

Radioimmunoassay

Thyroid Stimulating Hormone Immunoassay Using Heel Prick Plasma for Follow-Up Screening of Neonates to Confirm Hypothyroidism

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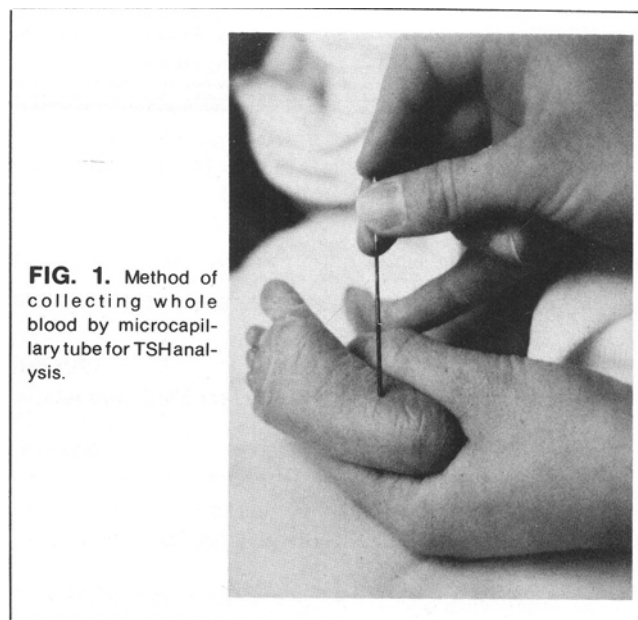
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A rapid, sensitive radioimmunoassay for TSH is described that requires a whole blood specimen collected by heel prick using an EDTA microhematocrit capillary tube. The technique was adapted from a commercially available kit and allows for hematocrit determination to correct day five T₄ spot screening and low plasma volume assaying (10 μ l). The collection procedure is atraumatic to the newborn when compared with the difficulties of venous sampling. This method is also advantageous for small volume venous samples and should be useful for follow-up on recalled newborns for positive diagnosis of neonatal hypothyroidism.

Thyroid hormone deprivation in the newborn causes irreversible mental retardation if diagnosis and treatment are not initiated, ideally within the first few weeks of life. Mass screening of neonatal hypothyroidism using T₄ and TSH by RIA has been established in a number of countries and is well documented (1,2). The recall rate has likewise been well established in these areas and ranges from 0.1 to 1%. To alleviate emotional trauma to anxious parents and logistics of sampling on recall and consequential follow-up after therapy, our method to sample and assay TSH in 10- μ l plasma from heel prick collection appears to be economical and rewarding for follow-up congenital hypothyroid (CHT) screening. The technique was adapted from Phadebas[®] spot TSH test kit (Pharmacia Diagnostics AB, Uppsala, Sweden, who supplied all materials).

Materials and Methods

Whole blood samples were obtained aseptically by heel prick or venipuncture. Heel prick samples were obtained by using a 50- μ l EDTA microcapillary tube (Fig. 1), sealed at one end, centrifuged, and assayed for circulating plasma TSH in a final volume of 160 μ l.



A single lyophilized hTSH standard (200 μ U/ml) was reconstituted with 1.0-ml human serum containing less than 1.5 μ U hTSH/ml after reconstitution. We advise using serum obtained from a hyperthyroid patient for stock purposes. Doubling dilutions were then prepared to cover the range 1.5–200 μ U/ml. The standards received from Pharmacia were calibrated against MRC 68/38. The MRC is the Medical Research Council (London, England), which is recognized as the International Reference Preparation of Human Thyroid Stimulating Hormone for Immunoassay.

The methodology is based on the sephadex[®] solid-

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phase technique (3,4). To 10- μ l plasma (unknown and standards), a 50- μ l sephadex anti-TSH (antibodies raised in rabbits) suspension is added. The suspension is stirred continuously during additions on a magnetic stirrer from a reconstituted volume of 4 ml. After a 4-hour incubation period under agitation, 100 μ l of I-125 TSH was added, followed by a further 24-hour incubation at room temperature under the same conditions, before washing with 0.15N saline and calculating % B/B_{1.5}.

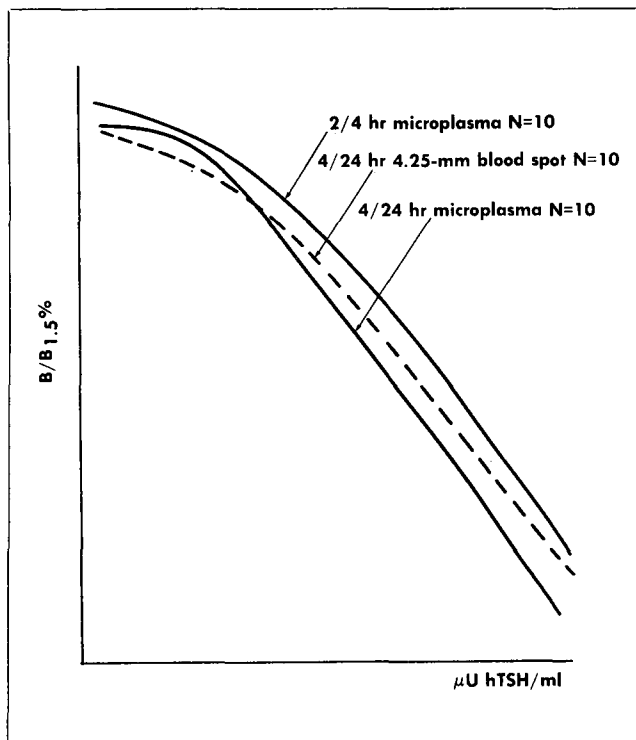


FIG. 2. Comparison of TSH standard curves obtained from short and extended incubation times of microplasma method and 4.25-mm blood spot method.

Results

Three standard curves are illustrated (Fig.2) depicting short (2/4 hr) and extended (4/24 hr) incubation times for the microplasma method—as well as the normal (4/24 hr) 4.25-mm blood spot method. The most clinically significant area lies between 6–30 μ U hTSH/ml for determining recall sampling. From the set of curves, note that within this stated range we obtain highest reproducibility using the microplasma method (4/24 hr). This is demonstrated by the intense slope obtained.

Table 1 shows results of a recently detected CHT neonate from the Peterborough District Hospital screening program. Analysis of blood spot TSH (Pharmacia) and T₄ (in-house) methods at day nine were 90 μ U hTSH/ml and 1.7 μ g/100 ml respectively. At day 14, recalled values were > 200 μ U hTSH/ml and 1.8 μ g/100 ml. Microcapillary plasma analysis at day 14 was > 200 μ U hTSH/ml. In the recalled assays, known TSH standards (Pharmacia) were incorporated for quality control purposes to acknowledge reproducibility of the particular methods. Data relative to the reproducibility of the method are presented in Table 2.

Discussion

The low sample plasma volume TSH assay described is rapid, sensitive, and reliable. The sensitivity in the 10–30 μ U hTSH/ml range has been improved compared with the current spot assay. The assay can be completed within 24 hr; however, results may be obtained within eight hours (2/4 hr incubations) with a slight loss in reproducibility. It appears that this technique in sampling and assaying for follow-up on recalled infants with borderline or positive hypothyroidism displays logistic and atraumatic characteristics for neonates undergoing repeat specimens in a normal functioning mass screening CHT program.

The major aim of our study was: to maximize sensitivity and maintain reproducibility in the 6–30 μ U hTSH/ml range; to present a new method of collecting whole blood samples where hematocrit factors can be applied to the original spot specimens; and to minimize the volume of collection under atraumatic conditions in the home by a visiting pediatrician or trained nurse.

TABLE 1. Results of TSH Dry Spot Screening and Recalled Spot/Serum and Plasma Specimens Analyzed by Three Distinct Methods.

	Screening Program	Recalled Specimens		Capillary Plasma
	Day 9/Dry Spot	Day 14		(10 μ l) sample/Day 14
	TSH μ U/ml	Dry Spot TSH μ U/ml	Serum TSH μ U/ml	TSH μ U/ml
Hypothyroid Neonate	90.0	>200	>200	>200
Control Standards*				
TSH μ U/ml				
2.0			4.7	5.0
2.5			2.0	5.0
6.3			5.5	8.1
56.0			76.0	69.0
105.0			98.0	100.0

*Control Standards: used for reproducibility

Acknowledgments

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