facturer, as well as our own laboratory control (ECMC Serum), in the evaluation. The ECMC Serum was from an individual donor; it had an RF titer of 640 by latex agglutination.

The WHO, CDC, and ICL-Scientific reference preparations and the kit controls were assayed by both ICS and FIAX to compare the observed values with the reference values assigned by the agency providing the controls. Due to the limited quantities of some controls and the volume needed for each assay, some reference specimens were assayed only two or three times.

Table 1 shows the discordant RF values obtained by the FIAX and ICS methods. The WHO reference serum assayed as 253 int. units/mL by ICS, 110 int. units/mL by FIAX. The CDC reference serum assayed as 360 int. units/mL by FIAX, 1215 int. units/mL by ICS. The manufacturers' package inserts indicated that the FIAX calibrators used to establish the standard curve for this assay had been calibrated with WHO reference serum, whereas the ICS calibration curve had been based on the CDC reference serum. Evidently, values observed for the WHO and CDC reference controls differ from their assigned values depending on the assay used and which reference control was used by the manufacturer for the calibration of the assay.

In addition, discordant results were obtained with the ICL-Scientific reference control when we assayed it with the ICS system, using kits of different lots. Mean values of 684 or 354 int. units/mL were obtained by ICS depending on the kit lot, as compared with 220 int. units/mL by FIAX. All values differed significantly from the labeled value. Data for the various controls showed no consistent relationship between ICS and FIAX values; the ratio of the ICS values to the FIAX values for RF ranged from 1.1 to 3.4.

If quantitative RF values are going to be used by physicians to monitor the patient's clinical condition, it is extremely important to standardize these procedures and to avoid the comparison of discordant results obtained by different techniques in different laboratories.

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Table 1. RF Values for Reference Controls

<table>
<thead>
<tr>
<th>Ref. serum</th>
<th>Ref. RF value, int. units/mL</th>
<th>Mean, int. units/mL</th>
<th>No. of runs</th>
<th>Mean, int. units/mL</th>
<th>No. of runs</th>
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</thead>
<tbody>
<tr>
<td>WHO*</td>
<td>100</td>
<td>253</td>
<td>3</td>
<td>110</td>
<td>11</td>
</tr>
<tr>
<td>CDC*</td>
<td>1000</td>
<td>1215</td>
<td>2</td>
<td>360</td>
<td>3</td>
</tr>
<tr>
<td>ICL-Scientific*</td>
<td>250</td>
<td>684d</td>
<td>10</td>
<td>220</td>
<td>18</td>
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<tr>
<td>ECMC</td>
<td>272</td>
<td>247</td>
<td>11</td>
<td></td>
<td></td>
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<tr>
<td>ICS</td>
<td>92-158</td>
<td>120</td>
<td>14</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>FIAX</td>
<td>20-48</td>
<td>69</td>
<td>2</td>
<td>35</td>
<td>25</td>
</tr>
</tbody>
</table>

* International Laboratory for Biological Standards, Statens Seruminstitut, Copenhagen, Denmark (17.1 mg = 100 int. units). * National Reference Preparation for rheumatoid factor, lot no. 79-0013, CDC, Atlanta, GA. * Lot no. 2264. * Values obtained with kits having different lot numbers.

Diagnostic Performance of a Commercial Assay for Thyrotropin, When Used as the Initial Test of Thyroid Function

To the Editor:

Highly sensitive thyrotropin (TSH) assays have been developed and used to demonstrate their great potential as a diagnostic tool for the investigation and management of thyroid disease (1, 2). Unfortunately, these methods have generally been too cumbersome or time-consuming for routine diagnostic application, and commercially available assays for TSH in plasma have been insufficiently sensitive for routine laboratories to take diagnostic advantage of known pathophysiological relationships, particularly in hyperthyroidism. Recently, several new sensitive assays have become commercially available that should enable diagnostic laboratories to routinely measure TSH across its full pathological range. These assays show excellent diagnostic discrimination between hyperthyroid and euthyroid patients in selected patient groups (3, 4); moreover, from TSH determined in basal samples only, these assays reliably predict the TSH response to thyrotoxin in hyperthyroid and euthyroid patients (3, 5).

We have assessed the diagnostic value of using a sensitive commercial TSH assay as the initial thyroid-function test, by comparing its performance for samples referred to the thyroid laboratory with that of our routine thyroid-function test strategy.

We used 505 consecutive samples referred from in-patients and consulting clinics: the samples were from patients being screened for new thyroid disease (376), undergoing treatment for thyroid disease—T4 replacement (83), T4 replacement plus anti-thyroid drugs (39), thyrotoxin stimulation test (5)—or being screened for the stability of previous thyroid disease not under current treatment. Case notes were retrospectively examined when the diagnostic classification defined by the in-house and the commercial test results conflicted.

Our routine laboratory strategy is to determine plasma T4 on all samples received. Additional tests are performed according to the T4 results and the clinical information accompanying the request. The main testing patterns are as follows:

- Initial information
- Low T4 (<85 nmol/L)
- High T4 (>140 nmol/L)
- T4 therapy, T4/anti-thyroid drugs value
- Eye symptoms
- Hypopituitarism
- Thyrotoxin marginal stimulation test
- Hyperthyroidism

We determined plasma T4 (reference range 60–160 nmol/L) and triiodothyronine (T3, reference range 1.2–2.8 nmol/L) with an established in-house radioimmunoassay of unextracted serum. The free thyroxin index (FTI, reference range 50–150) is calculated from T3-uptake (6, 7), and TSH is measured with the Abbott RIA bead reagent kit set (reference range <4.4 milli-int. units/L).

The sensitive assay we evaluated was the "TSH Sucrose Assay" (Boots-Celltech Diagnostics Ltd., Slough, Berks., U.K.), a two-site immunoradiometric assay (TSH-IRMA) involving two specific monoclonal antibodies, one of which is labeled with 125I. The analytical characteristics of the kit have been evaluated (3). The assays were carried out according to the manufacturer's instructions (8), and the kit performed to its specifications. The reference interval recommended by the manufacturer was used (0.2–5.5 milli-int. units/L), with <0.08 milli-int. unit/L indicating either a suppressed or undetectable concentration.

Patients' results were allocated to diagnostic groups (Table 1) on the basis of the clinical information accompanying the request plus the outcome of either the routine test strategy or the TSH-IRMA result.
Table 1. Diagnostic Classification by Biochemical Tests

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. of patients classified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Routine strategy</td>
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<tr>
<td>Euthyroid</td>
<td>343</td>
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<tr>
<td>Primary hypothyroidism</td>
<td>18</td>
</tr>
<tr>
<td>Hyperthyroidity</td>
<td>10</td>
</tr>
<tr>
<td>Borderline</td>
<td>0</td>
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<tr>
<td>T4 replacement</td>
<td></td>
</tr>
<tr>
<td>Adequate</td>
<td>56</td>
</tr>
<tr>
<td>Inadequate</td>
<td>20</td>
</tr>
<tr>
<td>Excess</td>
<td>7</td>
</tr>
<tr>
<td>Block replacement*</td>
<td></td>
</tr>
<tr>
<td>Adequate</td>
<td>33</td>
</tr>
<tr>
<td>Toxic</td>
<td>1</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>5</td>
</tr>
<tr>
<td>TRH response to thyrothrin</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
</tr>
<tr>
<td>FiSt</td>
<td>1</td>
</tr>
<tr>
<td>Blunt</td>
<td>1</td>
</tr>
<tr>
<td>Exclusions*</td>
<td>7</td>
</tr>
</tbody>
</table>

*Anti-thyroid drug plus T4 replacement therapy.

The TSH-IRMA results and the routine biochemical assessment concurred in the biochemical diagnosis of 316 cases of euthyroidism, in 18 cases of untreated primary hypothyroidism, and in nine cases of untreated hyperthyroidism. In 28 samples the biochemical diagnosis given by the routine thyroid function tests and the TSH-IRMA results conflicted. Seven of these were considered not significant because the difference between the inhouse and TSH-IRMA results was less than 2 milli-int. units/L to either side of the upper reference limit of either assay (an arbitrary limit was set in the absence of a laboratory-derived reference interval for the TSH-IRMA test). In 14 of the conflicting cases the TSH-IRMA result indicated various degrees of primary hypothyroidism (12 with TSH 5.6–10.0 milli-int. units/L; one with 12 milli-int. units/L; one with 23 milli-int. units/L).

In one case of overt hyperthyroidism (FTI 209, T3 6.5 nmol/L) the TSH-IRMA result was 0.14 milli-int. unit/L. It was undetectable when T4 was assayed at a later date; inspection of the original assay data revealed no technical errors. In three cases of thyrotoxicosis and one of euthyroid Graves’s disease the TSH-IRMA values were undetectable, whereas T4, FTI, and T3 were within their respective reference ranges. Suppressed TSH concentrations were also recorded by TSH-IRMA for two clinically euthyroid patients with no history of thyroid disease; one of these had parkinsonism and was receiving l-dopa medication; the other patient had anemia, mild renal insufficiency, and a short period of atrial fibrillation. Plasma T3 was normal in both patients, as were the FTI and T3 values, which were subsequently measured, given the TSH-IRMA results.

Of 83 patients on T4 replacement therapy, 26 (33%) had undetectable (<0.08 milli-int. unit/L) TSH by TSH-IRMA, whereas the routine tests suggested that only seven of these patients were taking too much T4. The TSH-IRMA detected biochemical evidence of under-replacement in the same 20 cases as did the routine tests, plus two more. TSH was also undetectable in a one-month-old breast-fed infant whose mother was on T4 replacement.

Four samples were from hypopituitary patients receiving T4 medication. The FTI was normal in all four; the TSH-IRMA was normal in two and low (<0.1 milli-int. unit/L) in two.

Of the 39 treated thyrotoxic patients taking combined anti-thyroid drugs and T4 replacement, 16 (41%) had undetectable TSH. Routine tests indicated high amounts of hormone in only one of these patients, the remainder having T4 and T3 concentrations within their reference ranges. Both the routine tests and the TSH-IRMA identified the same five patients as being hypothyroid.

The basal samples from five thyrothrin-stimulation tests gave normal TSH-IRMA values in four, and was undetectable in the single hyperthyroid case (flat response by routine TSH). By the routine TSH three responses were normal and one was blunted.

Seven responses were excluded from the study; they were from young children for whose age-group an appropriate TSH-IRMA reference interval was not available.

If a biochemical diagnosis of primary hypothyroidism is based only on an increase in plasma TSH, then the sensitive TSH assay identified at least 14 more cases than our routine strategy did. Whether this degree of hypothyroidism is clinically important, however, i.e., requiring treatment, is moot (9, 10). All cases of hyperthyroidism were identified by both approaches, although in one case of overt hyperthyroidism the TSH was only partially suppressed. This suppression confirms that additional confirmatory tests, such as total or free T4 and T3 in plasma, would be desirable in cases where the TSH is mildly suppressed.

Unexpected suppression of TSH was found in two patients—both with no clinical evidence of thyroid disease, the tests having been requested for screening purposes. One month later one of these patients yielded the same TSH-IRMA result and was without clinical indication of thyroid disease; the second patient has not been available for follow-up.

For samples being screened for the possibility of new thyroid disease, the use of our present laboratory strategy required 123 more tests (35% more) than if we were using the TSH-IRMA as a single screening test. The TSH-IRMA missed no cases of undiagnosed thyroid disease, detected by our current strategy, and identified additional ones that warranted closer clinical and biochemical evaluation.

Biochemical tests may be valuable in managing patients receiving thyroid hormone replacement because marginal under- or over-replacement can be difficult to assess clinically. Unfortunately, returning biochemical parameters to within their reference ranges is not necessarily indicative of clinical euthyroidism (11). A large proportion of hormone-replaced patients in this study had suppressed TSH values, confirming a similar finding by Semple et al. (12), but the suppression of TSH, suggesting biochemical hypothyroidism, did not appear to correlate with either clinical or biochemical concentrations of hormone, or the duration of replacement therapy. Several of the patients had had recent thyroid ablation, so their thyrothrin cells were probably still suppressed. In others the daily post-medication surge in plasma T4 could be sufficient to suppress the thyrothrin, and uncouple their reflection of peripheral thyroid status. If a normal TSH response to thyrothrin stimulation is considered the criterion of optimum hormone replacement (13), however, then nearly half of the patients in this study should be classified as over-replaced.

The TSH-IRMA assay we evaluated offers a rapid and economic diagnostic test that could replace more complicated strategies for the initial screening of samples for new thyroid disease. For confirming positive findings, and monitoring the treatment of thyroid disease, additional tests such as free T4 and free T3 will generally be necessary.

References


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Use of Cellulose Thin-Layer Chromatographic Plates to Detect Methimyalonic Acid in Urine

To the Editor:

Detection of methyl malonate in urine by use of Fast Blue B (o-dianisidine, or 3,5-dimethoxybenzidine) has been described (1). The technique is too cumbersome for use in screening. The more recent method of Coulombe et al. (2), in which urine is collected on filter paper for paper chromatography, was successfully applied to a general population for screening, but the method requires 3–4 h for the chromatographic part and 4–6 h for color development.

We present here an improved micromethod with which as little as 5 ng (0.1 μL of 50 mg/L) of methyl malonate can be detected.

Methyl malonate and Fast Blue B were purchased from Sigma Chemical Co., St. Louis, MO. A working 5 μL solution of Fast Blue B was prepared freshely each day in ethanol/water (75/25 by vol) and to 100 mL of this, 4 mL of glacial acetic acid was added. Cellulose thin-layer chromatographic plates (20 × 20 cm, Polygram cel 300, product of Machery-Mage & Co., Germany) were obtained from Brinkmann Instruments Co., Inc., Westbury, NY 10590. The plate was cut into 5 × 5 cm pieces for routine use. Aqueous standard solutions of methyl malonate (50, 100, 150, and 500 mg/L) and 0.1-μL urine samples were applied through a 0.6-μL Microcap (dispensable capillary glass tube, product of Drummond Scientific Co., distributed by Ace Scientific Supply Co., East Brunswick, NJ), 0.5 cm from the bottom. The chromatography was performed in an ordinary 200-mL glass beaker containing solvent solution (butanol/acetic acid/water, 12.5/5 by vol) to a height of 0.3 cm. The beaker was then covered with aluminum foil (3). After 30 min, when the solvent front had reached the top, the plate was removed, air-dried, sprayed lightly with a 5 g/L aqueous solution of Fast Blue B, air-dried again, and placed in a light-proof container for 30–60 min.

After this chromatography and staining, a distinct purple spot of methyl malonate (in concentrations of 50 mg/L or greater) appeared on the upper portion of the chromatogram (Rf = 0.87), with most of the other urinary components, which yield a brownish color, trailing far behind. If kept in the dark after drying, the color of the methyl malonate spot was stable for at least 4–6 h.

The methyl malonate spot appears more quickly if the plate is blown with air at about 60 °C for 5–7 min after staining. The color reaches its maximum intensity within 10–15 min, but the spot gradually becomes unidentifiable because of a rapid increase in the color of the background.

This method of detecting methyl malonate in urine with commercially available cellulose thin-layer plates is simple, rapid, and inexpensive. It requires only 0.1 μL of urine sample. No specific facility or equipment is needed. The chromatography takes 30 min, and color development 30–60 min.

The limit of detectability of methyl malonate is 50 mg/L, the same as described by Coulombe et al. (2), who recently successfully screened the entire neonatal population of Massachusetts.

We have successfully tested a urine sample from a patient with known methylmalonic acidemia, thus confirming that our method can be applied to mass screening. Possible interference by endogenous or exogenous compounds, or both, as well as the chance of false-positive and false-negative values remains to be investigated.

Auray-Blais et al. (4) described a micro-scale thin-layer chromatographic technique for urinary methyl malonate. In their method, the thin layer was prepared from slurry of silica gels or cellulose and dried overnight, and they could detect 1 μg of methyl malonate or more per applied sample. Our method can detect 5 ng (0.1 μL of a 50 mg/L sample). Thus, our method not only is more convenient, it has a higher sensitivity.

References


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Free Amino Acid Concentrations in Pericardial Fluid

To the Editor:

Pericardial fluid is considered to be a passive ultrafiltrate of plasma (1). Our review of the literature from 1976 revealed no reference to its free amino acid concentrations. We analyzed 30 samples of normal pericardial fluids from patients with no pericardial pathology or arterial or venous-circular hypertension who were undergoing open-heart surgery.

We used single-column ion-exchange chromatography (Chromaspeck, Rank-Hilger), with the following conditions. Resin: Rank-Hilger, 7 μm. Column length 50 cm; temperature 40 to 60 °C; flow = 17 mL/min; pressure 2–4 MN/min. Sample: sample injection volume of 60 g; volume of sample = 200 μL. Buffer Li citrate: acid (pH = 2.2); basic (pH = 11.5). Color reagent: ninhydrin. Incubation temp. 98 °C. Absor-