Louisiana Office of Public Health Laboratories	
Test Name	GSP Neonatal Biotinidase Kit Time Resolved Fluoroimmunoassy
PHL Location	Central Lab 1209 Leesville Avneue Baton Rouge, La. 70802
CPT Code	82261
Synonyms	Biotinidase
Brief Description of Test	This kit is intended for the quantitiative in vitro determination of human biotinidase activity in blood specimens dried on filter paper as an aid in screening newborns for biotinidase deficiency using the GSP instrument.
Possible Results	Normal Abnormal (Presumptive Positive)
Reference Range	>50 u/dL = Normal $\underline{<}$ 50 u/dL = Presumptive Positive
Specimen Type	Neonatal Dried Blood Spot
Specimen Container(s):	Standard letter size manila envelopes can be used for shipping
Minimum volume accepted:	Minimum of two completely filled blood spot circles.
Collection Instructions	Blood specimens should be taken directly from a heel prick onto filter paper. **See webaddress below http://www.ldh.louisiana.gov/index.cfm/page/488**
Causes for Rejection	Specimen > 14 days old, clotted or layered, serum rings, scratched or abraided, insufficient quantity for testing, not completely dry before mailing, blood applied to both sides of the filter paper, diluted discolored or contaminated, collection using capillary tubes containing EDTA, >12 months old, circles not completely filled.
Storage and Transport Instructions	Allow the blood specimen to air-dry in a horizontal position for at least 3 hours at ambient temperature (+18 to +25 ^{o}C), not in direct light. Do not heat or stack the specimens during the drying process. Transport or mail the specimen to the laboratory within 24 hours after collection, unless otherwise directed by the screening laboratory.
Limitations of the Procedure	Ampicillin (1.4 mg/dL and above), sulfisoxazole (7.5 mg/dL and above) at low biotinidase activity levels (35 U/dL) and ampicillin (2.8 mg/dL) at high biotinidase activity levels (150 U/dL) were found to interfere with this test by increasing measured biotinidase activity. Elevated ampicillin and sulfisoxazole levels near the biotinidase cut-off did not exhibit a significant effect. Glutathione levels above normal (> 30 mg/dL) can interfere with this test by increasing biotinidase activity. This could result in the misclassification of a patient

	with a biotinidase result near the cut-off value as ‘normal’ when in fact, the patient should be classified as ‘deficient’. A patient with known or clinically suspected elevated blood glutathione concentration should be screened with an alternative method and confirmed according to local requirements for follow-up testing. Unconjugated bilirubin (10 mg/dL) added to whole blood at low biotinidase activity levels (35 U/dL) were found to interfere with this test by increasing measured biotinidase activity. Elevated unconjugated bilirubin level near the biotinidase cut-off did not exhibit a significant effect. Conjugated bilirubin (2.5 mg/dL and above) and triglyceride (250 mg/dL and above) added to whole blood were found to interfere with this test by decreasing measured Biotinidase activity. Elevated conjugated bilirubin triglyceride (250 mg/dL and above) levels may cause a false positive screening result for a specimen with measured biotinidase activity near the cut-off.
Interfering Substances	Ampicillin (1.4 mg/dL and above), sulfisoxazole (7.5 mg/dL and above) at low biotinidase activity levels (35 U/dL) and ampicillin (2.8 mg/dL) at high biotinidase activity levels (150 U/dL) were found to interfere with this test by increasing measured biotinidase activity by 19.9%, 32.1% and 15.6%, respectively. Elevated ampicillin (2.8 mg/dL) and 13907242-1 (en) 19 sulfisoxazole (15 mg/dL) levels near the biotinidase cut-off did not exhibit a significant effect (< 15%). Glutathione levels above normal (> 30 mg/dL) can interfere with this test by increasing biotinidase activity by 16.1% or more. This could result in the misclassification of a patient with a biotinidase result near the cut-off value as ‘normal’ when in fact, the patient should be classified as ‘deficient’. A patient with known or clinically suspected elevated blood glutathione concentration (> 30 mg/dL) should be screened with an alternative method and confirmed according to local requirements for follow-up testing. Unconjugated bilirubin (10 mg/dL) added to whole blood at low biotinidase activity levels (35 U/dL) were found to interfere with this test by increasing measured biotinidase activity. Elevated unconjugated bilirubin level (20 mg/dL) near the biotinidase cut-off did not exhibit a significant effect (< 15%). Conjugated bilirubin (2.5 mg/dL and above) and triglyceride (250 mg/dL and above) added to whole blood were found to interfere with this test by decreasing measured biotinidase activity by 26.0% and 15.7%, respectively. Elevated conjugated bilirubin (2.5 mg/dL and above) and triglyceride (250 mg/dL and above) levels may

	cause a false positive screening result for a specimen with measured biotinidase activity near the cut-off.
References	Wolf, B. (2012): Biotinidase deficiency: “if you have to have an inherited metabolic disease, this is the one to have”. Genet. Med., **14**, (6), 565–575. [2] Kaye, C.I. and the Committee on Genetics (2006): Newborn Screening Fact Sheets. Pediatrics, **118**, e934–e963. [3] Clinical and Laboratory Standards Institute (2007): Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard – Fifth Edition; CLSI Document LA4-A5. CLSI, Wayne, Pennsylvania 19087-1898, USA. [4] Mei, J.V. et. al. from Newborn Screening Quality Assurance Program, Centers for Disease Control and Prevention, Atlanta, GA (2011): Evaluation of Filter Paper Contributions to Reduced Biotinidase Activity in Newborn Screening Specimens. Paper presented in Newborn Screening & Genetic Testing Symposium, San Diego, CA, November 7-10, 2011. [5] Adam, B.W., Hall, E.M., Sternberg, M., Lim, T.H., Flores, S.R., O'Brien, S., Simms, D., Li, L.X., De Jesus, V.R., Hannon, W.H. (2011): The stability of markers in dried-blood spots for recommended newborn screening disorders in the United States. Clin. Biochem. **44** (17-18), 1445-1450. [6] Freer, D.E (2005): Observation on Heat/Humidity Denaturation of Enzymes in Filter-Paper Blood Spots from Newborns. Clin. Chem. **51**, 1060-1062. [7] Westgard, J.O., Barry, P.L., Hunt, M.R., and Groth T. (1981): A multi-rule Shewhart chart for quality control. Clin. Chem. **27**, 493–501. [8] Clinical and Laboratory Standards Institute (2006): Statistical Quality Control for Quantitative Measurements Procedures: Principles and Definitions; Approved Guideline - Third Edition. CLSI Document C24-A3. CLSI, Wayne, Pennsylvania 19087–1898, USA. [9] Clinical and Laboratory Standards Institute (2004): Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. CLSI document EP5-A2. CLSI, Wayne, Pennsylvania 19087–1898, USA. [10] Clinical and Laboratory Standards Institute (2004): Protocols for Determination of Limits of Detection anf Limits of

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Additional Information	*NA*
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