



Biotinidase Deficiency Test

The Astoria-Pacific SPOTCHECK® biotinidase determination is used as an aid in screening for biotinidase deficiency in newborns using dried whole blood spots.

Biotinidase activity is determined colorimetrically by measuring the amount of p-aminobenzoic acid (PABA) released from biotinyl-p-aminobenzoate (Biotin-PAB). Samples are eluted in water and then incubated on-line for 90 minutes with Biotin-PAB in a pH 6 buffer. On-line dialysis separates the released PABA from other proteins in the sample. The PABA is then diazotized and coupled to a naphthol derivative to form an azo dye by the addition of sodium nitrite, acidic ammonium sulfamate and N-1-naphthylethylenediamine dihydrochloride (NED). The chromophore produced is measured at 550 nm.

The intensity of color produced in each sample is directly proportional to the amount of biotinidase activity. Samples with biotinidase activity develop a purple color. Samples deficient in biotinidase remain straw-colored. A standard curve prepared from a stock PABA solution is used to quantitate the results.

Whole blood, spotted on standardized filter paper, S&S® 903™ or equivalent is suitable for analysis. The procedure is designed for use with one 3/16 inch spot or two 1/8 inch spots but may be adapted to alternative punch protocols with appropriate validation.

Uridyltransferase (GALT) can be run with the biotinidase analysis simultaneously from the same extracted sample. For the GALT assay, two fluorometric detectors (active and blank channels) and two cartridges (active and blank) are required. The active cartridge includes an on-line incubator to enhance enzymatic response. Sample throughput for both tests is 90 per hour after an initial dwell time of approximately 90 minutes.

The SPOTCHECK® Biotinidase 50 Hour Reagent Kit is designed to be used on the Astoria-Pacific SPOTCHECK® Analyzer. The kit contains all necessary reagents needed for analysis and will provide approximately 50 hours of analyzer run time. Allowing for start up and calibrants, the approximate number of actual samples analyzed per kit is conservatively 2500.