATER?**  
**NO!** While some disorders may begin to be expressed and some damage may have already occurred, treatment begun at **any time** will always be beneficial to the infant. Additionally, the family should be made aware of the infant's metabolic disorder, its genetic implications, and given appropriate counseling.
6. **I WANT TO JOIN THE VOICE RESPONSE SYSTEM. WHAT SHOULD I DO?**  
You need to register with the Georgia Public Health Laboratory as a participant in the voice response system. Call Dr. M. Ramachandran at (404) 327-6800 for a copy of the registration form.

7. **WHAT IS A CHILD'S UNIQUE I.D.?**

It is the infant mother's social security number followed by 2 digits for the number of delivery (01 for first delivery and 02 for second delivery and so on) and 1 space for the order of delivery (A for Twin A; B for Twin B).

If mother's social security number is not available for any reason, use the mother's birth date and the month and last digit of the year of the infant's date of birth.

Example:

**mm/dd/yy/mm/y** 082178089-01-A for Twin A born on August (08) 99 (only the last digit 9) for the mother with the date of birth 8/21/78.

8. **I WANT A COPY OF THE INFANT'S NEWBORN SCREENING RESULTS.**

Route 1

If you are using the Voice Response System, you need to keep ready: (1) Your license number or submitter code number (2) Your 4 digit PIN number (3) The child's unique I.D. (Mother's SS#).

When you are ready call (404) 321-2293 or (404) 321-2294 or (404) 321-2297 and follow voice prompts.

Route 2

If you are not registered with the Public Health Laboratory for the Voice Response System, keep the following information ready: (1) Baby's last name (2) Date of birth (3) Mother's last name.

Call the Public Health Laboratory at (404) 327-7950 and ask for the test results. For all hard copy mailers, follow Route 2.

# **UNDERSTANDING METABOLIC DISORDERS**

SUMMARY TABLE METABOLIC DISORDERS  
PHENYLKETONURIA (PKU)  
TYROSINEMIA  
HOMOCYSTINURIA  
MAPLE SYRUP URINE DISEASE (MSUD)  
GALACTOSEMIA  
HYPOTHYROIDISM  
CONGENITAL ADRENAL HYPERPLASIA (CAH)

**SUMMARY TABLE  
METABOLIC DISORDERS**

DISORDER	BASIC DEFECT	SYMPTOMS	INCIDENCE	SCREENING CRITERIA	TREATMENT	FOLLOW UP NEEDS
PKU (Classic) Phenylketonuria	Lack of enzyme to properly convert the amino acid phenylalanine to tyrosine.	Severe mental retardation, eczema, seizures, behavior disorders, decreased pigmentation, distinctive "mousey" odor.	1/10,000 - 1/15,000 More common in caucasians but found in all ethnic groups	Elevated Phe 4mg/100 dL or greater	Life long - Low Phenylalanine diet. Possible Tyrosine supplementation	Lifelong dietary management; Careful monitoring of hyperphe variants; Careful management and preconception counseling and intervention for PKU women in the reproductive years.
Congenital Hypothyroidism (Primary)	Absent or hypoplastic gland. Dysfunctional gland. About 20% are genetic in origin.	Mental and motor retardation, short stature, coarse, dry skin and hair, hoarse cry, constipation. Increased incidence of other birth defects.	Overall 1/4,000 with ethnic variation 1/12,000 Black 1/1,000 Hispanic	Low T <sub>4</sub> , Radio Immune Assay (RIA) Elevated TSH	Replacement of L-thyroxine	Maintain L-thyroxine levels in upper half of normal range; Periodic bone age to monitor growth.
Galactosemia (Transferase deficiency)	Absent or low activity of enzyme to convert galactose into glucose. Many variants.	Neonatal death from severe dehydration, sepsis or liver pathology; mental retardation, jaundice, blindness, cataracts.	1/10,000 to 1/60,000	Elevated Galactose (Hill) Low or absent positive fluorescence (Beutler)	Eliminate galactose and lactose from the diet. Soy formulas in infancy; lactose free solid foods.	Provide early monitoring for speech and neurologic problems; Educate parents about hidden sources of lactose; monitor females for secondary ovarian failure; avoid medications with lactose fillers.

**SUMMARY TABLE  
METABOLIC DISORDERS**

DISORDER	BASIC EFFECT	SYMPTOMS	INCIDENCE	SCREENING CRITERIA	TREATMENT	FOLLOW UP NEEDS
Maple Syrup Urine Disease (MSUD)	Absent or low activity of enzyme needed to metabolize leucine, isoleucine and valine found in all natural protein.	Acidosis. Hypertonicity and seizures, vomiting, drowsiness, apnea, coma. Infant death or severe mental retardation and neurological impairment; Behavioral disorders.	1/90,000 to 1/200,000	Elevated Leucine 4mg/100dL or greater	Life Long-Diet low in leucine, isoleucine and valine. Thiamine supplement if responsive.	Educate family and friends regarding strict dietary regimen. Social and education evaluation; Behavioral counseling; Neurological monitoring; Prompt treatment of illness to minimize acidosis.
Homocystinuria	Deficiency of enzyme cystathionine-B-synthase which is needed for homocystine metabolism	Mental retardation, seizures, behavior disorders, onset thromboses, dislocated lenses, tall lanky body habitus.	1/200,000	Elevated Methionine 2mg/100 dL or greater	Methionine restricted diet. Cystine supplement; B <sub>6</sub> supplement if responsive.	Maintain lifelong low methionine diet; Monitor for thrombosis (check pulses, etc.); Ophthalmologic care; educational and psychological evaluation; Avoid unnecessary surgery.
Congenital Adrenal Hyperplasia (CAH)	Defect in the enzyme-21 Hydroxylase Many variant forms	Hyponatremia Hyperkalemia Hypoglycemia Dehydration and early death Ambiguous genitalia in females Progressive virilization in both sexes.	1/15,000 1/3,000 in native Eskimos	Elevated 17-hydroxy Progesterone; abnormal electrolytes	Replace corticosteroids. Plastic surgery to correct ambiguous genitalia	Maintain adequate corticosteroids; Elevate doses or give injectable doses in times of stress; Periodic bone age to monitor adequate treatment; Maintain pediatric endocrinology follow up appointments.

## **PHENYLKETONURIA (PKU) (HYPERPHENYLALANINEMIA)**

Defective metabolism of phenylalanine results in toxic accumulation of this amino acid and deficiencies in its metabolites.

### **Clinical Features:**

Infants with untreated PKU may appear normal in the first few months of life. While in utero, phenylalanine is maintained in the normal range by the mother's system. Cord blood tests are normal in these infants. About 24 hours after the first protein feeding the level of phenylalanine begins to rise to toxic levels. By one year of age, mental and motor retardation, microcephaly, poor growth rate, characteristic odor and seizures or tremors will be evident. Inadequate production of tyrosine (a precursor to pigment formation) results in lighter hair and skin than other family members. The skin may be oily and eczematous. If treatment is not initiated early, most individuals with PKU will achieve an I.Q. of less than fifty.

### **Causes:**

PKU is an autosomal recessive inherited disorder, usually caused by the lack of activity of phenylalanine hydroxylase. Parents are healthy "carriers", but have a one in four (25%) chance for an affected child with each pregnancy.

### **Variant Forms of PKU:**

1. There are several intermediate forms of hyperphenylalaninemia in which the phenylalanine levels are moderately elevated (3-15 mg/dL). Many of these children still require diet restrictions to maintain their blood phenylalanine level in the 2 to 6mg/dL range.
2. Biopterin deficiency (less than 5% of the patient population) is a defect in the production of a co-factor, tetrahydrobiopterin, which results in a PKU-like disorder. Although elevation of phenylalanine is characteristic in these infants the medical management is different from that for infants with PKU. Newborns with confirmed elevated phenylalanine levels should be referred for evaluation by a tertiary treatment center for biopterin cofactor defects and other variant forms.
3. Maternal PKU (MPKU) and hyperphenylalaninemia: Infants born of mothers with PKU or its variants may have elevated plasma phenylalanine in the first hours after birth (even though the infant is genetically normal). Women with hyperphenylalaninemia have an increased risk of miscarriage. Because phenylalanine is elevated throughout the pregnancy, surviving infants suffer stunting, mental retardation, microcephaly and congenital heart defects.



If the mother begins a medically supervised phenylalanine restricted diet before conception and maintains it throughout the pregnancy fetal loss or damage to the fetus may be prevented.

**Laboratory Tests:**

PKU is detected using a bacterial inhibition assay (the original "Guthrie test"). Elevated levels of phenylalanine stimulate the growth of bacteria around the blood spot after it is incubated in a special medium (the normal phenylalanine level is <3 mg/dL).

<u>RESULT</u>	<u>LIKELY CAUSES</u>	<u>ACTIONS</u>
>4-6mg/dL	1. PKU possible 2. Mother has PKU 3. Hyperalimentation	Lab phones results to follow-up team, who phones practitioner with instructions for retesting.
>6 mg/dL (infant of any age) no other tests abnormal	PKU probable	Lab phones follow-up team who phones the clinician and metabolic specialist. <b>Neonatal Emergency</b>

**Considerations:**

Infants should be screened >24 hours after breast or commercial formula feedings have begun. Infants confirmed to have significant hyperphenylalaninemia are placed on special PKU formula. The physician may repeat serum phenylalanine assays a few weeks after beginning this diet. Persistent hyperphenylalaninemia suggests bipterin deficiency. In these cases the metabolic specialist will arrange for co-factor testing.

**Avoiding False Positives and False Negatives:**

False negatives occur in PKU infants who are screened prior to receiving protein feedings, infants who have been taken off protein feedings or infants on antibiotics. False positives may be seen in very sick infants and infants on Total Parenteral Nutrition, goat's milk, or evaporated milk, e.g. Pet, Carnation.

**Treatment:**

Delaying treatment for only a few weeks diminishes intellectual outcome. Dietary treatment in consultation with a pediatric metabolic specialist should be started as soon as possible in any infant with phenylalanine levels over 6mg/dL and should be continued indefinitely, with careful monitoring of blood phenylalanine levels at frequent intervals. **Young women with PKU must maintain their low phenylalanine diet prior to and during pregnancy to prevent fetal loss and fetal brain damage.** The dietary treatment requires use of special formula and specially formulated foods obtained through metabolic centers and pharmacies. Parents require education in assessing food labels and in food preparation.

Families should receive genetic counseling regarding risk for other affected children.

**Expected Outcome:**

With proper dietary treatment, mental retardation is totally preventable and growth and development should be normal.

# TYROSINEMIA

In about 10 percent of newborns, significant tyrosinemia and secondary hyperphenylalaninemia may occur and last as long as 3 months. This is due to immaturity of liver enzymes, and high protein intake, or possibly suboptimal vitamin C intake of the mother or infant. These infants may show subtle learning defects in later childhood. Transient tyrosinemia may be permanently reversed by lowering the protein intake to about 2.0-2.5 g/kg/day and giving 100 mg of vitamin C for several days. After this regime is completed a repeat bacterial inhibition assay should be obtained to confirm that the result is normal.

## Clinical Features:

### Tyrosinemia, Type 1

The chief findings are hepatocellular damage leading to cirrhosis and liver failure, and renal tubular damage resulting in the Fanconi syndrome. Failure to thrive, hepatomegaly, rickets, thrombocytopenia and a profound clotting disorder, which may resist therapy with parenteral vitamin K, are frequent. There is a severe form with rapid deterioration and death in the first weeks of life and milder forms which permit survival until adult life. In older cases, hepatic carcinoma is frequent.

The differential diagnosis of this condition is difficult since it mimics other causes of liver disease.

### Tyrosinemia, Type 2

Corneal ulceration and unusual red, discrete raised hyperkeratotic pustules on the palms and soles are characteristics of hypertyrosinemia, Type 2. Almost half of the patients develop mental retardation. The eye and skin lesions resist all conventional therapy, but respond rapidly to a tyrosine-restricted diet.

## Laboratory Tests:

Elevation of tyrosine is detected with a bacterial inhibition assay. Normal tyrosine levels are <12 mg/dL.

<u>RESULT</u>	<u>LIKELY CAUSES</u>	<u>ACTIONS</u>
Tyrosine 12-19mg/dL	Normal Transient	Retest
Tyrosine >20mg/dL	1. Tyrosinemia 1 or 2 possible 2. Liver disease	Laboratory reports results to follow-up team who phones practitioners with instructions for retesting.

**Treatment:**

These cases should be transferred to a metabolic treatment center where a tyrosine restricted diet is the initial treatment of choice. Additional treatment depends on confirming the diagnosis and response to diet.

**Screening Practice Considerations:**

Detection depends on **protein** ingestion. Hyperalimentation and liver disease may cause false positive results.

# HOMOCYSTINURIA

The majority of cases of homocystinuria are due to deficiency of the enzyme cystathionine synthase, which causes an accumulation of methionine, homocysteine and various metabolites of homocystine.

## Clinical Features:

Clinical manifestations vary in degree, type and age of onset. They include thromboembolism, dislocation of the optic lens, osteoporosis, tall and lanky stature, seizures, psychiatric disturbances and mental retardation.

## Laboratory Test:

Elevation of methionine is detected by a bacterial inhibition assay. The normal methionine level is <1 mg/dL.

<u>RESULT</u>	<u>LIKELY CAUSES</u>	<u>ACTIONS</u>
Methionine >2mg/dL	Homocystinuria possible	Lab will contact follow-up team by phone

## Treatment:

Some individuals with cystathionine synthase deficiency respond to large doses of the vitamin pyridoxine (B<sub>6</sub>). Those not B<sub>6</sub> responsive are given a methionine restricted diet with cystine supplementation. Evidence indicates treatment will prevent mental retardation and prevent or minimize most other effects of homocystinuria.

## MAPLE SYRUP URINE DISEASE (MSUD)

This recessively inherited disorder is characterized by inability to metabolize the branched chain amino acids, leucine, isoleucine and valine.

### Clinical Features:

MSUD is a rare disorder associated with progressive neurological damage within a few days of birth. A high pitched cry, irritability, convulsions, spasticity and central nervous system depression leading to coma are usual. If not treated, the disease leads to death in 2-4 weeks. Biochemically, there is severe metabolic acidosis and hypoglycemia is frequent. Plasma leucine starts to rise usually within 24 hours of birth and within a few days ketoacids appear in the urine. These later possess a characteristic sweet maple syrup odor which gives the disease its name.

As with all hereditary disorders, there are less severe variants, the mildest of which may go undetected for months until some intercurrent illness unmasks the biochemical abnormalities.

### Laboratory Test:

Elevation of leucine is detected by a bacterial inhibition assay. Normal leucine levels are <2 mg/dL; even transient elevation of plasma leucine in the newborn is unusual.

<u>RESULT</u>	<u>LIKELY CAUSES</u>	<u>ACTIONS</u>
leucine>4 mg/dL	MSUD possible	Lab will contact follow-up team by phone <b>Neonatal Emergency</b>

### Treatment:

Any baby in whom the plasma leucine is 4 mg/dL or greater is considered to have MSUD until proven otherwise. A phone call is made to the attending physician to arrange appropriate investigations. Any infant with this disorder needs to be transferred to a major medical center as quickly as possible because the investigations and management are very complicated and death can occur rapidly in untreated cases. Treatment, which must be continued for life, is with a strict diet which controls the intake of the branched chain amino acids. Thiamine is also given in large amounts.

### Screening Practice Considerations:

Detection depends on **protein** ingestion. An affected infant must be detected early if major problems are to be prevented.

# GALACTOSEMIA

The major sugar of milk (and most non-soy commercial infant formulas) is lactose. This sugar is digested to galactose and glucose in the intestine. Galactosemia results from a deficiency in one of the enzymes necessary for the metabolism of galactose.

## **Clinical Features:**

The affected infant may appear normal at birth. Within a few days to two weeks after initiating milk feedings, the infant develops vomiting, diarrhea, lethargy, jaundice and liver damage. Untreated, the disorder may result in death, frequently associated with E. Coli septicemia. Infants surviving the above symptoms evidence developmental retardation, hepatomegaly, Fanconi's syndrome, growth failure and cataracts.

## **Causes:**

Galactosemia is a recessively inherited deficiency in one of the enzymes necessary for normal metabolism of galactose. The severe form of the disorder is due to almost total deficiency of the enzyme, galactose-1-phosphate uridyl transferase (GALT).

## **Variant Forms of Galactosemia:**

There are several genetic variants characterized by less severe reduction in the enzyme activity (e.g. Duarte variant). Although most of these individuals are asymptomatic, all should be evaluated because some will require dietary management and monitoring.

**Galactokinase Deficiency:** This rare enzymatic defect is also recessively inherited. It results in cataracts in infancy and possibly mild mental retardation. The life-threatening symptoms of severe galactosemia do not occur. Newborn screening does not detect this disorder.

## **Laboratory Tests:**

Two screening tests are used to screen infants affected with galactosemia (GALT deficiency).

1. The Beutler's test screens for galactosemia caused by deficiency of GALT. The test is based on the "fluorescence" produced by the reduction of the NADP by the normal enzyme cascade in red blood cells. Deficiency of either of the enzymes will produce a "no-fluorescence" test result. Homozygotes for transferase deficient galactosemia will show "no-fluorescence" while heterozygotes and Duarte variants demonstrate "decreased fluorescence" compared to normal controls. Heat and humidity can cause false positives by denaturing the enzyme as will a patient with G6PD deficiency. To rule out these possibilities a second "FLORIDA TEST" is performed on all presumptive positives from Beutler's test to verify the specimen integrity. A non-viable specimen will not show any fluorescence at all in this test.

The Laboratory also quantitates the galactose metabolites (galactose and galactose-1-phosphate) using a third test (Hill's test) in all inconclusives from the Florida test. Positive GALT deficiency is reported to the follow up team on the day of testing followed by total quantitation of metabolites the following working day.

RESULTS		INTERPRETATION	ACTIONS
Beutler Test	Hill Test		
Inconclusive	within normal limits	1. Variant forms of galactosemia 2. Heat denaturation of the enzymes 3. Improperly collected sample	Retest
Abnormal	$\geq 11$ mg/dL	1 & 2 as above	Lab will contact by letter or phone.
Abnormal	$\geq 14$ mg/dL	Severe galactosemia possible	Lab will contact follow-up team by phone. <b>Neonatal Emergency</b>

**Avoiding False Positives and False Negatives:**

Infants with galactosemia may have false negative screening results if they:

- are being treated with antibiotics,
- have been recently transfused,
- have not received milk or are taking soy formula.

False positives occur frequently as galactose is frequently mildly elevated (6-10 mg/dL) in normal neonates and may be high when there is liver dysfunction from other causes.

**Treatment:**

The galactosemia syndromes are treated by exclusion of lactose and galactose from the diet and should be done in consultation with a pediatric metabolic specialist. Soy formula is used. Parents require education in assessing food labels and in food preparation. Families with galactosemia should be referred for genetic counseling.



**Expected Outcome:**

Individuals adhering strictly to a galactose-free diet achieve satisfactory general health and do not develop liver disease, failure to thrive or cataracts. However, total elimination of dietary galactose does not ensure full normalcy, since galactose can be produced in the body from glucose. Some individuals may evidence mental decline, hyperactivity or speech and language defects. Developmental assessment should be closely monitored. Ovarian dysfunction is observed in some women.

## CONGENITAL HYPOTHYROIDISM

Congenital hypothyroidism occurs in babies who are born without the ability to produce adequate amounts of thyroid hormone. Thyroid hormone is important for normal growth and development. It is essential for normal brain growth. If untreated, congenital deficiency of thyroid hormone results in mental retardation and stunted growth.

### Clinical Features:

Infants with untreated congenital hypothyroidism may appear clinically normal up to three months of age, by which time some brain damage will likely have occurred. When symptoms or clinical signs are present, they may include prolonged neonatal jaundice, constipation, lethargy, poor muscle tone, feeding problems, a large tongue, mottled and dry skin, distended abdomen and umbilical hernia.

### Causes of Congenital Hypothyroidism:

The most common causes are total or partial failure of the thyroid gland to develop (aplasia or hypoplasia) or its development in an abnormal location (an ectopic gland). These types of hypothyroidism rarely recur in siblings. Less commonly, the hypothyroidism results from a hereditary inability to manufacture thyroid hormones, maternal medications during gestation (iodine, antithyroid drugs) or maternal antibodies. A rare form (1:80000) of hypothyroidism may occur with the primary defect being on the pituitary gland or hypothalamus.

### Laboratory Tests:

The initial screening test is the T<sub>4</sub> (thyroxine) assay. The 10 percent of samples with the lowest T<sub>4</sub> results are further tested by a screening TSH assay. Different combinations of results are possible:

<u>RESULT</u>	<u>LIKELY CAUSES</u>	<u>ACTIONS</u>
T <sub>4</sub> low/TSH elevated	1. Hypothyroidism probable	Lab will contact follow-up team by phone and send letter requesting more tests
T <sub>4</sub> <3.0 ug/dL, TSH pending	1. Hypothyroidism possible	Lab will contact follow-up team by phone and send letter requesting more tests
T <sub>4</sub> low/TSH Normal (on one or two specimens unless premature - then repeat third filter paper screening test)	1. Thyroid Binding Globulin (TBG) deficiency 2. False positive result 3. Pituitary gland problems with secondary hypothyroidism 4. Prematurity-see below	Lab will contact follow-up team by letter for further tests

### Thyroid Function in Premature Infants:

Premature infants may have a physiological reduction in blood T<sub>4</sub> levels. This is not due to a low TBG (Thyroid Binding Globulin) and the TSH levels are usually normal. Premature infants have about the

same incidence of hypothyroidism as full term infants. **Premature infants with abnormal thyroid tests need follow-up testing to ensure that the low T4 levels rise to the normal range as the infant matures.** Repeat screens may be submitted.

**Treatment:**

Treatment of congenital hypothyroidism is simple and effective. Thyroid hormone (Synthroid), in pill form, may be crushed and given with formula. The dosage of medication is based on the child's weight but must be individualized and adjusted (by monitoring T4/TSH levels) as the child grows. Pediatric endocrinology consultation should be obtained to determine recommendations for medication adjustment and follow-up for the child. Contact the Newborn Screening Follow-up team for Metabolic Disorders at the Division of Medical Genetics, Emory University at (404) 727-0486.

**Considerations:**

Congenital hypothyroidism is one of the most common disorders detected by newborn screening. The majority of hypothyroid infants are detected on the first specimen, however, a few have a normal first specimen and be abnormal later. In the presence of clinical symptoms, evaluation for congenital hypothyroidism should be performed despite normal screening results. The incidence of additional birth defects increased in these infants.

A low Thyroxine (T4) with a normal Thyroid-Stimulating Hormone (TSH) may indicate a thyroid binding globulin deficiency or a pituitary/hypothalamic problem. The former is of no clinical significance; the latter warrants immediate referral to a pediatric endocrinologist.

**Avoiding False Positive or False Negative Results:**

1. If specimen is collected within the first 3 hours after the birth the TSH will be markedly elevated.
2. A blood transfusion may alter the T4/TSH results.

**Expected Outcome:**

A child whose serum T4 is maintained in the normal range by careful adjustment of thyroid hormone medication should have normal growth, development, and intellectual potential. Goitrous hypothyroidism is more likely to be genetic. Families with this condition should be referred for genetic counseling.

## CONGENITAL ADRENAL HYPERPLASIA

Infants with Congenital Adrenal Hyperplasia (CAH) have a deficiency of an adrenal enzyme resulting in limited cortisol production and, in some cases, limited aldosterone production. The pituitary gland senses the cortisol deficiency and produces increased amounts of ACTH to demand cortisol production by the adrenals. The adrenal glands enlarge but continue to produce inadequate amounts of cortisol. The precursor products of cortisol, some of which are virilizing hormones, accumulate and are released into the circulation. As a result:

- \* because of cortisol deficiency the infant is unable to maintain adequate energy supply and blood sugar levels to meet the stress of injury or illness. Lethargy and coma may progress to death;
- \* because of aldosterone deficiency sodium and accompanying water are lost in the urine resulting in dehydration. Potassium accumulates in the blood, causing irritability or lethargy, vomiting and muscle weakness, including cardiac muscle irritability;
- \* because of rising levels of virilizing hormones female infants may develop clitoromegaly and labial fusion prenatally and be born with ambiguous genitalia.

### **Clinical Features:**

Male infants with CAH usually appear normal at birth but may develop symptoms within the first 2 weeks of life. Female infants may appear normal or may show the effects of virilizing hormones: an enlarged clitoris and fusion of the labia majora over the vaginal opening and may also exhibit symptoms within the first two weeks. Occasionally the female infant will appear to have a normal male penile structure with hypospadias. These females never have a palpable gonad in the labial/scrotal sac. Their ovaries, uterus and Fallopian tubes are normal.

**CAUTION: INFANTS WITH CAH DO NOT ALWAYS APPEAR ILL AT BIRTH OR IN THE FIRST FEW DAYS OF LIFE AND MAY PRECIPITOUSLY BECOME SERIOUSLY ILL AND CAN DIE AT TWO TO THREE WEEKS OF AGE.**

### **Causes of CAH:**

Several types of genetic defects cause the enzymatic deficiencies of CAH. All are autosomal recessive. The newborn screening test currently used is designed to detect the 21-hydroxylase enzyme deficiency. This enzyme deficiency is responsible for over 90% of all forms of CAH. Practitioners should remember that a normal newborn screening test does not rule out other and rarer enzyme deficiencies which cause CAH.

**Laboratory Tests:**

Screening is based upon a radioimmunoassay for a precursor steroid, 17-OHP. Affected infants have high levels and false positive results occur particularly in premature infants. Confirmation is by quantitative steroid assays in blood.

<u>RESULT</u>	<u>LIKELY CAUSES</u>	<u>ACTIONS</u>
1. Inconclusive		Repeat Screen
2. A) 17OH-P>45ng/ml (Normal Birth Weight) B) 17OH-P>80ng/ml (Premature/LBW/<48hrs)	CAH Probable	Letter to physician to repeat screen
3. A) 17OH-P>80ng/ml (Normal Birth Weight) B) 17OH-P>100ng/ml (Premature/LBW/<48hrs)	Abnormal Result	Lab will contact follow-up team who will call infant's physician.

**Treatment:**

The treatment for CAH is replacement hormone medications. Decisions about hormonal treatment should be made in consultation with a pediatric endocrinologist.

1. Glucocorticoids: Use cortisone or hydrocortisone. These medications may be given by mouth or by injection. If the child is vomiting, too lethargic to swallow or unconscious, hormone medication is given intermuscular(IM). The dose is calculated on the basis of weight or body surface area. Oral medications are usually given two or three times daily. IM medications may last several days. In times of vomiting, serious illness, injury or surgery, much higher doses of glucocorticoid are required. Dexamethasone is usually not used in infants because its strength can interfere with normal growth.
2. Mineralocorticoid: Use Florinef™ (fludrocortisone Acetate). Some infants with CAH are able to maintain normal levels of sodium and potassium without the use of this hormone. If it is necessary, it may be given once or twice a day. Over-medication causes hypertension, therefore blood pressure must be monitored. Some infants may also need salt added to their formula.

Medications need to be adjusted as the child grows. Serum adrenal hormone levels and renin may need to be monitored. Female infants who have virilization of the genitalia may need surgical correction. This is usually done in stages, with the first surgery before two years of age. Genetic counseling is indicated for future family planning.

**Considerations:**

The level of 17-OHP indicated on the screening test does not always correlate with the clinical severity of the disorder. Even a mild elevation of 17-OHP in a term baby warrants a clinical evaluation and

a serum sodium and potassium. Some infants with salt wasting and non-salt wasting CAH have a normal first screen and are subsequently detected on the second screen. Parents of all infants with CAH need education about their child's condition. Salt wasters need careful monitoring and lifelong medication adjustment. Those with virilization without salt wasting will also need hormonal therapy to ensure normal growth, pubertal development and fertility. Individuals with non-classical CAH, also identified by newborn screening, will need monitoring in childhood and may also need hormonal treatment as they grow older.

**Adrenal Function in Premature Infants:**

Premature infants usually have higher levels of 17-OHP than term infants. A high 17-OHP is therefore less likely to indicate CAH in a premature infant. The premature infant needs follow-up testing to ensure that the high 17-OHP level falls to the normal range. A 17-OHP persistently >80ng/ml seems too high on repeat screening and low serum sodiums with high serum potassiums and/or ambiguous genitalia should prompt a serum 17-OHP and pediatric endocrine consultation.

**Avoiding False Positive Results:**

Birth is a stressful event. Newborn screening done in the first 12 hours after birth is more likely to show high 17-OHP levels than screening done after the first day of life. The ideal time to screen is 48 hours after birth.

**Expected Outcome:**

Individuals with CAH, if maintained on appropriate doses of medication, will have normal growth, development and intellectual potential. Fertility is usually normal during adulthood.

# **UNDERSTANDING ABNORMAL HEMOGLOBIN**

HEMOGLOBINOPATHIES

S/BETA THALASSEMIA

BART'S HEMOGLOBIN AND ALPHA THALASSEMIA

BETA THALASSEMIA MINOR, INTERMEDIA, AND MAJOR

SUMMARY TABLE HEMOGLOBIN RESULTS

## HEMOGLOBINOPATHIES

Hemoglobinopathies are recessively inherited abnormalities in the structure of hemoglobin. Sickle cell diseases (SS, SC, S-beta thalassemia) affect about one in 1300 Georgia infants (about one in 400 infants of African American descent).

The most clinically significant abnormal hemoglobin condition is sickle cell anemia. In this condition the predominant hemoglobin is hemoglobin S (HbS). In the oxygenated state, HbS functions normally, but when this hemoglobin is deoxygenated, it forms crystal-like rods which deform the red blood cell into a brittle sickle shape. These malformed red blood cells are easily destroyed (resulting in hemolytic anemia) and tend to clump in and occlude small blood vessels (resulting in dactylitis, stroke, pulmonary infarction, splenic sequestration and painful ischemic damage to internal organs).

### **Clinical Features:**

The affected infant appears normal at birth. Anemia develops in the first few months of life as production of fetal hemoglobin decreases and production of HbS increases. The anemia is usually mild, needing no treatment. Enlargement of the spleen results from trapping of sickled red cells in the spleen. If this occurs acutely, severe anemia develops rapidly and transfusions are necessary. Splenic sequestration can result in death. Strokes and acute chest syndrome are also life threatening complications that can occur in childhood.

Infants and children with sickle cell anemia are particularly susceptible to infection due to *Streptococcus pneumoniae*, *Hemophilus influenzae type b*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *E. coli*, and *Salmonella species*. Infection may manifest as pneumonia, meningitis, osteomyelitis, septicemia or other infections. Prompt antibiotic therapy can be life-saving. Scientific studies have shown that **prophylactic oral penicillin started early and maintained throughout childhood decreases the number of episodes of infection and deaths from infection.**

Sickle cell anemia affects growth. By about four years of age, height and weight growth rates are slowed. Chronic hemolysis results in a high prevalence of gallstones. Microvascular occlusion causes retinopathy, nephropathy, leg ulcers and myocardial dysfunction.

### **Variants and other clinically significant hemoglobinopathies identified by newborn screening:**

Clinically significant sickling disease also results when the two genes code for the following beta hemoglobin combinations: S-C, S-D or S-E. Thalassemias are anemias caused by decreased synthesis of normal globin chains and therefore decreased production of hemoglobin A. Beta thalassemia genes may interact with genes for abnormal hemoglobin to cause serious hemoglobinopathies.

### **Trait Conditions:**

Individuals with one abnormal hemoglobin chain and one normal hemoglobin chain (heterozygotes) are carriers - often referred to as



"trait". An inconclusive report refers to the possibility of transfusion interference or presence of other structurally similar hemoglobin. Most hemoglobin carriers have few or no clinical symptoms. Carrier detection provides the opportunity to educate families, to test other family members and to refer for genetic counseling.

**Laboratory Tests:**

The initial screening will be done by isoelectric focusing (IEF). All abnormal results will be rechecked and run through fast flow high pressure liquid chromatograph (HPLC). All sickle cell cases will be identified by citrate agar electrophoresis (CAE) as well. It is expected that very low concentrations of HBS or HBC (less than 1% of the total hemoglobin) especially in a premature infant may escape detection by IEF. The physician should be aware of this possibility.

**Confirmatory Testing:**

Confirmation of suspected infants with sickle cell disease will be done at the Medical College of Georgia using a second liquid blood sample. Questions should be directed to Abdullah Kutlar, M.D., (706) 721-2171.

**Caution: Solubility testing (sickle dex, sickle prep) should never be used as a confirmatory test.**

**Considerations:**

**Treatment: If a neonate is identified with sickle cell disease, prophylactic penicillin should be immediately initiated. Parents need education about how to take a temperature, the care of acute illness and how to assess spleen size.** Consultation with a pediatric hematologist is advised.

In addition to routine required immunizations, infants with sickle cell disease should receive **the Pneumococcal polysaccharide vaccine, the Hepatitis B vaccine and the trivalent influenza virus vaccine (flu vaccine)**. In addition, some physicians may recommend that infants with sickle cell disease receive **the meningococcal vaccine**. Families with sickle cell disease or trait should be referred for genetic counseling.

**Expected Outcome:**

With appropriate medical care and management, death from infection and splenic sequestration can be prevented. Complications from sickle cell disease can be minimized.

# **SICKLE/ BETA THALASSEMIA**

## **WHAT IS HEMOGLOBIN S/BETA THALASSEMIA?**

Beta thalassemias are inherited disorders of  $\beta$  globin synthesis. In most, globin structure is normal but the rate of production is reduced because of decreased transcription of DNA, abnormal processing of pre-mRNA, or decreased translation of mRNA. Decreased hemoglobin synthesis causes microcytosis and unbalance synthesis of  $\alpha$  and  $\beta$  globin leads to ineffective erythropoiesis and hemolysis. Beta thalassemias are usually not detected by newborn screening but hemoglobin F only may be seen with thalassemia major or intermedia. Beta thalassemias also interact with structurally abnormal hemoglobins to produce significant diseases.

Compound heterozygotes who inherit beta thalassemia and Hb S have very significant clinical problems. The S gene from one parent and a beta thalassemia gene from the other parent interact to produce sickle cell disease called sickle/beta thalassemia. There are two forms of this disorder:

1. Sickle/ $\beta^0$  Thalassemia (S Beta zero thalassemia )
2. Sickle/ $\beta^+$  Thalassemia (S Beta plus thalassemia)

Sickle  $\beta^+$  thalassemia can usually be differentiated from sickle cell anemia. Differentiation of Hb S  $\beta^0$  thalassemia may require family studies, DNA analysis, or be delayed until age 2 years when routine hematology or Hb A<sub>2</sub> and F levels usually will allow differentiation. Patients should be treated as if they have sickle cell anemia until the diagnosis is certain. In Hb S  $\beta^0$  thalassemia, no Hb A is made so hemoglobin electrophoresis shows Hb S, increased Hb A<sub>2</sub> and increased Hb F. In Hb S  $\beta^+$  thalassemia, Hb A is reduced so hemoglobin electrophoresis shows Hb S, 5 to 25% Hb A, increased Hb A<sub>2</sub> and increased Hb than in homozygous sickle cell anemia.

The severity of the clinical manifestations show great variation between patients. Most individuals with Hb S  $\beta^+$  thalassemia have preservation of splenic function and less problems with infection, fewer pain episodes and less end-organ damage. Individuals with Hb S  $\beta^0$  thalassemia may have very severe disease, almost identical to homozygous sickle cell anemia. Hemoglobin levels may be higher on average, splenic function is lost later in childhood and splenomegaly is common into adulthood. Pain episodes, end-organ damage and prognosis may be similar. Bone and retinal disease may even be worse when compared to homozygous sickle anemia.

## **WHAT KIND OF SCREENING AND TREATMENT IS NEEDED?**

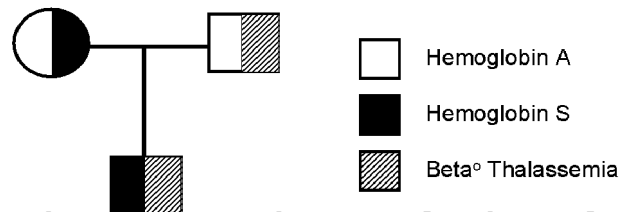
When doing genetic counseling and prenatal diagnosis for a structurally abnormal hemoglobin, one must always consider that one partner may be a carrier of a beta thalassemia gene. These carriers are very easily missed because hemoglobin levels may be normal or near normal and hemoglobin electrophoresis will only show subtle increases in Hb A<sub>2</sub> and Hb F that are often overlooked. A complete blood count with mean corpuscular volume (MCV) and red cell count should always be obtained before counseling couples if one has "normal" electrophoresis. The MCV

by automated cell counter is almost always low in beta thalassemia and the red cell count is often elevated. The percentage of Hb A<sub>2</sub> and Hb F is often diagnostic.

An example is provided to show how questions of non-paternity can arise if beta thalassemia is not considered. In this example, the mother has hemoglobin AS, that is, sickle cell trait. The father has "normal" hemoglobin A. The infant has only sickle hemoglobin. Because electrophoresis may indicate only hemoglobin A in a parent with thalassemia trait, the issue of non paternity may be falsely raised. Health care providers should take great care to avoid misinforming families on this issue.

See pedigree example below. What happened?

The mother's hemoglobin electrophoresis shows Hb AS.  
The father's hemoglobin electrophoresis shows Hb A.  
The newborn infant's hemoglobin electrophoresis shows Hb FS.



In the example, the father shows only hemoglobin A by hemoglobin electrophoresis, but he also has beta° thalassemia minor. This means that the hemoglobin A that is made is normal, but none is produced from one of the two beta genes. Therefore, the child who inherits the sickle gene from his mother and the thalassemia gene from his father is born with **only** fetal (F) and sickle (S), hemoglobin FS. In these cases, family studies must identify thalassemia carriers. To confirm beta thalassemia, family members should have CBC studies looking for a low mean red cell volume (MCV), hemoglobin electrophoresis and HPLC or other studies that quantify hemoglobin A<sub>2</sub> and F.

**Because S/beta thalassemia can be a life threatening disorder and diagnosis of beta thalassemia in the families can be difficult, a thorough evaluation by one of the comprehensive sickle cell centers or a skilled hematologist is needed.** Infants and young children with hemoglobin FS all need penicillin prophylactic therapy until their genotype is confirmed. Those with S/beta° thalassemia should remain on penicillin. There is controversy about the need for prophylactic penicillin in those with S/beta<sup>+</sup> thalassemia but many experts advocate penicillin for both groups.

# BART'S HEMOGLOBIN AND ALPHA THALASSEMIA

The carrier states for alpha thalassemia are very common in the African, Mediterranean and Asian populations. Cord blood testing may detect individuals who have inherited alpha thalassemia because small amounts of hemoglobin Bart's will be present.

## WHAT IS BART'S HEMOGLOBIN?

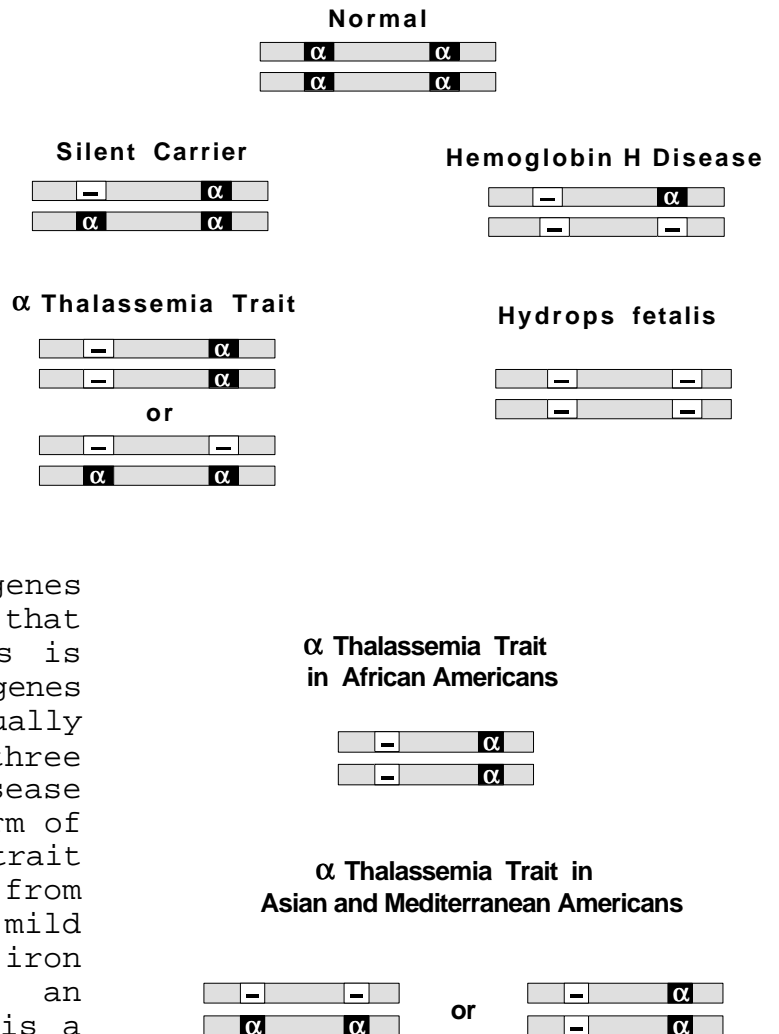
Bart's hemoglobin is four (4) gamma globin chains combined. Normal fetal hemoglobin is two (2) alpha and two (2) gamma globin chains combined. If less alpha globin chains are made, as happens with alpha thalassemia, the extra gamma chains combine to make Bart's hemoglobin.

## WHAT CAUSES ALPHA THALASSEMIAS?

The alpha thalassemias result from the loss of alpha globin genes. There are normally four genes for alpha globin production so that the loss of one to four genes is possible. The lack of all four genes causes hydrops fetalis and is usually fatal *in utero*. The loss of three genes causes hemoglobin H disease which is a moderately severe form of thalassemia. Alpha thalassemia trait (alpha thalassemia 1) results from loss of two genes and causes a mild anemia which resembles iron deficiency anemia. Finally, an individual who loses one gene is a silent carrier (alpha thalassemia 2) with no clinically detectable problems. Loss of one gene may cause small amounts of hemoglobin Bart's to be present in newborn blood samples.

In general, only the loss of one or two genes is seen in African Americans because only one gene deletion on an individual chromosome has occurred. Individuals from Southeast Asia and the Mediterranean area may have all four types of alpha thalassemia because both one and two alpha gene deletions (cis) on the same chromosome have occurred.

The percentage of hemoglobin Bart's in the cord blood sample may indicate the number of alpha genes that have been lost. If the percentage of hemoglobin Bart's is small (less than 10%), the infant most likely has lost one or two alpha genes and will be a silent



carrier or have alpha thalassemia trait. Hemoglobin Bart's between 5 to 10% indicates the presence of alpha thalassemia trait with loss of two alpha genes. If the hemoglobin Bart's is greater than 10% (usually 15 to 20%), a more severe form of alpha thalassemia may be present and further testing is indicated.

#### **WHAT SYMPTOMS OCCUR?**

Individuals with alpha thalassemia trait (hemoglobin Bart's less than 10%) will have a very mild anemia with microcytosis (small red blood cells) and no other clinical problems. This anemia is, however, frequently confused with iron deficiency anemia. Parents of infants with hemoglobin Bart's should be told their child has alpha thalassemia minor and this disorder will have no effect on the child's health. They should be told it is inherited so others in the family may have a similar disorder. They should be instructed to tell health professionals that alpha thalassemia runs in their family to prevent unnecessary tests or treatment with iron. If alpha thalassemia trait is detected in Oriental or Mediterranean infants, family studies should be initiated to detect the presence of more serious forms of alpha thalassemia. Infants with greater than 10% Bart's hemoglobin may have hemoglobin H disease and they should be referred immediately to either the Comprehensive Sickle Cell Center in Augusta, the Georgia Comprehensive Sickle Cell Center at Grady Hospital or a hematologist specializing in hemoglobinopathies.

Long term treatment of infants with alpha thalassemia with supplemental iron will not correct the anemia and may be harmful. Therefore, iron deficiency in children with hemoglobin Bart's should be documented by iron studies (serum iron and total iron binding capacity) or by serum ferritin determination. If iron deficiency is also present, then the child should be treated for six months and the iron supplements discontinued. W.I.C. approved formulas, which contain dietary amounts of iron, can be given to infants with alpha thalassemia without causing any problems.

## **BETA THALASSEMIA MINOR, INTERMEDIA AND MAJOR**

Beta thalassemias are a group of recessively inherited disorders characterized by a decrease or absence of synthesis of the beta globin chains. Individuals homozygous for a beta thalassemia gene often have Cooley's Anemia, also called thalassemia major. Other complex genetic changes result in many other forms of beta thalassemia. The word "thalassemia" means "from the sea" because it was first noted in Italians, Greeks, Turks and others who lived near the Mediterranean Sea.

### **WHAT CAUSES BETA THALASSEMIA?**

Beta thalassemias are inherited disorders of  $\beta$  globin synthesis. In most, globin structure is normal but the rate of production is reduced because of decreased transcription of DNA, abnormal processing of pre-mRNA, or decreased translation of mRNA. Decreased hemoglobin synthesis causes microcytosis and unbalance synthesis of alpha and  $\beta$  globin leads to ineffective erythropoiesis and hemolysis. Beta thalassemias are usually not detected by newborn screening but hemoglobin F only can be seen with thalassemia major or intermedia. Beta thalassemias also interact with a structurally abnormal hemoglobin to produce significant diseases.

### **WHAT ARE THE CLINICAL PROBLEMS IN BETA THALASSEMIAS?**

Individuals who are homozygous for a beta thalassemia may have no production of  $\beta$  globin ( $\beta^{\circ}$  thalassemia) or markedly reduced  $\beta$  globin production ( $\beta^{+}$  thalassemia). Clinical manifestations vary from  $\beta$  thalassemia major which is fatal in early childhood without transfusion to beta thalassemia intermedia where anemia is severe but transfusions are not required. On electrophoresis, Hb F is the major hemoglobin, Hb A<sub>2</sub> is increased and Hb A is absent ( $\beta^{\circ}$ ) or markedly reduced ( $\beta^{+}$ ).

Individuals heterozygous for a normal and beta thalassemia gene have beta thalassemia minor. They have mild anemia, microcytic erythrocytes, and splenomegaly. Hemoglobin electrophoresis shows Hb A with normal or slightly increased Hb F. Hemoglobin A<sub>2</sub> is elevated after age 2 years. The anemia is often confused with iron deficiency anemia.

Beta thalassemia intermedia is caused by inheriting beta<sup>+</sup> thalassemia genes from each parent or inheriting beta<sup>°</sup> thalassemia genes from each parent and also alpha thalassemia trait. Beta thalassemia intermedia is characterized by chronic anemia, enlarged spleen and liver, leg ulcers, bone changes and abdominal pain. However, usually the anemia is not severe enough to require routine blood transfusions.

Compound heterozygous individuals with beta thalassemia and Hb S have very significant clinical problems. Sickle  $\beta^{+}$  thalassemia can usually be differentiated from sickle cell anemia. Differentiation of Hb S  $\beta^{\circ}$  thalassemia may require family studies, DNA analysis, or be delayed until age 2 years when routine hematology or Hb A<sub>2</sub> and F levels usually will allow differentiation. Patients should be treated as if they have sickle cell anemia until the diagnosis is certain. In Hb S  $\beta^{\circ}$  thalassemia, no Hb A is made so hemoglobin electrophoresis shows Hb S, increased Hb A<sub>2</sub> and increased Hb F. In Hb S  $\beta^{+}$  thalassemia, Hb A is

reduced so hemoglobin electrophoresis shows Hb S, 5 to 25% Hb A, increased Hb A<sub>2</sub> and increased Hb than in homozygous sickle cell anemia.

The severity of the clinical manifestations show great variation between patients. Most individuals with Hb S β<sup>+</sup> thalassemia have preservation of splenic function and less problems with infection, fewer pain episodes and less end-organ damage. Individuals with Hb S β<sup>0</sup> thalassemia may have very severe disease identical to homozygous sickle cell anemia. Hemoglobin levels may be higher on average, splenic function is lost later in childhood and splenomegaly is common into adulthood. Pain episodes, end-organ damage, and prognosis may be similar. Bone and retinal disease may even be worse when compared to homozygous sickle anemia.

#### **HOW ABOUT SCREENING AND DIAGNOSIS?**

Electrophoresis will not diagnose beta thalassemia in the newborn, but important clues may be provided. Infants who have only Hb F at birth may have beta thalassemia. Most are normal, but follow-up of all infants with F only is indicated. Similarly, infants with Hb FS or FE may have a structural variant and beta thalassemia and should be followed appropriately.

In children over 2 years and adults the Complete Blood Count (CBC) with mean cell volume (MCV) is the most sensitive screening test. Referral should be considered in any older child or adult with undiagnosed anemia where the MCV is less than 80, when there is increased A<sub>2</sub>, or persistent elevation of fetal hemoglobin.

When doing genetic counseling and prenatal diagnosis for a structurally abnormal hemoglobin, one must always consider that one partner may be a carrier of a beta thalassemia gene. These carriers are very easily missed because hemoglobin levels may be normal or near normal and hemoglobin electrophoresis will only show subtle increases in Hb A<sub>2</sub> and Hb F that are often overlooked. A complete blood count with mean corpuscular volume (MCV) and red cell count should always be obtained before counseling couples if one has "normal" electrophoresis. The MCV by automated cell counter is almost always low in beta thalassemia and the red cell count is often elevated. Quantifying the percentage of Hb A<sub>2</sub> and Hb F is often diagnostic.

**TREATMENT AND FOLLOW UP:**

Patient with beta thalassemia major must be managed in centers that understand the complex management issues in these patients. Blood transfusions are necessary to sustain life, but always lead to chronic iron overload that can cause heart, liver, and endocrine failure. Chelation therapy is always required. Bone marrow transplants have cured many cases. Mortality is most frequently associated with cardiac arrhythmias and congestive heart failure from iron storage in heart muscle tissue. Prenatal diagnosis of beta thalassemia is available using DNA techniques.

Beta thalassemia minor usually requires no special medical management. Iron overload can be a problem if oral or parenteral iron is administered. Because beta thalassemia minor can mimic iron deficiency, this is all too common a problem.



**CONCLUSION:**

Most disorders in Georgia's newborn screening program are not single diseases, but rather a group of variable disorders affecting slightly different parts of a single metabolic pathway. Other variations result from partial inactivation of a particular enzyme. Hemoglobinopathies are also a group of disorders resulting from different alterations in the protein. Methods of testing vary widely from disorder to disorder and screening criteria also vary depending upon the types of disorders included in the state's screening program. Health care providers should be aware of these variations in order to understand current regulations for initial screening, retrieval and retesting.

## SUMMARY TABLE OF HEMOGLOBIN RESULTS

### Hemoglobinopathies

Result	Explanation
FA	no abnormal hemoglobin detected
FA, Bart*s	alpha thalassemia, further testing of parents, child may be needed
FAS	sickle cell trait (carrier)
FS	sickle cell disease SS
FSC	sickle cell disease SC
FSA	sickle cell disease S b thalassemia plus
FS variant	sickle cell disease S variant
FAC	hemoglobin C trait (carrier)
FAE	hemoglobin E trait (carrier)
FE	hemoglobin EE or E b thalassemia
FC	hemoglobin CC or C b thalassemia
FA variant	variant trait (carrier)
FA variant, Bart*s	variant trait a thalassemia
F	undetermined hemoglobin
Unsatisfactory sample	repeat testing
FSA	sickle cell disease, sickle b+ thalassemia
FS + variant	sickle cell disease, hemoglobin S + variant
FAC	hemoglobin C trait (carrier)
FAE	hemoglobin E trait (carrier)
FE	hemoglobin EE or FE b thalassemia
FC	hemoglobin CC or C b thalassemia
FA variant	variant trait (carrier)
F only	possible b thalassemia, Repeat testing required.

## SICKLE HEMOGLOBIN

Hemoglobin S (Hb S) has a substitution of valine for glutamic acid in the sixth position of the  $\beta$  globin chain. Hb S occurs in high frequency in populations previously exposed to falciparum malaria including those from Africa, India, the Mediterranean area and Saudi Arabia. Sickie hemoglobin polymerizes with deoxygenation causing distortion of the erythrocyte and many clinical problems. Sickling of erythrocytes is facilitated by increased temperature (fever), decreased pH (acidosis) and high MCHC (dehydration).

The common sickle cell syndromes result when the gene for sickle hemoglobin is inherited from both parents (Sickle Cell Anemia), when a gene for sickle hemoglobin is inherited from one parent and a gene for hemoglobin C is inherited from the other (Hemoglobin SC Disease) or when a gene for sickle hemoglobin is inherited from one parent and a gene for beta thalassemia is inherited from the other (Hemoglobin S  $\beta$  Thalassemia). There are some differences between these syndromes, but all have similar clinical manifestations. Past statements stressing the benign nature of Hb SC disease and Hb S  $\beta$  thalassemia are generally incorrect.

Clinical manifestations of all syndromes may include moderate to severe hemolytic anemia, increased severity of certain infections, tissue infarction with organ damage and failure and recurrent pain episodes. The hemolytic anemia is generally well tolerated but does lead to premature gall stones in many patients. The anemia may become life-threatening during aplastic crises or splenic sequestration crises. Early recognition and treatment with transfusions are important in both. Splenectomy is done for splenic sequestration in older children and with recurrence. Children and some adults have increased incidence of sepsis, meningitis and other serious infections with *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Salmonella species* and *Mycoplasma pneumoniae*. Tissue infarction may cause significant morbidity and mortality. Strokes occur in children secondary to brain infarctions. Involvement of the bone may cause pain episodes, predispose to osteomyelitis and lead to aseptic necrosis of the femur and humerus. Obstruction of retinal vessels may lead to vitreous hemorrhage or retinal detachment with resulting loss of vision. As patients age, cumulative damage to the lungs and kidneys make pulmonary and renal insufficiency common problems. Pain episodes cause life-long morbidity.

Diagnosis of sickle syndromes at birth with proper care stressing good nutrition, immunization, early treatment of infection, splenic sequestration, aplastic crisis and prophylactic administration of penicillin greatly improves the prognosis in sickle cell patients. Prenatal diagnosis is available for carriers at risk of have affected children.

Individuals who inherit a gene for sickle hemoglobin from one parent and one for hemoglobin A from the other are genetic carriers of sickle syndromes. This is often called sickle trait and is not associated with any hematologic abnormalities. Carriers may have episodes of hematuria (blood in the urine) and may have more urinary tract infections. Rarely, pain episodes or splenic infarctions have been seen with extreme lack of oxygen. Sudden death may be slightly more common at the extremes of human endurance.

## HEMOGLOBIN C

Hemoglobin C (Hb C) has a substitution of lysine for glutamic acid in the sixth position of the  $\beta$  globin chain. Hb C occurs in higher frequency in individuals with heritage from Western Africa. Association of Hb C with the erythrocyte membrane causes red cell dehydration and resulting increase in MCHC. This leads to a shortened red cell survival in Hb C homozygotes and sickling complications in compound heterozygotes for Hb S and Hb C.

Individuals with Hb CC disease inherit a Hb C gene from each parent. They have a mild hemolytic anemia, microcytosis, and target cell formation. There may be occasional episodes of joint and abdominal pain which are attributed to Hb CC disease. Splenomegaly is common. Aplastic crises and gall stones may occur.

Compound heterozygotes with Hb SC disease inherit a Hb C gene from one parent and a Hb S gene from the other. In general, they have a sickle syndrome which is very similar to sickle cell anemia. The hemolysis is usually less severe so the hemoglobin level is higher. Splenomegaly is much more common in older children and adults even though function is lost. There may be more problems with retinal disease and aseptic necrosis. Other manifestations are similar.

Hemoglobin C carriers inherit Hb C from one parent and Hb A from the other. They have no anemia but will usually have target cells on blood smear and may have a slightly lower MCV. There are no other clinical problems.

Individuals who are compound heterozygotes for Hb C and  $\beta$  thalassemia inherit a Hb C gene from one parent and a  $\beta$  thalassemia gene from the other. If they inherit  $\beta^+$  thalassemia there is 65 to 80% Hb C, 15 to 20% Hb A and increased Hb F. If they inherit  $\beta^0$  thalassemia on electrophoresis there is no Hb A and increased Hb F with Hb C. Individuals with Hb C  $\beta^+$  thalassemia have a mild anemia, low MCV and target cells. Individuals with Hb C  $\beta^0$  thalassemia have a moderately severe anemia, splenomegaly and may have bone changes.

## BARTS HEMOGLOBIN AND ALPHA THALASSEMIA

The carrier states for alpha thalassemia are very common in the African, Mediterranean and Asian populations. Cord blood testing may detect individuals who have inherited alpha thalassemia because small amounts of hemoglobin Barts will be present.

The alpha thalassemias result from the loss of alpha globin genes. There are normally four genes for alpha globin production so that the loss of one to four genes is possible. The lack of all four genes causes hydrops fetalis and is usually fatal *in utero*. The loss of three genes causes hemoglobin H disease which is a moderately severe form of thalassemia. Alpha thalassemia trait (alpha thalassemia 1) results from loss of two genes and causes a mild anemia which resembles iron deficiency anemia. Finally, an individual who loses one gene is a silent carrier (alpha thalassemia 2) with no clinically detectable problems. Loss of one gene may cause small amounts of hemoglobin Barts to be present in newborn blood samples. In general, only the loss of one or two genes is seen in African Americans. Individuals from Southeast Asia and the Mediterranean area may have all four types of alpha thalassemia.

The percentage of hemoglobin Barts in the cord blood sample may indicate the number of alpha genes that have been lost. If the percentage of hemoglobin Barts is small (less than 10%), the infant most likely has lost one or two alpha genes and will be a silent carrier or have alpha thalassemia trait. Hemoglobin Barts between 5 to 10% indicates the presence of alpha thalassemia trait with loss of two alpha genes. If the hemoglobin Barts is greater than 10% (usually 15 to 20%), a more severe form of alpha thalassemia may be present and further testing is indicated.

Individuals with alpha thalassemia trait (hemoglobin Barts less than 10%) will have a very mild anemia with microcytosis (small red blood cells) and no other clinical problems. This anemia is, however, frequently confused with iron deficiency anemia. Parents of infants with hemoglobin Barts should be told their child has alpha thalassemia minor and this disorder will have no effect on the child's health. They should be told it is inherited so others in the family may have a similar disorder. They should be instructed to tell health professionals that alpha thalassemia runs in their family to prevent unnecessary tests or treatment with iron. If alpha thalassemia trait is detected in Oriental or Mediterranean infants, family studies should be initiated to detect the presence of more serious forms of alpha thalassemia. Infants with greater than 10% Barts hemoglobin should be referred to tertiary care centers for further evaluation.

Long term treatment of infants with alpha thalassemia with supplemental iron will not correct the anemia and may be harmful. Therefore, iron deficiency in children with hemoglobin Barts should be documented by iron studies (serum iron and total iron binding capacity) or by serum ferritin determination. If iron deficiency is also present, then the child should be treated for six months and the iron supplements discontinued. W.I.C. approved formulas, which contain dietary amounts of iron, can be given to infants with alpha thalassemia without causing any problems.

## BETA THALASSEMIAS

Beta thalassemias are inherited disorders of  $\beta$  globin synthesis. In most, globin structure is normal but the rate of production is reduced because of decrease transcription of DNA, abnormal processing of pre-mRNA, or decreased translation of mRNA. Decreased hemoglobin synthesis causes microcytosis and unbalance synthesis of  $\alpha$  and  $\beta$  globin leads to ineffective erythropoiesis and hemolysis. Beta thalassemias are usually not detected by newborn screening but hemoglobin F only can be seen with thalassemia major or intermedia. Beta thalassemias also interact with structurally abnormal hemoglobins to produce significant diseases.

Individuals who are homozygous for a beta thalassemia may have no production of  $\beta$  globin ( $\beta^0$  thalassemia) or markedly reduced  $\beta$  globin production ( $\beta^+$  thalassemia). Clinical manifestations vary from  $\beta$  thalassemia major which is fatal in early childhood without transfusion to beta thalassemia intermedia where anemia is severe but transfusions are not required. On electrophoresis, Hb F is the major hemoglobin, Hb A<sub>2</sub> is increased and Hb A is absent ( $\beta^0$ ) or markedly reduced ( $\beta^+$ ).

Heterozygotes for a normal and beta thalassemia gene have beta thalassemia minor. They have mild anemia, microcytic erythrocytes and splenomegaly. Hemoglobin electrophoresis shows Hb A with normal or slightly increased Hb F. Hemoglobin A<sub>2</sub> is elevated after age 2 years. The anemia is often confused with iron deficiency anemia.

Compound heterozygotes of beta thalassemia and Hb S have very significant clinical problems. Sickle  $\beta^+$ thalassemia can usually be differentiated from sickle cell anemia. Differentiation of Hb S  $\beta^0$  thalassemia may require family studies, DNA analysis or be delayed until age 2 years when routine hematology or Hb A<sub>2</sub> and F levels usually will allow differentiation. Patients should be treated as if they have sickle cell anemia until the diagnosis is certain. In Hb S  $\beta^0$  thalassemia, no Hb A is made so hemoglobin electrophoresis shows Hb S, increased Hb A<sub>2</sub> and increased Hb F. In Hb S  $\beta^+$  thalassemia, Hb A is reduced so hemoglobin electrophoresis shows Hb S, 5 to 25% Hb A, increased Hb A<sub>2</sub> and increased Hb than in homozygous sickle cell anemia.

The severity of the clinical manifestations show great variation between patients. Most individuals with Hb S  $\beta^+$  thalassemia have preservation of splenic function and less problems with infection, fewer pain episodes and less end-organ damage. Individuals with Hb S  $\beta^0$  thalassemia may have very severe disease identical to homozygous sickle cell anemia. Hemoglobin levels may be higher on average, splenic function is lost later in childhood and splenomegaly is common into adulthood. Pain episodes, end-organ damage and prognosis may be similar. Bone and retinal disease may even be worse when compared to homozygous sickle anemia.

When doing genetic counseling and prenatal diagnosis for structurally abnormal hemoglobins one must always consider that one partner may be a carrier of a beta thalassemia gene. These carriers are very easily missed because hemoglobin levels may be normal or near normal and



hemoglobin electrophoresis will only show subtle increases in Hb A<sub>2</sub> and Hb F that are often overlooked. A complete blood count with mean corpuscular volume (MCV) and red cell count should always be obtained before counseling couples if one has "normal" electrophoresis. The MCV by automated cell counter is almost always low in beta thalassemia and the red cell count is often elevated. Quantitation on Hb A<sub>2</sub> and Hb F is often diagnostic.

## HEMOGLOBIN F

Hemoglobin F (fetal hemoglobin) is the predominant hemoglobin before birth and a normal minor hemoglobin during adult life composing less than 2 percent of total hemoglobin. This percentage is based on a heterogeneous distribution of Hb F with most erythrocytes having no Hb F and a small percentage having higher percentages. Hb F is elevated in a number of anemias, sickle cell anemia and thalassemias with a heterogeneous distribution. High levels with homogeneous distribution are found with hereditary persistence of fetal hemoglobin (HPFH) inherited alone or in combination with Hb S. HbS-HPFH compound heterozygotes have hemoglobin F levels of 15 to 30% and generally have mild clinical disease.

Fetal hemoglobin declines over the first six months of life to near adult levels. Decline is slower in individuals with sickle syndromes, with plateau at adult levels by age 2 years. Increases can be seen during pregnancy, severe anemia and with leukemia. Individuals with  $\epsilon$  thalassemias usually have persistent elevations for life. Those with sickle cell anemia have levels from 2% to 20% with some suggestion that higher levels are associated with reduction in some complications.

Hb F is formed from two alpha and two gamma chains. The gene is duplicated with the product of each differing in a single amino acid glycine or alanine in position 136. Gamma chain hemoglobin variants may be present at birth and rapidly disappear in the first months of life. Because these hemoglobins are not present long after birth, they are of no clinical importance to the individual or family. Differentiation of homozygous Hb SS, Hb S-HPFH and Hb S- $\epsilon$  thalassemia has implications in genetic counseling and may be important to the education and management of the affected individual.

Hemoglobin F only in a newborn sample can reflect normal biologic variation in hemoglobin F synthesis. Hb F only at birth can also be seen in infants with beta thalassemia major and intermedia. Follow-up testing by a tertiary center is indicated until beta thalassemia syndrome can be excluded.

Hb F can be quantitated by alkaline denaturation, high performance liquid chromatography and radial immunodiffusion. Distribution can be determined by acid elution of a smear (Kleihauer-Betke test) or by immunofluorescent techniques using Hb F specific antibodies.

## HEMOGLOBIN E

Hemoglobin E is a structurally abnormal hemoglobin caused by substitution of lysine for glutamic acid at the 26 position of the  $\beta$  globin chain which has a  $\beta^+$  thalassemia phenotype. The substitution causes abnormal processing of pre-mRNA to functional mRNA, resulting in decreased synthesis of Hb E. The Hb E gene is very common in many areas of Southeast Asia, India and China.

Heterozygotes for Hb E and Hb A have no anemia, a low MCV, and target cells on blood smear. Hemoglobin electrophoresis will show about 75% Hb A and 25% Hb E.

Homozygotes for Hemoglobin E may have normal hemoglobin levels or they may have slight anemia. The MCV is low and many target cells are present on blood smear. There is a single band in the Hb C / A<sub>2</sub> position on cellulose acetate electrophoresis and increased Hb F (10 to 15%). There are no significant clinical problems.

Individuals who are compound heterozygotes for Hb E and  $\beta^0$  thalassemia may have a severe disease with severe anemia, microcytosis, splenomegaly, jaundice and expansion of marrow space. Hemoglobin electrophoresis will show Hb E and significant increase in Hb F (30 to 60%). Treatment of severely affected individuals is similar to homozygous beta thalassemia.

Individuals who are compound heterozygotes for Hb E and  $\beta^+$  thalassemia have mild to moderate disease with anemia, microcytosis, splenomegaly and jaundice. Hemoglobin electrophoresis will show Hb E (40%), Hb A (1 to 30%) and significant increase in Hb F (30 to 50%).

## HEMOGLOBIN "D"

There are a number of hemoglobins termed Hb D based on migration on hemoglobin electrophoresis. In general, they are of significance because they migrate in the same position as Hb S on cellulose acetate, alkaline electrophoresis. They move with Hb A on citrate agar, acid electrophoresis. The mobility on isoelectric focusing is variable.

Heterozygotes for Hb D and Hb A are normal. Homozygosity for Hb D is associated with normal hemoglobin levels, decreased osmotic fragility and some target cells. Double heterozygotes for Hb D and  $\beta$  thalassemia have mild anemia and microcytosis.

There are D hemoglobins that interact with Hb S. Hb D-Los Angeles (also called D-Punjab has a substitution of glutamine for glutamic acid at  $\beta$  121. Individuals who are compound heterozygotes for Hb S and Hb D-Los Angeles have moderately severe hemolytic anemia and pain episodes. They may have all of the complications that are seen in sickle cell anemia.

Hb D-Ibadan has a beta 87 substitution of lysine for the normal threonine. Compound heterozygotes for Hb S and Hb D-Ibadan have less anemia and usually do not have other complications.

Hb D will migrate with Hb S on cellulose acetate electrophoresis, however, a solubility test will be negative. Confirmation of suspected Hb S requires electrophoresis with citrate agar, HPLC, isoelectric focusing or other techniques before counseling is offered because of this potential for a false positive initial testing result.

## HEMOGLOBIN "J"

There are 58 hemoglobins designated Hb J by electrophoretic mobility (fast band) on cellulose acetate electrophoresis. The vast majority of these are of no clinical significance. There are 6 that are unstable and Hb J-Cape Town has increased oxygen affinity. The potential for interaction with Hb S is not defined.

The electrophoretic pattern observed may vary significantly depending on whether the alpha or  $\beta$  chain is involved in the mutation and with the stability of the J variant. Hb J-Baltimore is the most common found in Northern European and some African Americans.

Most have no clinical significance and extensive testing and counseling is not generally indicated. Extended testing at a reference laboratory may be indicated if erythrocytosis (high hemoglobin) or anemia is present.

## HEMOGLOBIN G - PHILADELPHIA

Hemoglobin G Philadelphia (Hb G-Phil) is an alpha chain variant which is often associated with deletion alpha thalassemia of the cis (linked) alpha gene. The frequency is increased in African Americans, making this the most common alpha gene variant in this population. The electrophoretic mobility is the same as Hb S on cellulose acetate, causing occasional misdiagnosis of sickle trait.

This hemoglobin variant has no clinical consequences. Individuals should be reassured that there are no clinical problems.

Numerous electrophoretic bands can occur when Hb G-Philadelphia is present. When Hb AA is present, there is usually 25 to 40% Hb G-Phil and a faint band of G<sub>2</sub> in the carbonic anhydrase location. Abnormal density of the Hb S band is present with Hb AS and Hb G-Phil. Four bands are seen with Hb AC and G-Phil. In newborns, hybrids with Hb F are also observed providing potential for multiple bands when other variants are also present.

Rare examples of hemoglobin H disease have been described in association with Hb G-Phil. In these two families, the G-Phil gene mutation linked to a deletion alpha thalassemia was inherited with a two gene deletion alpha thalassemia. Genetic counseling is probably not indicated unless the family is known to carry a two gene deletion, cis alpha thalassemia 1 phenotype.

## HEMOGLOBIN CONSTANT SPRING

Hemoglobin Constant Spring (Hb CSpr) is an alpha chain variant with an elongated alpha globin chain of 30 amino acids. This is caused by a mutation that alters the mRNA termination codon.

Individuals with Hb CSpr may have increased hemoglobin Barts in newborn screening samples. Electrophoretic mobility is between carbonic anhydrase and Hb A<sub>2</sub> on cellulose acetate. The percentage is usually about 1% in heterozygote carriers, 5 to 7% in homozygotes, and 3 to 5% in hemoglobin H disease caused by Hb CSpr with trans two gene deletion alpha thalassemia.

Heterozygotes with Hb CSpr and two normal trans alpha genes are hematologically normal. Homozygotes for Hb CSpr have a mild hemolytic anemia and may have splenomegaly. Individuals with hemoglobin H disease from Hb CSpr and two gene deletion alpha thalassemia have a more severe disease with more Hb H and Bart's than three gene deletion alpha thalassemia.

Hb CSpr is common in Southeast Asia and found in high frequency in American immigrants from some of these areas. Hb CSpr also has been found in Greeks. Counseling is indicated in individuals from these geographic areas because of the high incidence of cis alpha thalassemia 1 which puts couples at risk for having infants with Hb H disease (see alpha thalassemia).

## **HEMOGLOBINS "N"**

This group of 6 fast hemoglobins has electrophoretic mobility between Hb J and Hb H on cellulose acetate electrophoresis. Hb N-Baltimore is the most prevalent N hemoglobin in African Americans. There are no associated hematological abnormalities and counseling is not indicated.



## HEMOGLOBIN O-ARAB

Hemoglobin O-Arab has significance in sickle syndromes because it interacts with Hb S to produce clinical manifestations approaching the severity of Hb SS disease. The amino acid substitution is lysine for glutamic acid in the  $\beta$  121 position.

Heterozygote carriers have no clinical manifestations. Compound heterozygotes for Hb S and Hb O-Arab have hemoglobins in the 7 to 8 gm/dl range with reticulocytosis, jaundice, splenomegaly, episodes of pain and many other complications seen in Hb SS disease. Compound heterozygotes for Hb O-Arab and  $\beta$  thalassemia have manifestations similar to thalassemia intermedia.

Electrophoretic mobility is in the Hb A<sub>2</sub> / C position on cellulose acetate and between Hb S and Hb A on citrate agar, pH 6.2. Migration on isoelectric focusing is with Hb E and Hb C Harlem.

These hemoglobins occur in individuals from North Africa, Arabia, Bulgaria and the eastern Mediterranean area. Counseling of carriers is indicated because of the potential for interaction with Hb S and  $\beta$  thalassemia producing significant disease.

# ***GEORGIA LAW***

## GEORGIA HEALTH CODE ANNOTATED CITATIONS RELATED TO THE GEORGIA GENETIC SCREENING PROGRAM

- 31-12-5. State-wide network for medical genetics services.
- (a) The department and appropriate medical centers shall cooperate in the development of a state-wide network for medical genetics.
  - (b) The network shall be available state-wide and will be responsible for training of personnel in genetics, research in inborn errors of metabolism, and quality control of laboratory services for genetics. This system shall also provide counseling regarding genetically caused disorders. (Code 1933, § 88-1203, enacted by Ga. L. 1978, p. 2262, § 2.)
- Code commission note.** - This section was enacted as § 88-1203, by GA.L. 1978, p. 2262, § 2. Since this section number had previously been enacted by the General Assembly, this section was codified at § 88-1201.3.
- 31-12-6. System for prevention of mental retardation resulting from inherited metabolic disorders.
- (a) The department shall promulgate rules and regulations creating a system for the prevention of mental retardation caused by Phenylketonuria, Galactosemia, Tyrosinemia, Homocystinuria, Maple Syrup Urine Disease, Hypothyroidism, Congenital Adrenal Hyperplasia and such other inherited metabolic disorders as may be determined in the future to cause mental retardation if undiagnosed and untreated. The system shall have five components: screening newborns for the disorders; retrieving potentially affected screenees back into the health care system; accomplishing specific diagnoses; initiating and continuing therapy; and assessing the program.
  - (b) The entire process for screening, retrieval, and diagnosis must occur within the first three weeks of an infant's life, and the system shall be structured to meet this critical need.
  - (c) The department shall be responsible for the screening of all newborns for the disorder and shall be responsible for assessment of the program.
  - (d) The department shall, to the extent state or federal funds are available for such purposes, including but not limited to funds provided under Title V of the Social Security Act maternal and child health funds, provide for retrieving potentially affected screenees back into the health care system; accomplishing specific diagnoses; initiating and continuing therapy; and assessing the program.

- (e) Because the rudiments of such a system already exist, the department shall utilize appropriate existing resources whenever possible and shall cause the coordination and cooperation of agencies and organizations having resources necessary for the creation of an effective system. (Code 1933, § 88-1202, enacted by GA. L. 1978, p. 2262, § 1.)

**Code commission note.** - This section was enacted as § 88-1202 by GA. L. 1978, p. 2262, § 1. Since this section number had previously been enacted by the General Assembly, this section was codified at § 88-1201.2.

**U.S. Code.** - Title V of the Social Security Act, referred to in subsection (d) of this section, is codified as 42 U.S.C.A. §§ 701-716.

**The 1989 Amendment,** effective July 1, 1989. inserted "congenital adrenal hyperplasia" in the first sentence in subsection (a).

- 31-12-7. Rules and regulations regarding tests for phenylketonuria, sickle cell anemia, and sickle cell trait; counseling.
- (a) The department, or its successor agency or department, shall adopt and promulgate appropriate rules and regulations governing tests for Phenylketonuria, Sickle Cell Anemia, and Sickle Cell Trait so that as nearly as possible all newborn infants who are susceptible or likely to have Phenylketonuria, Sickle Cell Anemia, or Sickle Cell Trait shall receive a test for Phenylketonuria, Sickle Cell Anemia, or Sickle Cell Trait, or all of such conditions as soon after birth as successful testing and treatment therefore may be initiated; provided, however, that this Code section shall not apply to any infant whose parents object thereto on the ground that such tests and treatment conflict with their religious tenets and practices.
- (b) If any such child is found to have Phenylketonuria, Sickle Cell Anemia or Sickle Cell Trait, it shall be the duty of the examining physician or the department to inform the parents of such child that the child is so afflicted and, if such child has Sickle Cell Anemia or Sickle Cell Trait, that counseling regarding the nature of the disease, its effects, and its treatment is available without cost from the department and the county board of health or county department of health.

(c) It shall be the duty of the department and each county board of health and county department of health, or their successor agencies or departments, to furnish counseling and advice to any persons requesting such counseling regarding Sickle Cell Anemia or Sickle Cell Trait, its characteristics, symptoms, traits, effects, and treatment. Such counseling shall be furnished without cost to the person requesting it. (Code 1933, § 88-1201.1, enacted by Ga. L. 1966, p. 140, § 1; Ga. L. 1972, p. 962, § 1.)

\* 31-12-5 through 31-12-7 Control of Hazards, Diseases, Etc., Georgia Health Code Annotated Citations, pp. 239-240.

**DHR RULES  
AND  
REGULATIONS**

# RULES OF DEPARTMENT OF HUMAN RESOURCES PUBLIC HEALTH

## CHAPTER 290-5-24 TESTING FOR INHERITED DISORDERS IN THE NEWBORN

### TABLE OF CONTENTS

290-5-24-.01 Definitions	290-5-24-.03 Enforcement
290-5-24-.02 Provisions	

290-5-24-.01 **Definitions.** Unless a different meaning is required by the context, the following terms as used in these rules shall have the meaning hereinafter ascribed to same:

- (a) "Department" means the Department of Human Resources of the State of Georgia;
- (b) "Adequate blood test" means any test or procedure capable of detecting the screening criteria established by the Department;
- (c) "Approved laboratory" means a laboratory licensed by the Department to perform tests in metabolic studies which include tests for Phenylketonuria, Galactosemia, Tyrosinemia, Homocystinuria, Maple Syrup Urine Disease, Hypothyroidism, Congenital Adrenal Hyperplasia and the subcategory which includes sickle cell hemoglobin testing;
- (d) "Phenylketonuria" means an inherited error in the metabolism of phenylalanine;
- (e) "Galactosemia" means an inherited error in the metabolism of galactose;
- (f) "Tyrosinemia" means an inherited error in the metabolism of tyrosine;
- (g) "Homocystinuria" means an inherited error in the metabolism of methionine;
- (h) "Maple Syrup Urine Disease" means an inherited error in the metabolism of leucine, isoleucine and valine;
- (i) "Hypothyroidism" means a deficient amount or activity of thyroid hormone;

- (j) "Abnormal test result" means any test result different from the screening criteria as determined by the Department for Phenylketonuria, Galactosemia, Tyrosinemia, Homocystinuria, Maple Syrup Urine Disease and Hypothyroidism (thyroxine), Congenital Adrenal Hyperplasia or revealing the presence of sickle hemoglobin;
- (k) "Physician" means a person lawfully licensed in this State to practice medicine and surgery pursuant to Chapter 43-34 of the Official Code of Georgia Annotated;
- (l) "Hospital" means any institution designed, equipped and staffed to receive two or more persons for diagnosis, treatment and other health services under the supervision of a practitioner for periods continuing twenty-four (24) hours or longer, and in which professional policies are adopted by the governing body after consultation with the active professional staff;
- (m) "Appropriate medical facility" means a facility designated by the Department with a full-service consultative program which is capable of performing diagnostic tests, appropriate therapy and genetic counseling for inherited metabolic disorders and abnormal hemoglobin;
- (n) "Patient Required Information" means information as specified on forms provided by the Department for this purpose;
- (o) "Sickle Cell Anemia" refers to sickle cell syndromes in which an individual has inherited sickle cell hemoglobin from one parent and, either sickle cell hemoglobin, or another abnormal hemoglobin, from the other parent resulting in a clinically significant sickling disorder.
- (p) "Sickle Cell Trait" means a condition in which the individual has inherited sickle cell hemoglobin from one parent and normal hemoglobin from the other (heterozygous). Other traits occur when the individual has inherited another abnormal hemoglobin from one parent and normal hemoglobin from the other.
- (q) "Sickle cell hemoglobin" means an abnormal hemoglobin which results from an inherited defect and which produces the sickling phenomenon in erythrocytes;
- (r) "Counsel" means the giving of information and advice appropriate to the individual situation;



- (s) "Congenital Adrenal Hyperplasia" means an inherited error in the metabolism of steroid hormones;

Authority OCGA SECS. 31-2-4, 31-12-5, 31-12-6, 3-12-7.  
290-5-24-.02 Provisions.

- (1) Every live born infant shall have an adequate blood test for all disorders defined in Rule 290-5-24-.01 unless its parents for religious reasons object to such testing.
- (2) When a live birth occurs in a hospital the physician shall have a specimen of the infant's blood taken prior to the infant's discharge from the hospital.
- (3) The infant's blood for these tests shall be collected not earlier than forty-eight (48) hours after birth and no later than when the infant is one week old.
- (4) If the infant born in a hospital is discharged before forty-eight (48) hours after birth, a blood specimen shall be collected prior to discharge. In this case the newborn must be tested again prior to one week of age. The administrator or a designated representative shall provide written notice of this requirement to the parents, guardian, or other legally responsible person.
- (5) The blood sample and the required patient information must be sent to an approved laboratory on the day of collection for an adequate test.
- (6) When a live birth occurs in a facility other than a licensed hospital, it shall be the responsibility of the person in charge of the facility or the person in attendance, to give written notice to the parents, guardian or other legally responsible person of the legal requirements for the newborn to be tested and to advise where testing can be obtained.
- (7) Laboratories performing blood tests for the detection of Phenylketonuria, Galactosemia, Tyrosinemia, Homocystinuria, Maple Syrup Urine Disease, Hypothyroidism, Congenital Adrenal Hyperplasia and Sickle Cell hemoglobin for the purpose of satisfying the legal requirements for testing newborns shall report all such test results to the attending physician and the hospital where the birth occurred; the results shall be made a part of the clinical record. Such laboratories shall report all results for Phenylketonuria, Maple Syrup Urine Disease, Tyrosinemia, Homocystinuria, Galactosemia, Hypothyroidism, Congenital Adrenal Hyperplasia and Sickle Cell hemoglobin to the Central Laboratory of the Department on the day the testing is completed and this report shall include the patient's required information.
- (8) In the event of an abnormal test result for Phenylketonuria,

Maple Syrup Urine Disease, Tyrosinemia, Homocystinuria, Galactosemia, Hypothyroidism and Congenital Adrenal Hyperplasia and Sickle Cell hemoglobin the laboratory doing the testing shall notify within twenty-four (24) hours the appropriate medical facility, who will telephone the attending physician. If the appropriate medical facility is unable to reach physician, it will then contact the parents by telephone. If the parents cannot be reached or are non-responsive, the appropriate medical facility will contact the local health department for assistance.

Authority OCGA SECS. 31-2-4, 31-12-5., 31-12-6, 31-12-7.  
290-5-24-.03 Enforcement. The administration and enforcement of these rules and regulations shall be as prescribed in Chapter 31-5 of the Official Code of Georgia Annotated.

Authority OCGA 31-2-4, 31-12-5, 31-12,6, 31-12-7.

# **RESOURCES**

## **RESOURCES FOR NEWBORN SCREENING AND CLINICAL INFORMATION**

### **Contact Persons for Metabolic Disorders :**

Louis J. Elsas, II, M.D.  
Paul M. Fernhoff, M.D.  
R. Dwain Blackston, M.D.  
Nicola Longo, M.D., M.P.H.  
Emory University School of Medicine  
Department of Pediatrics  
Division of Medical Genetics  
2040 Ridgewood Drive  
Atlanta, Georgia 30322  
(404)778-5000  
Ask for the geneticist or the pediatric endocrinologist on call.

David B. Flannery, M.D.  
*\* PKU, MSUD, Homocystinuria, Tyrosinemia and Galactosemia*  
(706) 721-2809  
William H. Hoffman, M.D.  
*\* Hypothyroidism and CAH*  
(706) 721-4158  
Department of Pediatrics  
Medical College of Georgia  
Augusta, Georgia 30912  
(706) 721-2390

### **Contact Persons for Sickle Cell and Other Hemoglobinopathies:**

Medical College of Georgia  
Kathleen McKie, M.D.  
Pediatric Sickle Cell Center  
HF 1107  
Augusta, GA 30912-3730  
(706) 721-0174

Georgia Sickle Cell Center at Grady Hospital  
Jim Eckman, M.D.  
P.O. Box 109  
80 Butler Street  
Atlanta, GA 30335  
(404) 616-5982  
24 hour number: (404) 616-3572

**Contact Persons for Specimen Collection and Testing:**

Laboratory Manager  
Newborn Screening Laboratory  
Division of Public Health  
Department of Human Resources  
1749 Clairmont Road  
Decatur, Georgia 30033-4050  
(404)327-7951

M. Ramachandran, Ph.D.  
Laboratory Clinical Program Director  
Newborn Screening Laboratory  
Division of Public Health  
Department of Human Resources  
1749 Clairmont Road  
Decatur, Georgia 30033-4050  
(404)327-6800

**Order collection kits from:**

Georgia Public Health Laboratory  
Laboratory Support Services  
1749 Clairmont Road  
Decatur, Georgia 30334  
(404)327-7920

**Confirmation Testing for Sickle Cell Diseases:**

Leslie Holley  
Medical College of Georgia  
Hemoglobin Laboratory  
1120 15th Street  
AC 1004  
Augusta, Georgia 30192  
(706) 721-9640

**Counseling Extended Family Testing for Sickle Cell Traits:**

Jean Brannan  
Executive Director  
Sickle Cell Foundation of Georgia  
2391 Benjamin E. Mays Drive, S.W.  
Atlanta, Georgia 30311  
(404)755-1641

**Information on the Genetics Program, including posters, videotapes and technical assistance:**

Mary Ann Henson, R.N., M.S.N.  
Genetics Program Manager  
Children's Health Services Unit  
2600 Skyland Drive, NE  
Atlanta, Georgia 30319  
(404)679-0527

Supported in part by project #131008-01-0 from the Maternal and Child Health Program (Title V, Social Security Act). Health Resources & Services Administration, Department of Health and Human Services