THE INVESTIGATION OF FACTORS INFLUENCING THE MEASUREMENT OF THYROID STIMULATING HORMONE IN DRIED BLOOD SPOTS ON FILTER PAPER

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Abstract. We investigated sample collection variables that may influence the measurement of the thyroid stimulating hormone (TSH) including: time after birth, season, different ways for blood spot drying and varied elution time from filter paper. TSH was measured with an enzyme-linked immunoassay (EIA) method on dried blood spots collected from newborns and/or external quality control materials from CDC. We found that TSH results were stable if specimens were collected from newborns 72 hours after birth. We obtained different results when TSH was measured during different seasons. The results also changed as the specimens were dried in different ways. The length of time for eluting from the DBS also exerted influence on the TSH measurement. In order to assure newborn screening quality, all factors influencing the results should be considered and the best condition for testing chosen. The specimen should be collected from babies at 3-6 days of age and air-dried at room temperature. Different cut-offs may be necessary for different seasons of the years.

INTRODUCTION

Measurement of thyroid-stimulating hormone (TSH) on specimens collected from newborns is used for congenital hypothyroid screening in most countries (American Academy of Pediatrics, 1987; Ward *et al,* 1998). Since TSH levels are low in the blood and specimens are collected on filter paper, there are many factors which can influence the results. These factors can lead to false positives and false negatives. In order to study the different variables, samples collected from April of 1998 to December of 2000 were studied.

MATERIALS AND METHODS

To assess the impact of collection time, specimens were collected from the heels of the babies born in Jining from April 1998 to December 2000 at 12, 24, 48, 72, 96, 120, and 240 hours after birth. To look for drying conditions, specimens were dried in air, direct sunlight (40-60°C) and on a heating radiator (60-80°C), stored in refrigerator at 2-8°C. TSH was measured using the enzyme-linked immunoassay method (Golden Bridge International, Inc). Spots were punched from standard specimens, and placed in avidin-coated micro-wells, and 100ul elution buffer was added to each well. The plates were rocked for 1-12hours, washed four times, and 100ul TSH antibody reagent was pipetted into each well. The plates were rocked again for 2-4hours and washed four times. The developer was put into the wells, mixed for 30 minutes, and a reaction stopper added to each well. The optical density (OD) was measured at 450nM and 630nM within 30 minutes. TSH concentrations were calculated by linear milli-absorbance using a serum cut-off of 20uIU/ml.

RESULTS

Influence of time of collection from birth on TSH levels

The TSH level was found to be higher when the sample was collected on the 12th hour of life, decreased after the 24th hour, and went to normal levels at the 48th hour (Table 1).

Effect of season on TSH levels

TSH levels when measured in winter is higher than the other seasons (Table 2).

Effect of drying method on TSH values

TSH values were highest in air dried samples

Table 1. TSH levels according to time of collection from birth.

Sample collection time (hr of life)	No. of samples (n)	TSH level		High TSH	
		Mean	Standard deviation	No. of samples	ratio (%)
12	386	13.65	3.87	98	25.39
24	237	10.32	4.12	21	8.86
48	413	8.28	3.96	19	4.60
72	681	5.23	2.89	7	1.03
96	546	5.09	2.36	3	0.55
120	480	4.54	2.41	2	0.42
240	386	5.49	1.98	2	0.52

Table 2. Measured TSH levels in relation to season when collected.

Statistical indicator	Spring (Mar-May)	Summer (Jun-Aug)	Autumn (Sep-Nov)	Winter (Dec-Feb)
No. of Samples	4874	13275	11427	4310
Mean	5.24	5.12	4.65	5.15
95% limit	10.06	9.62	11.52	13.24
99% limit	19.81	17.23	19.12	21.34

Table 3. Measured TSH levels according to method of drying filter paper.

Dried ways	No. of samples	not eluted completely (%)	mean of TSH	SD
Air dried (18-25^{o}C)	23	0 (0)	36.54	3.21
Direct sunlight (40-60^{o}C)	23	18 (78.2)	19.21	5.45
Heated on radiator (60~80^{o}C)	23	23 (100)	11.75	6.59
Moisture	23	0 (0)	24.96	9.25

Table 4. Measured TSH levels according to time of elution from filter paper.

Elution time (h)	No. of samples	Mean	SD	CV (%)
1	36	35.38	3.81	10.77
2	36	49.65	4.19	8.44
3	36	53.97	4.24	7.97
4	36	58.23	4.11	7.06
5	36	8.98	4.09	6.93
8	36	57.23	4.38	7.55
12	36	56.85	5.02	8.81

(Table3).

Effect of Elution time from the samples on TSH values

The samples are from the External Quality Control materials. The TSH measurements changed when eluted from the spots at different time (Table 4).

DISCUSSION

Mass screening for CH is now encouraged widely in China. In most laboratories, TSH is a major hormone for screening with ELISA.This study was performed to guide and help other laboratories assure their test accuracy and effectiveness in avoiding false-negatives. It was confirmed that TSH levels went up 12 hours after birth, decrease until 24-48 hours and are stable at 48 hours, a finding inconsistent with other researches (Fisher, 1985). This suggests that blood should best be collected between 3-6 days after birth. According to test results obtained during different seasons, it is better to have seasonal cut-off values. Specimens should be air-dried at room temperature before transportation. Since other methods of drying affect elution during the assays, an acceptable time should be determined and adhered to in all assays. Specimens that cannot be properly eluted during incubation should be kept from testing.

REFERENCES

American Academy of Pediatrics. American Thyroid Association. Pediatrics 1987,80:745-9.

Fisher D.Thyroid Physiology in the perinatal period and during childhood. In:Ingbar SH,Braverman LE, ed. The Thyroid. 5th ed. Philadelphia: Werner's, 1985: 1387-895.

Ward LS, Macjet RM, Magalphaes RF, *et al.* Comparison of two strategies for the early detection of congenital hypothyroidism. *Rey Assoc Med Bras* 1998; 44:81-6.