

CP 3.1.1 Biopterin Testing

POLICY:

1. Testing for Tetrahydrobiopterin (BH4) deficiency is done on all newborns whose recall serum phe level is positive and who **DO NOT** have siblings with diagnosed PKU (if siblings have been proven to have PKU, it is genetically extremely unlikely that this baby has Biopterin Deficiency)
2. Biopterin follow-up testing is conducted by the Genetic Disease Laboratory at no extra charge to the family. GDL will enter test results in SIS.
3. Biopterin metabolites are measured by testing blood and urine samples. Both are required for follow-up testing and both urine and blood spots can be obtained any time even if patient is on a phe-restricted diet.

GENERAL INFORMATION AND GUIDELINES FOR RECALL POSITIVES:

BH4 deficiency is a progressive, frequently fatal neurological disorder associated with elevation of blood phenylalanine level. BH4 is a co-enzyme for phenylalanine hydroxylase (PAH), as well as tyrosine and tryptophan hydroxylase. As a cofactor for PAH, BH4 deficiency can cause hyperphenylalaninemia. As a cofactor for tyrosine and tryptophan hydroxylase BH4 deficiency can result in the lack of neurotransmitters (dopamine, serotonin, norepinephrine and epinephrine) and impaired synthesis of DOPA (an amino acid derived from tyrosine that is involved in the formation of dopamine). The deficiency is caused by the lack of enzymes that either regenerate or synthesize BH4. If hyperphenylalaninemia is due to BH4 deficiency, phenylalanine restriction initiated after a positive confirmatory test for phenylketonuria (PKU) does not result in alleviation of clinical symptoms. Untreated patients exhibit mental retardation, seizures, abnormal tone/posture, drowsiness, irritability, abnormal movements, recurrent hyperthermia without infection, and hypersalivation, or swallowing difficulties. The goal of clinical treatment is to control hyperphenylalaninemia by restriction of phenylalanine and/or BH4 administration, and to replace missing neurotransmitters by administration of L-Dopa and 5-hydroxytryptophan.

Enzymes Involved in BH4 Synthesis

- guanosine triphosphate cyclohydrolase (GTPCH) is one of the enzymes involved in the synthesis of BH4.
- 6 pyruvoyltetrahydrobiopterin synthase (PTPS) is also involved in the synthesis of BH4 and is the most common cause of BH4 deficiency.
- dihydropteridine reductase (DHPR) is the enzyme involved in the regeneration or recycling of BH4.
- Pterin-4a-carbinolamine dehydratase (PCD)

1. Urine Sample

The activity of enzymes **Guanosine Triphosphate (GTP) Cyclohydrolase** and **6 pyruvoyltetrahydrobiopterin synthase (PTPS)** can be measured in urine via analysis of neopterin and biopterin content. The metabolites of these enzymes decompose when exposed to light (including artificial light). Therefore, the specimen must be frozen and shielded from light.

- Low biopterin excretion, high neopterin excretion indicate PTPS deficiency
- Very low excretion of both biopterin and neopterin indicate (GTP)cyclohydrolase-I deficiency

3.1.1 Biopterin Testing

2. Blood sample

The activity of the enzyme **dihydropteridine reductase (DHPR)** can be measured using direct assay of dried blood spots. Testing of dried blood spots eliminates the need for repeating the urine test when tetrahydrobiopterin is low in the initial sample. Due to the instability of dihydropteridine reductase, the specimen must be sealed in a plastic bag and shipped in dry ice.

The specimens (blood and urine) can be obtained anytime, even after the initiation of a phenylalanine-restricted diet.

Baby must **not** have received blood transfusion within the last 90 days. A transfusion may invalidate DHPR results.

Associated Forms/Documents:

3.1.2 Instructions for Collecting and Shipping BD Specimens for Biopterin Testing

3.1.3 Biopterin TRF

PROTOCOL:

Resp Person	Action
ASC NBS Coord .	<ul style="list-style-type: none"> • Follows the protocol for PKU – Follow-up of Positives (Follow-up of Initial Positive Results) through the receipt of second screen positive result and facilitation of referral to a CCS-approved metabolic center for diagnosis and treatment. • Provides confirmatory specimen collection/processing instructions to the PCP and/or CCS Center upon request (See 3.1.2 <i>Instructions for Collecting and Shipping Bio-Specimens for Biopterin Testing</i>). • If test is positive for biopterin deficiency, indicates such in SIS, following Case Resolution Protocol (3.20). If negative, resolves case as PKU per confirmation of diagnosis from MD/SCC.
Metabolic Center	<ul style="list-style-type: none"> • Contacts PCP to discuss health status of newborn and follow-up for newborn referred to SCC. Determines if immediate visit at SCC is necessary. Schedules appointment as appropriate. • Obtains urine/blood specimens (see 3.1.3 <i>Biopterin Specimen Collection Form</i>) as per GDL instructions. Provides information and instruction to family regarding specimen collection. • At initial visit assists parent/guardian with completion of CCS application. • Faxes completed CCS application to local CCS office. • Bills per family's insurance and CCS guidelines. • Enters appointment status, Metabolic Service Record (MSR), and case notes, if appropriate, into SIS.