HemoCue glucose value was ~10\% lower than the glucose value of the 0\% MetHb sample; and at a measured MetHb value of 30\% (i.e., approximately the concentration in our patient), the HemoCue glucose value fell by one-third. Minimal change was seen with the other glucose meters or, as described above, with the main laboratory method. These results confirm that MetHb interferes with the HemoCue glucose analyzer. The HemoCue glucose result for the 0\% MetHb sample was higher than any of the results found with the other methods, again in keeping with other studies (1–3). The measured MetHb values agree well with the nominal values.

We have demonstrated that MetHb interferes with the HemoCue B-Glucose Analyzer, and we speculate that this error originates either from direct interference with the color development of the tetrazolium salt or, more specifically, from the absorption of the MetHb species at >630 nm (14). Previously, Zijlstra et al. (15) suggested photometric interference for the differences they observed between adult and neonatal blood with the HemoCue analyzer.

We recommend that the HemoCue B-Glucose Analyzer should not be used for patients with MetHb concentrations >10\% and that an alternative method should be used for glucose determination in such patients.

References


Limitations of Steroid Determination by Direct Immunoassay, Joëlle Taieb, Clarisse Benattar, Anne Sophie Birr, and Albert Lindenbaum (Department of Biochemistry and Hormonology, Hôpital Antoine Béclère, 157 rue de la Porte de Trivaux, 92141 Clamart cedex, France; *author for correspondence: fax 33-1-45374745, e-mail joelle.taieb@abc.ap-hop-paris.fr)

Rapid steroid hormone immunoassays often agree poorly, especially at normal and low concentrations (1–4). These problems result from low assay specificity, inadequate standardization, and poor optimization of the methods over the large range of concentrations seen clinically (5–7). These systems are often unsuitable for clinical applications that require a low detection limit, such as the following: (a) estradiol measurements in men [<110 pmol/L; (<30 pg/mL)] or children [from <18 pmol/L to 165 pmol/L (<5 pg/mL to 45 pg/mL)] (8) and evaluation of down-regulation by gonadoliberin analogs before in vitro fertilization and embryo transfer (IVF-ET) programs; (b) progesterone determinations during ovarian stimulation, with values <3.2 nmol/L (<1 ng/mL) on the day of human chorionic gonadotropin administration predictive for pregnancy in IVF-ET (9, 10); (c) testosterone assays for children [from <0.35 nmol/L to 5 nmol/L (<0.1 ng/mL to 1.5 ng/mL)] and women [<2.4 nmol/L (<0.7 ng/mL)] (11, 12). Furthermore, limits of detection determined with the zero calibrator are generally far below the lowest concentration that can be reliably quantified in human serum [functional sensitivity (13, 14) or limit of quantitation (LOQ) (15)].

In this study, we analyzed and compared detection limits and functional sensitivities for nine estradiol (E2) and eight progesterone (P) immunoassays.

Between 1997 and 2001, we tested nine automated multianalyte systems for E2 and/or P measurements: ACS-180 (Bayer Diagnostics, Advia-Centaur (Bayer Diagnostics), Vitros ECI (Ortho-Clinical Diagnostics), Architect i2000 (Abbott Laboratories), Kryptor (Brahms), Immuno-1 (Bayer Diagnostics) for E2 and P; IMx (Abbott Laboratories), Elecsys 2010 (Roche Diagnostics) for E2 and AxSYM (Abbott Laboratories) for P. All of these nonisotopic immunoassays are based on competitive methods and involve detection by direct (Architect i2000, Advia-Centaur, ACS-180) or indirect (Vitros ECI) chemiluminescence, elecrochemiluminescence (Elecsys 2010), fluorescence (IMx, AxSYM), spectrophotometry (Immono-1), or Trace® technology (Kryptor). We have also studied one direct RIA for E2 and P (Coatia®; BioMérieux).

We determined the detection limit, defined as the concentration at 2 SD above the mean signal value of the zero calibrator (free of analyte) from each assay (measured 10 times within a single analytical run), with respect to the concentration for another calibrator concentration. If no zero calibrator was included in the calibration set (most of the systems studied required master curve calibration carried out by the manufacturer and required only two calibrators to adjust the master curve), we asked the
manufacturer to supply it. We determined the functional sensitivity (not usually determined by the manufacturer), defined as the lowest concentration of analyte that can be measured with a run-to-run imprecision (CV) of 20% (13). The interassay precision profile was used to determine, for each analyte and each assay, the concentration corresponding to CV of 20%. This profile was determined with pools of sera covering the calibration curves, analyzed (once for each analytical run) over 30 days with two different lots of reagent, according to the protocol of Spencer et al. (14).

Detection limits were 11 pmol/L (3 pg/mL; Kryptor) to 77 pmol/L (25 pg/mL; Architect i2000) for E2 and 0.19 nmol/L (0.06 ng/mL; Kryptor) to 0.17 mg/mL (0.54 nmol/L; AxSYM) for P (Table 1). All immunoassays except E2 Architect and E2 IMx had detection limits close to 37 pmol/L (10 pg/mL) for E2 and close to 0.32 nmol/L (0.1 ng/mL) for P. The values obtained in our study were close to those given by the manufacturers (cited in package inserts). Only E2 Architect i2000 had a detection limit (77 pmol/L; 25 pg/mL) different from that given by the manufacturer (<66 pmol/L; <18 pg/mL). However, Architect i2000 did not give results under the detection limits programmed into the analyzer: E2, <66 pmol/L (<18 pg/mL); P, <0.32 nmol/L (<0.1 ng/mL).

Functional sensitivities were 20 pmol/L (5.5 pg/mL; Kryptor) to 169 pmol/L (46 pg/mL; Architect i2000) for E2 and 0.32 nmol/L (0.1 ng/mL; Kryptor) to 1.43 nmol/L (0.45 ng/mL; ACS-180) for P.

The functional sensitivities of direct E2 and P immunoassays were two- to fourfold higher than the detection limits of these tests. A detection limit is generally defined as the lowest concentration of analyte that can be measured within 1 h). Although these automated systems are easy to use, with short cycle times and low costs, their use should be avoided for sera in which low concentrations are expected. We did not analyze testosterone in this study, but all the problems raised for E2 and P also apply to testosterone, especially in the concentration range found in women (12). We hope that manufacturers will soon agree to include the determination of functional sensitivity in package inserts to enlighten users concerning the limitations of these assays.

### Table 1. Detection limits and functional sensitivities of nine E2 and eight P immunoassays.

<table>
<thead>
<tr>
<th></th>
<th>ACS-180</th>
<th>Advia-Centaur</th>
<th>Architect i2000</th>
<th>AXSYM</th>
<th>Coatria 125I</th>
<th>Elesys 2010</th>
<th>Immuno-1</th>
<th>IMx</th>
<th>Kryptor</th>
<th>Vitros ECI</th>
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<tbody>
<tr>
<td><strong>E2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Detection limit, pmol/L (pg/mL)</td>
<td>33</td>
<td>33</td>
<td>77</td>
<td>ND</td>
<td>26</td>
<td>10</td>
<td>33</td>
<td>73</td>
<td>11</td>
<td>48</td>
</tr>
<tr>
<td>Functional sensitivity, pmol/L (pg/mL)</td>
<td>110</td>
<td>110</td>
<td>169</td>
<td>ND</td>
<td>73</td>
<td>62</td>
<td>55</td>
<td>144</td>
<td>20</td>
<td>59</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td></td>
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</tr>
<tr>
<td>Detection limit, nmol/L (ng/mL)</td>
<td>0.48</td>
<td>0.32</td>
<td>&lt;0.32</td>
<td>0.16</td>
<td>ND</td>
<td>ND</td>
<td>0.32</td>
<td>ND</td>
<td>0.19</td>
<td>0.48</td>
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<tr>
<td>Functional sensitivity, nmol/L (ng/mL)</td>
<td>1.43</td>
<td>1.27</td>
<td>0.64</td>
<td>0.80</td>
<td>ND</td>
<td>ND</td>
<td>0.57</td>
<td>ND</td>
<td>0.32</td>
<td>1.11</td>
</tr>
</tbody>
</table>

* Conversion factors to SI units, ×3.67 (E2) and ×3.18 (P).

* ND, not determined.
study was recently established between an academic laboratory and industry for the standardization of steroid immunoassays (21–23).

We thank the following manufacturers for supplying the assay reagents and systems free of charge: Abbott Laboratories, Bayer Diagnostics, Bio-Merieux, Brahms, Ortho-Clinical Diagnostics, and Roche Diagnostics.

References