increased (177 ± 65, range 69–281 µg/L), with only 3 patients having values higher than the upper limit of the reference interval (202 µg/L). The mean value of the PICP:PINP ratio was 0.79 ± 0.5. PINP correlated with PICP (r = 0.68, P < 0.05) and with sAlkP (r = 0.58, P = 0.07).

The other 13 patients (6 men and 7 women, mean age 66.5 years, range 44–83 years) had low or quiescent disease activity, characterized by few active ostotic lesions and mean sAlkP concentrations (112 ± 30 U/L) generally within the upper limit of the reference interval (125 U/L). They experienced little discomfort and had received no previous treatment for Paget disease. In these patients, the mean ± SD PINP concentration was 88 ± 37 (range 31–171) µg/L and PICP was 163 ± 68 (71–331) µg/L. The PICP:PINP ratio was 1.91 ± 0.5. PINP correlated with PICP (r = 0.56, P = 0.05) and with sAlkP (r = 0.95, P < 0.001).

Comparison between these patient groups indicates that concentrations of intact PINP are significantly greater in active Paget disease (P < 0.002) but PICP is little changed. In active Paget disease the concentrations of both propeptides are markedly discordant, with intact PINP circulating in up to sixfold molar excess over PICP. In less active disease states this discordance is less marked or absent.

In active Paget disease, propeptide nonstoichiometry could result either from aberrant propeptide liberation, from localized destruction or removal of PICP (e.g., by macrophagic uptake) within the pagetic lesion, or from altered receptor-mediated clearance in the liver.

A more radical explanation may involve release into the circulation of an intact PINP-like antigen, but not PICP, from a type I collagen variant produced by affected bone cells in the diseased lesion. Interestingly, at least three variants of type I collagen have recently been described (for review see ref. 1).

The large discrepancy between PINP and PICP in active disease, but not in quiescent states, awaits biological explanation. Meanwhile, the intact PINP appears to be a dynamic marker of bone collagen turnover and is potentially useful in clinical evaluation of patients with active Paget disease of bone.

References

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Meeting Report: First and Second Estradiol International Workshops

To the Editor:

In recent years, the laboratory medicine community (i.e., clinic, laboratory, and industry) has become increasingly interested in a better description of test system specifications and improved method harmonization. In particular, industry is strongly interested in recommendations from the clinic and laboratory. In Europe, this interest is most obvious in the field of serum steroid hormones, primarily for serum estradiol-17β (E2). A recent pair of workshops illustrate an approach for progress in this area. The First E2 International Workshop, held February 22–25, 1995, at Baden bei Wien, Austria, was organized by L. Vankrieken (Belgium) in cooperation with G. Bolelli and M. Capelli (both from Italy). The workshop was sponsored by the participants from industry, whereas the organizing panel comprised individuals from clinics and laboratories (see below).

The first day of the initial workshop highlighted the importance of E2 measurements in various clinical situations, such as assisted reproductive technology and hormone replacement therapy, and during childhood, during the menstrual cycle, and in men. The next day focused on the analytical side of E2 determination. The need for better method harmonization was made evident from external quality-assessment data and from the needs of multicenter clinical trials. A proposal for method harmonization by use of a network of European steroid reference laboratories was presented. The day closed with a lively round-table discussion, which evinced a variety of controversial viewpoints among the participants. For example, laboratorians and clinicians expressed the wish for more specific, precise, and sensitive assays, whereas industry emphasized that developing such assays would need additional research and investments and would be justified only if the clinical need were unanimously recognized. Further, industry argued that the volume of analyses for specific applications might be too low to be cost-effective (e.g., E2 measurements in pediatrics). In addition, an extremely controversial discussion arose about the meaning of such terms as limit of detection, limit of quantification, sensitivity, etc. At the end of the workshop, many felt that the clinical and analytical topics deserved more in-depth discussions.

For this reason, a follow-up meeting was organized by L. Thienpont and A. Vereecken (both from Belgium), the Second E2 International Workshop, held November 15–18, 1995, in Brugge, Belgium (for participants, see below). The workshop was opened by a plenary lecture by R. Ekins, “Current trends in immunoassay development,” followed by sessions in three working groups dealing with the topics: 1) Definitions related to limit of detection, limit of quantification, sensitivity, etc.; 2) Prerequisites for development, validation, and quality control of accurate routine methods; and 3) Clinical requirements of routine methods. The overall intention of these working groups was to elaborate recommendations for a scientifically sound but practical strategy for development of E2 test systems complying with the analytical state-of-the-art, current clinical requirements, and legislative regulations. To this end, the working group sessions
1. The within-day and between-day precision profiles (confidence limits included) and description of the conditions and materials used to generate them. The working range shall be derived by the user on the basis of these profiles.

2. The least amount that can be detected with a stated confidence, with indication of the nature of the materials used. (Note: there was no consensus about whether to use analytical sensitivity or limit of detection).

Working Group 2

1. The concept of traceability according to the ISO definition is supported, as well as the use of a traceability model consisting of reference methods in combination with reference materials and a reference serum panel, to reach a harmonization of routine methods.

2. In the present state of the art for E2, the reference method is ID-GC-MS, although other reference method principles may become available in the future (note: ID-GC-MS is also considered a Definitive Method). The determination of reference method values can be performed within a Network of Reference Laboratories, provided that (a) the network does not function as an authoritative body, (b) full confidentiality is assured, (c) results are published only after agreement, and (d) use of the service is on a free-access basis.

3. There was agreement that appropriately designed external quality-assessment programs have a role to play in postmarket surveillance.

Working Group 3:

1. Referring to the recommendations for publication in international journals, the use of pmol/L is advocated for expression of E2 concentrations.

2. Analytical requirements for single measurements of E2 (menstrual cycle, untreated postmenopausal females): the CV shall be <25% for a working range from 150 to 2000 pmol/L. The total error shall be <50% at a concentration of 150 pmol/L. The limit of detection shall be <100 pmol/L.

3. Analytical requirements for repeated measurements (ovulation induction/in vitro fertilization/assisted reproductive technology): the CV shall be <10% for a range from 1000 to 10 000 pmol/L, and <25% for a range from 150 to 1000 pmol/L and for concentrations >10 000 pmol/L. The total error (calculated from the bias and the CV at a probability level of 90%) shall be <15% at concentrations from 1000 to 10 000 pmol/L.

started with the state-of-the-art information on the topics under consideration, which then served as a basis for discussion and formulation of provisional recommendations. During the next day, the results of each working group were presented to the plenum for subsequent general discussion. This finally resulted in common recommendations, of which the most important excerpts are stated in Table 1.

At the conclusion of the workshop, a Third E2 International Workshop was proposed, to address the evaluation/standardization of E2 test systems. Manufacturers engaged themselves to present results of an evaluation of the accuracy of their routine test systems for a panel of 22 native sera that would be certified for their E2 concentration by isotope dilution–gas chromatography–mass spectrometry (ID-GC-MS) [1]. This evaluation of E2 test systems might form the basis for a subsequent international standardization through the use of an expanded, clinically well-characterized reference panel of patients' specimens, also certified by ID-GC-MS. However, these latter steps are to be done in cooperation with the IFCC/WHO/AACC/NCCLS/JCCLS, etc.

In summary, at a time of increasing concern about legislative regulation and decreasing budgets in healthcare, it was comforting to find a common platform for discussing the needs of modern test systems. In particular, it was encouraging that strong motivation exists to take a major step toward method harmonization by application of split-sample measurements with the ID-GC-MS Reference Method. In addition, the participants expressed hope that the E2 Workshop series could serve as an example for progress with other steroid hormones.


E2 Secretariat: S. van de Kerckhof. The organizers: Linda Thienpont (see below), Annie Vereecken (Lab. for Clin. Pathol., Antwerp, Belgium), Leo Vankrieken (Dept. of Obstet. and Gynaecol., Faculty of Med., Catholic Univ. of Louvain, Brussels, Belgium; current address: Diagnostic Products Corp., Los Angeles, CA, and Humbeek, Belgium), Maurizio Capelli (Lab. Centro di Sezione di Endocrinologia e Tecnica Immunometab. Policlinico S. Orsola, Bologna, Italy), and Gianfranco Bolelli (Clin. Osteo. e Ginecol., Univ. di Bologna, Italy).

Reference

1. Thienpont LM, Verhaeghe PM, Van Brussel KA, De Leeheer AP. Quantification of estradiol-17β in human serum by isotope dilution–gas
Effect of Preanalytical Conditions on Measurements of Free Thyroxine and Total Triiodothyronine

To the Editor:

We note with interest Keffer’s article [1] on preanalytical considerations in testing thyroid function, particularly the section on specimen-based influences. Our work [2] on stability of total thyroxine (T₄) and thyroid-stimulating hormone (TSH) in whole blood, although indicating statistically significant differences at various time intervals, suggests that the minimal changes were probably observed because of the very tight level of precision and would be of little clinical consequence. We have now extended our study, with hospital ethical committee approval, to include free T₄ (FT₄) and total triiodothyronine (T₃).

Samples were collected into lithium heparin tubes (LH/10 Monovette; Sarstedt, Leicester, UK) from 12 volunteers. One aliquot was centrifuged, separated from blood cells immediately, and stored at −20°C; five other aliquots were allowed to stand on the laboratory bench (ambient temperature, 23°C) for 6, 24, 48, or 168 h before separation and freezing. A further aliquot was stored without separation from cells at 4°C for 168 h before separation. Each sample of plasma was measured in quadruplicate in two batches. We measured FT₄ with the Amerlex MAB IM5051 kit (Johnson & Johnson Diagnostics, Amersham, UK) and T₃ with an in-house RIA using an anti-sheep primary antibody with polyethylene glycol-assisted donkey anti-sheep second-antibody separation and ¹²⁵I label (Amersham International, Cardiff, UK).

We used analysis of variance with Dunnet’s test and Tukey’s Studentized Range test to compare concentrations of hormone in blood stored for 6, 24, 48, or 168 h at room temperature or stored for 168 h at 4°C with the concentrations obtained in plasma separated at time 0. No significant difference in the concentration of FT₄ was seen at any time interval (P > 0.05), nor in T₃ concentrations at 6 h and 168 h compared with those at time 0. However, concentrations of T₃ after 24, 48, and 168 h at 4°C were significantly different (P < 0.05) from those at time 0 (see Table 1).

As in our previous study [2], it is unlikely that the statistically significant differences observed would alter the clinical interpretation of the thyroid status of the subjects tested. Reference ranges for FT₄ are 11–22 pmol/L and for T₃ are 1.4–3.2 nmol/L.

These present results and those previously reported [2] strongly support the opinion that concentrations of T₄, FT₄, T₃, and TSH in blood remain unaltered when collected in lithium heparin and stored as whole blood at room temperature or at 4°C for up to 1 week before separation of plasma for analysis. Although others (see references listed in 2) have recommended analyzing serum samples, we conclude that a clotted or heparinized sample will suffice.

References

Table 1. Mean FT₄ and T₃ concentrations in blood from 12 subjects after storage for various intervals before separation of plasma.

<table>
<thead>
<tr>
<th>Time at room temp., h</th>
<th>FT₄, pmol/L</th>
<th>T₃, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14.95</td>
<td>2.44</td>
</tr>
<tr>
<td>6</td>
<td>15.21</td>
<td>2.49</td>
</tr>
<tr>
<td>24</td>
<td>15.13</td>
<td>2.59b</td>
</tr>
<tr>
<td>48</td>
<td>15.29</td>
<td>2.75b</td>
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<tr>
<td>168</td>
<td>15.10</td>
<td>2.41</td>
</tr>
<tr>
<td>168</td>
<td>15.20</td>
<td>2.70b</td>
</tr>
</tbody>
</table>

At the indicated concentrations of FT₄ and T₃, between-batch CVs were 4.4% and 10%, respectively.

* At 4°C.

b Significantly different from 0 h storage, P < 0.05.

Interference by Thiocyanate on Electrochemical Biosensors for Blood Glucose

To the Editor:

A recent Case Report by Randell and St. Louis [1] described an interference by thiocyanate on glucose measured with the Stat Profile 5 Plus (Nova Biomedical, Waltham, MA). Thiocyanate is a metabolic byproduct of nitroprusside, a drug widely used to treat congestive heart failure. A possible cause for the interference, as concluded by these authors, is oxidation of thiocyanate at the platinum anode of the glucose sensor along with hydrogen peroxide, the desired end product of the sensor bioreaction. Randell and St. Louis further stated, “The interference by thiocyanate on glucose electrodes may also occur with electrodes produced by other manufacturers using similar methodology.” This statement requires investigation, the purpose of this communication.

Basically, three strategies are used to eliminate interferences with electrochemical biosensors for glucose, as well as other analytes of interest in clinical chemistry: (a) designing perm-selective membranes to restrict diffusion of interfering substances to the electroactive sensor surface, (b) operating the sensor at a potential low enough not to oxidize the interfering substance but still oxidize the species of interest (hydrogen peroxide in this case), and (c) using a correcting electrode as part of the sensor design to measure and remove the contribution of

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