Rapid and Automated Assay for Thyrotropin in Guthrie Cards on the ACS:180, Eurico Camargo Neto* and Jaqueline Schulte (Laboratório Nobel RIE and Centro de Triagem Neonatal, Rua Chaves Barcelos, 36/1706, 90.030-120 Porto Alegre, RS, Brazil; *author for correspondence: fax 55 51 225 6026, e-mail nobelrie@voyager.com.br or eneto@voyager.com.br)

Congenital hypothyroidism causes a decreased growth rate and skeletal development and can lead to severe mental retardation (1). In the nearly three decades since measurement of thyroxine in filter paper dried blood disks (Guthrie cards) was introduced as a method to screen newborns for congenital hypothyroidism (2), new procedures for thyroxine and thyrotropin (TSH), such as ELISA, fluoroimmunoassay, or chemiluminescence (3) have been developed or adapted from commercial kits, most of them requiring overnight incubation. Previously, we described a manual adaptation of a commercial immunochemiluminometric assay kit based on an acridinium label to measure TSH in dried blood spots (4). This method is used routinely in our laboratory, and we have detected 176 congenital hypothyroidism cases in the 480 000 newborn samples tested. Studies concerning its correlation with the DELFIA™ neonatal TSH kit (Wallac Oy) were also described (5). Here, we present results obtained with a rapid and automated third-generation TSH chemiluminescent assay for serum samples adapted to measure TSH in Guthrie cards (ACS:180™, Chiron Diagnostics). This fully automated chemiluminescence immunoassay system uses paramagnetic particles as the solid phase (6).

We measured TSH in 2200 dried blood spot samples obtained in filter paper (Schleicher and Schuell, cat. no. 903) by heel stick from newborns routinely referred to our service. The samples were dried at room temperature, and the TSH concentrations were measured using our routine chemiluminescent method (4) and also by the technique described here. We used calibrators at 2.2, 22.0, 55.0, 110.0, 220.0, and 550.0 mIU TSH/L serum equivalent and controls at 33.0 and 132.0 mIU TSH/L serum equivalent provided with the DELFIA™ neonatal TSH kit, lot nos. 383869 and 383389, respectively. These values, originally obtained in whole blood for a 55% hematocrit, were corrected to serum equivalents. Controls supplied by the Neonatal Screening Quality Control Assurance Program (Centers for Disease Control and Prevention, CDC, Atlanta, GA; lot nos. 511, 512, and 513, with values of 25.0, 40.0, and 80.0 mIU/L of serum, respectively) and five positive samples detected previously by our in-house method were also used. The ACS:180 was calibrated according to the instructions of the manufacturer. A lot-specific calibration curve provided with the kit was entered in the instrument software. Low and high concentration liquid calibrators provided by the manufacturer (Chiron) were used for the adjustment of the software to the stored calibration curve. After calibration, eight eluates of dried blood spot TSH filter paper calibrators (ICN; lot no. BS9503, with values of 0, 9.0, 18.0, 36.0, 71.0, 136.0, 244.0, and 438.0 mIU/L serum equivalent) were run as blood spot controls. The eluates were obtained by punching a 5.0-mm diameter blood spot from the filter paper, placing it in 500 μL of phosphate buffer, pH 7.4, in a special sample tube, and shaking it in a multitube vortex-type mixer for 40 min at room temperature. There is a direct relationship between the amount of TSH present in the sample and the relative light units (RLUs) detected by the equipment. The mean results of RLU obtained with each one of the calibration curve eluates were calculated after 10 analytical runs and introduced into the memory of the ACS:180 as a new TSH calibration curve specific for blood spot eluates. Eluates of ICN controls (lot no. BC9501, 10.0 and 71.0 mIU/L) were used for the necessary two-point calibration described above. Sample cups, including samples, DELFIA calibrators and controls, and CDC controls, were put in a special carousel holding 60 tubes, and the ACS:180 was allowed to proceed automatically, according to the manufacturer’s recommendation. The CDC controls were also measured 10 times on 1 day. The detection limit was calculated as the concentration of TSH corresponding to the RLU 2 SD from the mean RLU obtained with 20 replicates of the ICN zero calibrator. TSH concentrations of 872 and 1314 mIU/L were obtained by eluting the blood from two and three blood spots of the 438 mIU/L calibrator, respectively, in 500 μL of phosphate buffer and assaying the eluates to evaluate the linearity of the procedure.

Analysis of 2200 samples, including controls and five samples positive for congenital hypothyroidism by the two methods (Fig. 1A) produced the following linear regression equation: y = -0.270 + 1.015x (r = 0.966). The results in subjects without hypothyroidism (Fig. 1B) were easily distinguished from those for positive controls (>20.0 mIU/L; Fig. 1A). The five positive newborn samples assayed by the manual routine method (4) and by the ACS:180 presented, respectively, results of 74 and 81, 101 and 124, 232 and 253, 467 and 504, and 602 and 648 mIU TSH/L. The highest imprecision (CV) observed was 10.2%, in the second sample. Results for the three CDC controls with stated values of 25, 40, and 80 mIU/L were 23.7 ± 1.7, 39.8 ± 3.9, and 80.0 ± 5.8 mIU/L, with CVs of 7.8%, 9.8%, and 4.7%, respectively (n = 10 days). Intraassay CVs (from duplicate analyses) were 11%, 13%, and 7.2%. The detection limit was 2.9 mIU/L serum equivalent of TSH, and the assay was linear up to 1100 mIU/L. The mean results of TSH obtained with duplicates of the DELFIA calibrators and controls showed CVs <12%, except for a CV of 21% at 2.2 mIU/L, a value very near the detection limit of the assay. According to Elvers and Loebers (7), “Wallac uses a filter paper SS #2992 as matrix and assigns the TSH concentration based on the calculated TSH added to the plasma and mixed with red blood cells. The calibrators from ICN [used to calibrate the ACS:180™ in this work] were added directly to whole blood in filter paper SS #4903 and standardized against an in-house blood spot reference. These differences can explain the finding of concentrations which are 10–20% low...
when the assay is calibrated with SS #903 and the specimens tested on SS #2992 filter paper”.

The increased interest in newborn screening for congenital hypothyroidism, particularly in developing countries, supports the manufacture of reagents, adaptation of commercial kits, or in-house preparation of reagents for the measurement of TSH in very small volumes of serum eluted from Guthrie spots. Many authors recommend a low cutoff for this test, ~20 mIU/L serum equivalent \((8, 9)\), to avoid false-negative cases caused by the delayed increase of TSH in neonates with hypothyroidism (10). The excellent functional sensitivity of the third-generation chemiluminescent serum TSH kit tested here allowed measurement of TSH in blood eluates from Guthrie cards with 2.0 \(\mu\)L of serum, using a 5-mm filter paper dried blood spot disk \((7, 11)\) and working with a wide range of counts (RLUs) between the lowest and the highest calibrator \((\sim 2000–150,000)\). This alternative protocol requires only a daily two-point calibration and the introduction of a new calibration curve in the equipment software when changing lot numbers of assay reagents. The assay on the ACS:180 provides the first result in 15 min, can detect TSH in eluates of filter paper blood spots in a concentration range from 2.9 to 1100 mIU/L, gives up to 180 results per hour, and allows the dilution of the eluate when the result is greater than the highest point of the calibration curve. We believe that the method allows quantification of the small amounts of TSH eluted from Guthrie cards and that the method is suitable for use in the large-scale protocols of newborn screening programs.

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References


Molecular Diagnosis of Finnish Type Infantile Neuronal Ceroid Lipofuscinosis by Restriction Fragment Length Polymorphism and Oligonucleotide Ligation Assay, Eeva-Liisa Romppanen, Pirjo Valtonen, Tarja Mononen, and Ilkka Mononen* (Kuopio University Hospital, Department of Clinical Chemistry, P.O. Box 1777, FIN-70211 Kuopio, Finland; * author for correspondence: fax 358-17-173186, e-mail ilkka.mononen@messi.uku.fi)

Infantile neuronal ceroid lipofuscinosis (INCL; McKusick 256730) is a recessively inherited neurodegenerative disorder of infancy. It leads to severe progressive psychomotor deterioration, visual loss, and early death (1). The patients’ electrocardiograms become isoelectric by the age of 3 years, and patients usually die at the age of 9–11 years. The defective enzyme in the disease is the lysosomal enzyme palmitoyl-protein thioesterase (PPT; EC