Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 422–423.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1170

JLCR

Short Research Article

Development of an immunoradiometric assay (IRMA) kit for determination of thyroid stimulating hormone in dry blood spot of newborns†

VIRGINIA N. N. BORZA*, ELENA V. NEACSU, CRISTIAN I. POSTOLACHE and LIDIA C. MATEI
'Horia Hulubei' National Institute for Physics and Nuclear Engineering IFIN-HH, Magurele, Romania

Received 26 June 2006; Revised 7 November 2006; Accepted 18 November 2006

Keywords: thyroid stimulating hormone; newborns; immunoradiometric assay; monoclonal antibodies

Introduction

Congenital hypothyroidism will lead to mental retardation if untreated. Treatment must begin very early, because half of the human postnatal brain growth is completed by six month of age.[1] The measurement of thyroid stimulating hormone (TSH) by immunometric assay is the most sensitive test for early detection of congenital hypothyroidism.

The aim of this work is the development of an IRMA kit for determination of TSH in dry blood spots of newborns, as an aid in screening newborns for congenital hypothyroidism.

Results and discussion

The kit we have developed includes two anti-TSH monoclonal antibodies with great affinity for antigen; one antibody was radiolabeled with ^{125}I, while the second antibody was immobilized on solid phase (polystyrene tubes). Standards of TSH and wash reagent are the other components of the kit. The radioiodination of the anti-TSH monoclonal antibody was carried out by the chloramine-T method[2]; the reaction mixture was purified by gel chromatography on a Sephadex G-150 column. The specific activitiy of the labeled antibody (tracer) was 15–20 mCi/mg.

The immobilization of the anti-TSH antibody to the wall of the polystyrene tubes was performed by an adsorption process, from a 0.05 M phosphate buffer solution, pH 7.4.

The standards were prepared as dried blood spots on Schleicher & Schuell filter paper, no.903; 7 standards, with concentration of TSH in the range 0–448 μIU/ml, were prepared in blood with hematocrit 55%.[3] The wash reagent was a 0.05 M phosphate buffer solution, pH 7.4.

The test is based upon a principle of a sandwich immunoradiometric assay using one single 6 mm disk from each of the TSH blood standards, controls and patient samples together with 500 μl of tracer. This kit for neonatal TSH determination was verified by the evaluation of sensitivity, intra-assay precision and inter-assay precision. Also, the results obtained for neonatal TSH determinations with this kit were compared with the results obtained with a Delfia Wallac Oy, Turku, Finland kit.

Sensitivity was calculated as the concentration of TSH, equivalent to the mean count rate for 10 replicates of zero standard plus 3SDs. The sensitivity of the assay is 1.6 μIU/ml blood.

Intra and interassay precision were evaluated by calculating the coefficient of variation (CV).

Intra-assay precision was evaluated on 3 serum samples that were tested 10 times in a single assay, and inter-assay precision was evaluated on 3 serum samples, each of them being tested in duplicate, in 10 different assays (Table 1). The antibodies used in the kit present no cross reaction with structurally similar substances: LH, FSH, HCG.

*Correspondence to: Virginia N. N. Borza, 'Horia Hulubei' National Institute for Physics and Nuclear Engineering IFIN-HH, Magurele, Romania. E-mail: virginia_borza@yahoo.com
†Proceedings of the Ninth International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds, Edinburgh, 16–20 July 2006.

Table 1 Assay precision

Intra-assay precision			Inter-assay precision		
Sample	Mean value (μIU/ml blood)	CV%	Sample	Mean value (μIU/ml blood)	CV%
1	25	9.9	1	26.2	9.7
2	104.6	8.2	2	108.3	13.9
3	199.1	9.1	3	221.5	12.8

Table 2 The frequency of TSH concentration values

TSH concentration (μIU/ml blood)	≤ 2	2–3	3–4	4–5	5–6	6–7	7–8	15–16
Number of samples	74	13	4	2	5	1	1	1

The normal range was established by measuring the TSH concentration of 101 dry blood samples taken from neonate heels 2–5 days after birth (Table 2).

The TSH concentration of the same samples evaluated with Delfia kit, ranged from ≤ 2 to 15.5 μIU/ml; only one sample has the concentration of TSH of 15.5 μIU/ml.

Based on these results as well as the recommendation published by American Academy of Pediatrics for categorization of TSH values,[4] interpretation of our results can be summarized as: normal: ≤ 8 μIU/ml blood, borderline: 8–20 μIU/ml blood, hypothyroid: >20 μIU/ml blood.

REFERENCES

1. Chabarolle JP, Rossier A. *Arch Dis Child* 1978; **53**: 494.
2. Greenwood FC, Hunter WM, Gloves JS. *Biochem J* 1963; **89**: 114.
3. Salinas AM, Gonzales K, Colque C, Barragan LF, Arias R. *Proceedings of an International Symposium on Radioimmunoassay and Related Procedues: Perspectives in developing countries*, Vienna, 26–30 August 1991; 225–233.
4. American Academy of Pediatrics. Newborn screening for congenital hypothyroidism: recommended guidelines. *Pediatrics* 1993; **92**: 1203.

DOI: 10.1002.jlcr