Biotinidase Deficiency: *BTD* Gene Sequencing

**Condition Description**

Biotinidase deficiency (BTD) is an autosomal recessive inborn error of biotin metabolism [1]. It is clinically and biochemically similar to the disorder early-onset or holocarboxylase synthetase deficiency which is caused by mutations in the holocarboxylase synthetase gene (HCLS) (refer to the holocarboxylase synthetase deficiency test for more information) [2]. Biotin is an essential water-soluble vitamin that serves as a coenzyme for four carboxylases in humans (acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase, and b-methylcrotonyl-CoA carboxylase) [3]. Its serum level depends on dietary biotin intake and the recycling of endogenous biotin. Biotinidase is the enzyme that catalyzes the cleavage of biotin from biocytin or biotinylpeptides, which are the products of carboxylase degradation. BTD is classified as either profound or partial based on the serum biotinidase activity (0-10% and 10-30%, respectively). Profound BTD can present between the ages of one week and ten years, with a mean age of three and one-half months [4]. In an untreated state it is usually characterized initially by seizures, hypotonia, ataxia, developmental delay, vision problems, hearing loss, and cutaneous abnormalities such as alopecia, skin rash, and candidiasis. With age, motor limb weakness, spastic paresis, and decreased visual acuity occur. Individuals with partial BTD may have hypotonia, skin rash, and hair loss, particularly during times of stress. Once vision problems, hearing loss, and developmental delay occur, they are usually irreversible even with biotin therapy. Early recognition and biotin supplementation results in rapid clinical improvement. Newborn screening allows early presumptomatic treatment that can prevent neurological delays [5]. The age of onset is one of the distinguishing factors, with BTD typically presenting after 3 months of age and holocarboxylase synthetase deficiency typically presenting before 3 months. The symptoms in these disorders are similar and clinical differentiation is often difficult. Organic acid abnormalities are similar in BTD and holocarboxylase synthetase deficiency and may be reported as consistent with multiple carboxylase deficiency on tandem mass spectrometry utilized in neonatal screening. Definitive enzyme determinations are required to distinguish between the two disorders [6]. Biotinidase activity is normal in serum of individuals with holocarboxylase synthetase deficiency; therefore, the enzymatic assay of biotinidase activity used in newborn screening is specific for biotinidase deficiency and does not identify children with holocarboxylase synthetase deficiency. Both biotinidase deficiency and holocarboxylase synthetase deficiency are characterized by deficient activities of the three mitochondrial carboxylases in peripheral blood leukocytes prior to biotin treatment. In both disorders, these activities increase to near-normal or normal after biotin treatment. BTD is caused by mutations in the *BTD* gene (3p25) and about 100 mutations have been described worldwide to date [7-8]. Genotype-phenotype correlations are limited [9-10]. Gene sequence analysis is available to test for mutations in the *BTD* gene (test code JJ). For patients with mutations not identified by full gene sequencing, a separate deletion/duplication assay is available using a targeted CGH array (test code JJ).

**Indications**

- Confirmation of a clinical/biochemical diagnosis of biotinidase deficiency
- Carrier testing in adults with a family history of biotinidase deficiency

**Methodology**

PCR amplification of 4 exons contained in the *BTD* gene is performed on patient genomic DNA. Direct sequencing of amplification products is performed in both the forward and reverse directions using automated fluorescence dideoxynucleotide sequencing methods. Patient gene sequences are compared to a normal reference sequence. Sequence variations are then classified as mutations, benign variants unrelated to disease or variations of unknown clinical significance. Variants of unknown clinical significance may require further studies of the patient and/or family members. This assay does not interrogate the promoter region, deep intronic regions or other regulatory elements. Large deletions are not detected by this analysis.

**References**

### Detection

The majority of patients with clinical and biochemical diagnosis of BTD will have an abnormal DNA test.  
Clinical Sensitivity: 97/98 mutations identified in 49 patients [8], 26/26 mutations identified in 13 patients [12].  
Analytical Sensitivity: ~99%

### Specimen Requirements

Submit only 1 of the following specimen types

- Preferred specimen type: Whole Blood

#### Type: Whole Blood

Specimen Requirements:

- In EDTA (purple top) tube:  
  - Infants (2 years): 3-5 ml  
  - Older Children & Adults: 5-10 ml

Specimen Collection and Shipping: Refrigerate until time of shipment. Ship sample within 5 days of collection at room temperature with overnight delivery.

#### Type: Saliva

Specimen Requirements:

- Oragene™ Saliva Collection kit (available through EGL) used according to manufacturer instructions.

Specimen Collection and Shipping: Store sample at room temperature. Ship sample within 5 days of collection at room temperature with overnight delivery.

### Special Instructions

Submit copies of diagnostic biochemical test results with the sample. Sequence analysis is required before deletion/duplication analysis by targeted CGH array. If sequencing is performed outside of EGL Genetics, please submit a copy of the sequencing report with the test requisition.

### Related Tests

- Biotinidase enzyme assay (BX), urine organic acid analysis (OA) and plasma acylcarnitine analysis (AR) are used in the diagnosis of a patient with BTD.
- **Custom diagnostic mutation analysis (KM)** is available to family members if mutations are identified by sequencing.
- A deletion/duplication assay is available separately for individuals where mutations are not identified by sequence analysis. Refer to the test requisition or contact the laboratory for more information.
- Prenatal testing is available for known familial mutations only. Please call the Laboratory Genetic Counselor before collecting a fetal sample.