A few years ago, PSA was reported to be present in serum in three different forms. The predominant molecular form is complexed to α1-antichymotrypsin, whereas a minor fraction circulates in a free noncomplexed form. These two forms are both measured by PSA assay. Only a very small proportion of PSA circulates bound to α2-macroglobulin; this third form, however, is a nonimmunoreactive complex.

The free, noncomplexed form of PSA is reported to constitute a minute proportion of the serum PSA in patients with CAP, but to be significantly greater in subjects affected by BPH. On the basis of this observation the simultaneous measurement of total PSA (tPSA) and free PSA (fPSA) has been suggested. The computed ratio fPSA/tPSA is considered a useful tool to better discriminate between BPH subjects and CAP patients and therefore to improve the early diagnosis of CAP [4].

Many immunoassays for fPSA measurement have been developed and are now commercially available. To evaluate the analytical performance of these assays, the international External Quality Assessment (EQA) program “Oncocheck” for tumor markers (AFP, CEA, CA 19-9, CA 15-3, CA 125, tPSA) organized by Service de Radioactivite et Radioanalyse, University of Lyon in cooperation with our Institute and CIS BioInternational has been extended to fPSA assay [5,6]. About 300 laboratories participated in the 1996 EQA cycle, assaying tPSA; among these about 70 laboratories also assayed fPSA in control samples.

The most popular methods used by participants in the EQA for fPSA assay were IRMA Hybritech; IRMA Cis, CIS BioInternational; and ICMA Immulite, Diagnostic Products Corp. Each of these methods was used by about 20 laboratories.

Control samples were prepared by diluting a serum pool (tPSA concentration ~2000 μg/L) obtained from patients affected by CAP with normal human serum (tPSA concentration <0.5 μg/L); different dilutions were made to cover the entire assay range.

During the 1996 EQA cycle, 22 control samples (freeze-dried) were distributed and assayed; their average concentrations (consensus mean of all reported results) ranged from 1.99 to 28.1 μg/L for tPSA and from 0.15 to 2.07 μg/L for fPSA. The average between-laboratory agreement (or total variability, CV) of fPSA determinations was 28.0%. This variability was decomposed by ANOVA technique in the between-method and within-method components [7,8].

The within-method component (an estimate of the precision of the “average” method) was 21.8%, accounting for 60% of variability. This figure indicates that the methods for fPSA assays are affected by poor precision when compared with the within-method precision of tPSA (14.9%, computed from results of the same control samples).

The between-method component (which reflects the systematic differences in results produced by different methods) was 17.6%, accounting for the remaining 40% of the total variability. In fact, average fPSA results produced by the three most popular methods are consistently different from each other. This last observation is clearly
appreciated from regression analysis reported in Fig. 1; it can be calculated, from regression equations, that 1 m\(\text{g/L}\) of fPSA measured by IRMA Hybritech corresponds to 1.22 m\(\text{g/L}\) of IRMA Cis and 0.78 m\(\text{g/L}\) of ICMA Immulite (22% of overestimation and underestimation, respectively). This scarce agreement indicates poor relative accuracy of the methods and can be explained both by differences in antibody specificities and (or) by differences in calibrators.

The precision of the individual fPSA methods was estimated by averaging the CVs of all results produced by the method during the whole EQA cycle for the same control sample (assayed in different laboratories and in different occasions). This between-laboratory and between-assay CV was found to be 18.1% for IRMA Cis, 26.0% for IRMA Hybritech, and 26.9% for ICMA Immulite. The corresponding CVs observed in the same control samples for results of tPSA were markedly lower: 11.4% for IRMA Cis, 11.5% for IRMA Hybritech, and 17.2% for ICMA Immulite. The worse precision in measuring fPSA (with respect to tPSA) can be explained by the lower concentration of fPSA (on average 7–8% of tPSA in the control samples distributed in this survey) and suggests that fPSA methods are affected by scarce analytical sensitivity. This is confirmed by the behavior of precision in relation to fPSA concentration (precision profile). Samples with fPSA >0.5 \(\mu\text{g/L}\) show approximately constant CVs ranging from 14.9% to 19.1% (IRMA Hybritech), 12.3% to 16.4% (IRMA Cis), and 13.4% to 18.9% (ICMA Immulite). On the contrary, lower-concentration samples (<0.5 \(\mu\text{g/L}\)) exhibit precision that markedly worsened up to about 40% for all three methods.

To discriminate BPH from CAP patients, the fPSA determination is not used alone, but combined with tPSA as the ratio fPSA/tPSA. For this reason we evaluated the variability of the ratio fPSA/tPSA reported by laboratories grouped by method. The CVs of the ratio have been computed for two control pools (distributed in three occasions as hidden replicates) with mean concentrations of tPSA of 4.25 and 8.94 m\(\text{g/L}\) (see Table 1). The variability of the ratio fPSA/tPSA was 21–36% in the lower pool and 15–25% in the higher pool for the three methods considered. This large variability is similar to that found for fPSA measurement; the finding was expected since the CV of the ratio reflects both the CV of the numerator and the CV of the denominator (according to the well-known relation CV_{ratio} = \sqrt{CV_{fPSA}^2 + CV_{tPSA}^2}) and the CV of fPSA is much larger (about twofold) than that of tPSA.

Moreover, the between-method differences of the ratio fPSA/tPSA (mean values reported in Table 1 for the three methods) are larger than those of fPSA alone. This is due to the fact that, for all three methods, fPSA values do not appear directly correlated with the corresponding tPSA values; for instance, IRMA Cis yields the highest fPSA associated with the lowest tPSA values. As a consequence the cutoff value of the ratio fPSA/tPSA (used for clinical

<table>
<thead>
<tr>
<th>Pool</th>
<th>Method</th>
<th>fPSA, (\mu\text{g/L})</th>
<th>tPSA, (\mu\text{g/L})</th>
<th>(n)</th>
<th>Mean, %</th>
<th>CV, %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>P075</td>
<td>IRMA Cis</td>
<td>0.38</td>
<td>3.99</td>
<td>52</td>
<td>9.64</td>
<td>20.8</td>
<td>6.8–15</td>
</tr>
<tr>
<td>IRMA Hybritech</td>
<td>0.27</td>
<td>4.69</td>
<td>56</td>
<td>5.85</td>
<td>24.3</td>
<td>2.1–8.6</td>
<td></td>
</tr>
<tr>
<td>ICMA Immulite</td>
<td>0.24</td>
<td>4.09</td>
<td>41</td>
<td>5.52</td>
<td>35.6</td>
<td>2.1–11.8</td>
<td></td>
</tr>
<tr>
<td>P076</td>
<td>IRMA Cis</td>
<td>0.75</td>
<td>8.16</td>
<td>49</td>
<td>9.22</td>
<td>15.3</td>
<td>5.6–12.4</td>
</tr>
<tr>
<td>IRMA Hybritech</td>
<td>0.61</td>
<td>9.42</td>
<td>55</td>
<td>6.58</td>
<td>20.1</td>
<td>4.5–10.8</td>
<td></td>
</tr>
<tr>
<td>ICMA Immulite</td>
<td>0.52</td>
<td>8.78</td>
<td>41</td>
<td>5.44</td>
<td>25.0</td>
<td>3.4–9.0</td>
<td></td>
</tr>
</tbody>
</table>

a Mean values and CVs obtained from results of two pools (P075 and P076) each distributed in three occasions as hidden replicates.

b Mean of all results reported by users of the considered method (Cis: 70 results for tPSA and 59 for fPSA; Hybritech: 129 results for tPSA and 61 for fPSA; Immulite: 58 results for tPSA and 44 for fPSA).

c The ratio has been computed only for the laboratories that reported both fPSA and tPSA assayed by the same method.

Fig. 1. Regression analysis of mean fPSA results reported by users of IRMA Cis (■) and ICMA Immulite (■) against results of the users of IRMA Hybritech, for 22 control samples assayed during the 1996 cycle of Oncocheck EQA.

The regression equations are: Cis = 0.059 + 1.16Hybritech, \(r = 0.99\); Immulite = 0.023 + 0.75Hybritech, \(r = 0.99\).
decision) has to be calculated in each laboratory according to the methods used for fPSA and tPSA assay.

In conclusion, if the ratio fPSA/tPSA is to become a reliable tool in the clinical management of prostatic diseases, the precision of fPSA determination needs to be improved, particularly in the low range (<0.5 μg/L); in addition, a better standardization of different methods is desirable.

References

New Immunoelectrochemiluminometric Assay to Measure Serum Thyrotropin, Pilar Sotorrio,* Aurelia Quiro´s, and José María Izquierdo (Depto. de Bioquim. Clin., Hosp. General de Asturias, 33005 Oviedo, Spain; *author for correspondence: fax 34 85 27 36 57)

The log/linear relation between serum thyrotropin (TSH) and free thyroxine (FT₄) makes TSH, when measured by a highly sensitive method, a more sensitive indicator of thyroid dysfunction than FT₄ [1].

Nonisotopic immunoassays are the methods of choice to measure serum TSH, as they are at least equally sensitive to IRMAs, can be automated, are generally faster and more convenient than IRMAs, and do not use radioisotopes. Within the former group of methods, the immunochemiluminometric assay is increasingly popular because of its inherent sensitivity, but instrumental and triggering modes may jeopardize it. A new immunoelectrochemiluminometric assay (IECMA) has recently come on the market [2]. It is based on electrogenerated chemiluminescence that, aiming to avoid the instability of luminophores, does not proceed in free solution but on to the surface of a platinum electrode [3, 4]. We describe the evaluation of such a method using the random access analyzer Elecsys® 2010 (Boehringer Mannheim Diagnostics).

The method consists of two immunological “sandwich” reactions linking, at one side, sample TSH to a solid phase of paramagnetic streptavidin-coated microparticles by means of a biotinylated monoclonal TSH-specific antibody and, at the other side, a Ru-labeled TSH-specific antibody. Separation of bound and unbound TSH is made by a magnet, and luminescence from a luminophore is triggered electrochemically.

The immunological reaction consists of a first incubation where 50 μL of sample, the biotinylated antibody, and the Ru-labeled antibody react for 9 min, when antibodies capture the TSH present in the sample to form a sandwich complex. In a second step, streptavidin-coated paramagnetic microparticles are added, and during a second 9-min incubation time the biotinylated antibody attaches to the streptavidin-coated surface of the microparticles. The reaction mixture containing the immune complex, with the sample TSH linked to the microparticles, is then aspirated into a measuring cell, and is magnetically captured on the surface of a platinum electrode. Unbound substances are washed away by a buffer that at the same time provides tripropylamine (TPA), an electrochemically active substance.

The chemiluminescence reaction that leads to the emission of light results from the interaction of TPA and the Ru complex (containing the sample TSH) at the surface of the electrode [4]. Both substances remain stable until application of voltage creates an electric field, which starts the reaction. This consists of an electrochemical oxidation of both luminophore and amine. The oxidation product of TPA produces an unstable, highly reducing intermediate. The oxidized luminophore Ru(2–2’-bipyridine)³⁺ accepts an electron from the deprotonated TPA radical, leading to an excited state of the former and to an ultimate emission of photons at 620 nm. Light emission is directly proportional to the Ru concentration and, consequently, to the TSH concentration in the sample. The Ru ground state is generated many times and therefore multiple light generation cycles are performed during the measuring process, with parallel gain in sensitivity. Concentrations of TSH are calculated from a calibration curve generated by a two-point calibration (0.00 and 1.30 mU/L), and a master curve is provided via the reagent bar code. The makers state that the calibrators were recalibrated against the 2nd IRP WHO Reference Standard 80/558.

The Elecsys 2010 Immunoassay System has a control unit and an analyzer unit. The latter consists of three areas: sample/reagent, consumables, and measuring. The sample/reagent area consists of a sample disk with 18 positions for a maximum of 15 analytes, a sample/reagent pipettor, and a bar-code reader. The measuring area consists of a 32-position incubator (37 °C) and a sipper probe, which aspires the reaction mixture, buffer, and cleaning fluid through the measuring cell and is washed externally by means of a sipper rinse station; the detection unit contains a photomultiplier, a peltier system, a flow-through measuring cell, a magnet drive assembly, and an amplifier. The control unit consists of a touch-screen monitor, a keyboard, and a floppy disk drive. The Elecsys...