Storage Conditions and Stability of Thyrotropin and Thyroid Hormones on Filter Paper

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We evaluated the effects of temperature and humidity on thyroid hormones (T4, T3) and thyrotropin (TSH) measured in blood spots dried on filter paper. RIAs for T4 and T3 blood spots were optimized to measure these analytes over the neonatal and euthyroid adult reference intervals. Sensitivities of the T3 and T4 assays were 0.5 and 10 nmol/L, respectively. A blood-spot immunoradiometric assay for TSH involving magnetizable particles was developed with a sensitivity of 6 milli-int. units/L. Control sera stored at −20°C, 4°C, room temperature, 37°C, and at ambient external temperatures for 36 days showed no significant change in measured concentrations of TSH or T3 during 30 days or for T4 at −20 and 4°C. T4 markedly declined in blood spots stored at room temperature (either high or low humidity), 37°C, or ambient temperature. TSH and T3 in blood spots evidently are stable at temperatures likely to be encountered during storage or transport, but blood spots for T4 assay must be stored between −20 and 4°C.

Additional Keyphrases: sample handling - screening - thyroid status - iodine deficiency

In many developed countries, screening for hypothyroidism in neonates is routinely done with blood from heel pricks, spotted onto filter paper and dried. The prevalence of sporadic cretinism varies from 1/2500 to 1/5000 (1-4). However, in severely iodine-deficient areas, the prevalence of biochemical hypothyroidism is up to 12% of newborns (5, 6). In these areas the blood-spot method may become the procedure of choice to screen for iodine deficiency and to monitor prophylactic programs (5-7). A major advantage of blood spots on filter paper is the ease with which such samples can be transported and stored. In remote areas, samples are often exposed to various climatic conditions for variable lengths of time before being assayed. To obtain results comparable with those for fresh serum, one must know the effects of temperature and humidity on thyroid hormone concentrations measured in blood-spot samples. There is little such information available (4, 8-12) concerning TSH and T4, and none for T3.1 Because we plan to use blood-spot sampling, both in neonates and adults, in remote iodine-deficient regions, we used sensitive blood-spot RIAs to study the effects of storage conditions on results for T4, T3, and TSH (13). We chose various environments, so as to evaluate the full range of conditions likely to be encountered.

Materials and Methods

Assays. Sensitive RIAs were developed and validated for blood-spot measurements of T4 and T3. In all assays blood spots were taken from the specimen at an equal distance from center and edge. For T4, a 3-mm (diameter) blood spot of standards, controls, and unknowns was added in duplicate to assay tubes. Before incubation [125]T4 (New England Nuclear; 1- [125]Ithyroxin, 1250 Ci/g), T4 sheep antisera, and salicylate-glycine buffer (pH 8.6) were added to each tube. We incubated the tubes at 37°C in a shaking water bath for 4 h, then separated bound from free hormone by use of polyethylene glycol 4000 (BDH Chemicals Pty. Ltd.) and refrigerated centrifugation (4°C, 2500 × g). The supernate was aspirated and the radioactivity in the pellet was counted in an "Auto gamma" counter, with use of RIA data-reduction software (Multigamma 1260; LKB, Sweden).

The RIA for T3 was similarly performed except that two 3-mm blood spots were added to each tube and sheep T3 antibody and blood spots were pre-incubated in assay buffer for 2 h at room temperature. [125]I]T3 was then added and the tubes were incubated for a further 2 h at room temperature. Separation and analysis were similar to the T4 assay.

Both T3 and T4 antisera were raised in sheep in our laboratory as described previously (14). TSH was measured in blood spots by an immunoradiometric method involving magnetic particle separation of the immune complex (15).

Samples. Control samples were made by adding known concentrations of T4, T3, and TSH to heparinized blood from a normal volunteer.

Plasma hormone concentrations of these samples were determined by our routine diagnostic RIAs (15). Control blood spots:

- TSH control 1, 226 milli-int. units/L. Control 2, 80 milli-int. units/L.
- T3 control 1, 2.74 nmol/L. Control 2, 5.33 nmol/L.
- T4 control 1, 68 nmol/L. Control 2, 245 nmol/L.

The control samples were spotted onto filter paper cards (S & S 2992; Schleicher and Schuell, Keene, NH 03431) and air dried for several hours. We then assayed 10 replicates from each of these controls, using RIAs for blood-spot T3 and T4, and the immunoradiometric assay for TSH to obtain mean values and standard deviations for controls from the first day. Cards of blood spots for each control were then placed in plastic bags, sealed, and wrapped in foil. A set of controls for each hormone was stored under the following conditions:
- −20°C, with silica gel in the bag
- 4°C, with silica gel in the bag
- room temperature (25°C) in a high-humidity tank, and not sealed
- room temperature (25°C) in a high-humidity tank, but double sealed with silica gel in the bag
- 37°C with silica gel in the bag
- ambient temperature (4 to 30°C) on the window ledge outside the laboratory

The silica gel was regularly changed to maintain low humidity. Controls stored under these conditions were assayed on subsequent days 4, 8, 22, and 30 for T3 and T4, and on days 4, 8, 17, 22, and 36 for TSH. We used one-way analysis of variance to determine any changes in assay values for controls stored at −20°C during the 30 (or 36)-day period. We used Student's t-test to compare assay results for control samples stored at −20°C with those for raw
It has been suggested (4) that both T4 and TSH are stable at room temperature for at least a week, and for a month at 4 °C. Reportedly, TSH concentrations decline if samples are stored at room temperature for more than six weeks, but are stable at -20 °C for up to nine months (8-12). The present

**Assays.** The sensitivity of the T4 RIA was 10 nmol per liter of serum, with intra-assay CVs ranging from 1 to 6% and between-assay CVs from 6 to 18% (Figure 1). The T3 RIA sensitivity was 0.5 nmol/L, with intra-assay CVs from 1 to 6% and between-assay CVs from 2 to 8% (Figure 2).

**Samples.** One-way analysis of variance of controls stored at -20 °C showed no statistically significant differences (P > 0.05) among values for TSH, T4, and T3 as measured on different days after storage was begun. The controls at -20 °C were then used in each assay to compare other storage conditions on different days. Figure 3 shows mean values for T4, T3, and TSH under the different storage conditions, expressed as a percent of the -20 °C controls. There was no significant change in value for T3 or TSH under any of the conditions of storage during 30 days. T4 values, however, markedly declined under all conditions (P < 0.05), except at 4 °C, as compared with the samples at -20 °C.

**Discussion**

It has been suggested (4) that both T4 and TSH are stable at room temperature for at least a week, and for a month at 4 °C. Reportedly, TSH concentrations decline if samples are stored at room temperature for more than six weeks, but are stable at -20 °C for up to nine months (8-12). The present
study is the first detailed report of the stability of T4 and TSH during a month. We are not aware of any other reports regarding stability of T3 in blood spots.

Clearly, T4 was most affected by suboptimal storage conditions, while T3 and TSH were relatively stable for as long as one month under our most adverse conditions. Samples exposed to high humidity, high temperature (37°C), or variable temperature (4–30°C) showed a 50% decline in apparent T4 within four days, then a less-dramatic decline. Interestingly, TSH, a large polypeptide, was found to be more stable in storage than T4. T3, a molecule very similar to T4, appeared to be more stable. Perhaps T4 is more susceptible to spontaneous de-iodination than T3.

In national neonatal screening programs, blood spots are likely to be exposed to environmental conditions similar to those we have studied here. A reliance on T4 estimations alone may cause an increase in falsely positive diagnoses.

With the recent improvement in sensitivity of the TSH assay (13, 15), and the now-proven stability of TSH, it would appear to be the method of choice for neonatal screening. This assay is sufficiently sensitive for use in both adults and neonates.

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References
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Obviating Interferences in the Assay of Urinary Oxalate

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Evaluation of the Sigma Kit for determination of urinary oxalate highlighted two major limitations of which users must be aware: the pH of urine samples before extraction is critical, and ascorbic acid interferes positively. I give details, and describe simple modifications to the manufacturer's protocol that overcome these problems.

Much research into the investigation of renal stone disease has centered around analysis for calcium or oxalate, or both, in urine (1–4). More attention has been paid recently to analysis for oxalate in urine (5–8), possibly owing to elimination of difficulties previously associated with its estimation. Many methods for oxalate measurement are described in the literature, but lack reliability (9, 10), or require expensive, specialized apparatus (11–13). Thus the availability of a simple, quick method (in the form of the Sigma kit), not requiring specialized equipment, has created much interest in oxalate analysis. The method is a simple equilibrium colorimetric procedure, in which oxalate, initially extracted from urine, is assayed by measuring the amount of hydrogen peroxide produced in an oxidation reaction catalyzed by oxalate oxidase (EC 1.2.3.4). My evaluation of the Sigma oxalate kit, however, highlighted two major problems that needed to be overcome before the kit could go into routine use: variation in extraction efficiency at different urinary pH, and positive interference by ascorbic acid.

I describe here how these problems were investigated, and