Growth hormone (GH) assays: influence of standard preparations, GH isoforms, assay characteristics, and GH-binding protein

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The impact of the adoption of the new biosynthetic growth hormone (GH) WHO International Reference Preparation (IRP 88/624), and the recommendation to report results in µg/L instead of mU/L, is described. Conversion factors were determined by comparing both the linear and nonlinear relations of the GH values. The Pharmacia polyclonal IRMA (p-IRMA) and the DELFIA® monoclonal time-resolved immunofluorometric assay (trIFMA) with kit calibrators calibrated either against the pituitary-derived WHO IRP 80/505 or the new 88/624 were evaluated. Conversion factors of 4.17 mU/L = 1 µg/L for the p-IRMA and 4.31 mU/L = 1 µg/L for the trIFMA were necessary. Different cross-reactivity patterns for the deaminated and dimer 22-kDa, 20-kDa, and 17-kDa GH isoforms were found. Expected GH recovery was similar when the measured values were adjusted according to the results of the cross-reactivity study.

INDEXING TERMS: conversion factors • immunoassays • antibodies

The use of different calibrator preparations [i.e., recombinant growth hormone (GH), pituitary GH, or calibrated sera] and different antibodies, and the occurrence of matrix effects seem to be the most important causes of the striking between-assay differences observed in GH measurements [1, 2]. Many different assay systems have been developed for measurement of GH concentrations, involving a variety of either polyclonal or monoclonal antibodies and calibrator preparations consisting of purified pituitary GH, biosynthetic GH, or calibrated serum calibrators [1, 3–6].

In general, assay systems involving polyclonal antibodies yield similar results, whereas discrepancies are more striking when monoclonal antibodies are used. Furthermore, it is well established that GH in the pituitary and in circulation consists of a variety of molecular isoforms, including monomers such as 27-, 22-, 20-, 17-, and 5-kDa GH [7], and oligomers such as “big” and “big big GH” (>45 kDa) [8], which have different cross-reactivities in various GH immunoassays. In addition, binding of GH to the GH-binding protein (GHBP) is a potential source of interference in GH assay systems, although Jan et al. have reported only minor effects of increasing GHBP concentrations in assays [9].

The licensing authorities of the US, Europe, and Japan, and all major manufacturers of GH [10] have recently suggested that there should be a change from the pituitary-derived WHO International Reference Preparation (IRP) to the first biosynthetic GH standard, IRP 88/624. In this study we have analyzed the impact of the change from the pituitary-derived WHO IRP to the new biosynthetic WHO IRP 88/624 standard on GH measurements in different immunoassays, as recently requested by the Committe for the Standardization of Growth Hormone Assays [11]. In addition, the importance of two other suspected sources of variability in GH measurements (i.e., GH isoforms and GHBP) was evaluated.
Materials and Methods

Plasma GH concentrations were determined by three different assays: a polyclonal antibody-based IRMA (p-IRMA), a monoclonal antibody-based IRMA (m-IRMA) (both generously donated by Pharmacia & Upjohn, Uppsala, Sweden), and a monoclonal antibody-based time-resolved immunofluorometric assay (trIFMA) (generously donated by Wallac, Turku, Finland), which has been described in detail previously [12]. In this study, the numbers given with the abbreviated names for the assays indicate which WHO IRP has been used to calibrate the calibrator supplied by the manufacturer (Table 1).

Sensitivity. According to the manufacturers, the detection limits of the commercially available p-IRMA and trIFMA were 0.4 and 0.03 mU/L, respectively. In our laboratory the detection limit is defined as the apparent concentration two standard deviations below the counts of maximum binding. Twenty zero calibrators were measured in a single assay. The detection limits were 0.3 and 0.03 mU/L for the p-IRMA and trIFMA, respectively.

Specificity. The cross-reactivity of 22-kDa recombinant GH, 22-kDa dimer GH, 22-kDa deaminated GH, 22-kDa pituitary GH, and 20-kDa recombinant GH (generously donated by Novo Nordisk, Gentofte, Denmark), and 17-kDa (GH 44–191) and 5-kDa GH (GH 1–43) (generously donated by Urban J. Lewis, La Jolla, CA) were assessed in the p-IRMA and trIFMA.

Reference Preparations
Recently, European manufacturers decided to change the unit for the WHO biosynthetic 88/624 standard preparation from mU/L to μg/L [10]. We have also investigated conversion factors related to the change of pituitary-derived WHO IRP 66/217 to the purified pituitary derived WHO IRP 80/505, and the units are expressed as mU/L.

GH Recovery
GHBP (generously donated by Genentech, South San Francisco, CA) at 16, 31, 63, 125, 250, and 500 pmol/L was added to serum samples, and GH concentrations were measured by the p-IRMA and trIFMA.

GH (20-kDa) at 1.8, 3.6, 7.2, 14.4, 28.8, 57.5, 115, and 230 mU/L, and 17-kDa and 5-kDa GH at 8.5, 17, 34, 68, 136, 272, 544, and 1088 mU/L were added to serum samples, and GH concentrations measured by the p-IRMA and the trIFMA.

GHBP Measurements
Total GHBP was measured by a ligand-mediated immunofunctional assay as previously described [13] with reagents from Genentech. The detection range was 15.6–1000 pmol/L. The intraassay CV was 7%.

Study Subjects
Ten girls participated in the study, which was performed at the Children’s Hospital, Göteborg, Sweden. All girls were healthy, well nourished, and had normal thyroid, liver, and kidney functions. Their ages ranged from 9.9 to 14.0 years and their pubertal stages between 1 and 4 as determined by pubic hair and breast development according to Tanner and Whitehouse [14]. Compared with the Swedish reference values [15], their height standard deviation scores were between −0.94 and 1.80.

The samples used in this study were obtained in connection with other investigations [16]. The study was approved by the Ethical Committee of the Medical Faculty, University of Göteborg. Informed consent was obtained from each girl and her parent.

Study Protocol
A nonthrombogenic catheter (Carmeda, Stockholm, Sweden) was used to obtain the samples, as described previously [16]. The rate of withdrawal was 0.5–2 mL/h, and the volume of the tubing system was 0.1–0.2 mL. The heparinized tubes were changed every 20 min for 24 h, giving a total of 72 samples per individual. The blood samples were kept at room temperature and centrifuged within 24 h. After centrifugation, the plasma samples were frozen and stored at −20 °C until assayed for GH.

Statistical Analysis
Data were analyzed by both linear and nonlinear regression. Values below the detection limit were omitted. For the linear relation, results are given as A (intercept), B (regression coefficient), and CI (95% confidence interval
for B). For the nonlinear relation, results are given as A (intercept), B₁ (regression coefficient), CI (95% confidence interval for B₁), and B₂.

**Results**

**Effects related to assay reagents.** To determine the conversion factors that should be applied when changing the calibration of the standard preparation from the reference WHO IRP 80/505, expressed in mU/L, to WHO IRP 88/624, expressed in μg/L, GH concentrations in the same samples were compared when read against different calibration curves. The calibration curves consisted of serial dilutions of GH from the same source, calibrated against either of the two reference preparations. The samples consisted of 14 24-h profiles from 10 girls. The results obtained when the GH concentrations measured in the same samples were expressed in terms of calibrators calibrated against WHO IRP 80/505 and 88/624 were compared. For the p-IRMA, GH, 1 μg/L, measured with a calibrator calibrated against 88/624, corresponded to 4.17 (CI, 4.14–4.20) mU/L GH, measured with a calibrator calibrated against 80/505. For the trIFMA, the same comparison showed that 1 μg/L GH was equivalent to 4.31 (CI, 4.27–4.34) mU/L GH.

When plotting the GH concentration given on the kit calibrator vials, calibrated against WHO 88/624 and 80/505, against the measured counts per minute, the calibrator shift did not change the position of the calibration curve for the p-IRMA (Fig. 1, left panel), whereas the calibration curve for the trIFMA shifted slightly (Fig. 1, right panel). As manufacturers intend to continue to give the concentrations of the kit calibrators both as mU/L and μg/L for some time, determining the conversion factors was important. The same samples were therefore measured twice, once with the same kit calibration curve calibrated against WHO IRP 88/624 with the units given in mU/L, and once with the units given in μg/L. For the p-IRMA-88 a conversion factor of 4.17 mU/L = 1 μg/L was obtained, whereas for the trIFMA-88 the conversion factor was 3.47 mU/L = 1 μg/L.

To determine the conversion factor between WHO IRP 80/505 and WHO 66/217 for the p-IRMA and m-IRMA, the same 24-h profiles were assayed for GH with the different assays and calibrators. Both the biosynthetic kit calibration curves, calibrated against the purified pituitary-derived WHO IRP 80/505, and the older pituitary kit calibrator calibrated against pituitary-derived WHO IRP 66/217, were expressed in mU/L (Fig. 2a, b). For the p-IRMA, 1 mU/L GH measured by the p-IRMA-66 was equivalent to 1.61 (CI, 1.58–1.63) mU/L GH measured by the p-IRMA-80. For the m-IRMA, a nonlinear relation was found in which 1 mU/L GH measured by the m-IRMA-66 is equivalent to the function $y = 0.35 + 1.19x + 0.009x^2$ mU/L GH measured by the m-IRMA-80.

**Methodology effects.** To evaluate the impact of different assay characteristics, the GH concentrations in the 24-h

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![Fig. 1](https://academic.oup.com/clinchem/article-abstract/43/6/950/5640978) Calibration curves for p-IRMA (left panel) and trIFMA (right panel) obtained with the same biosynthetic calibrator calibrated against either the WHO IRP 80/505 (dotted lines) or the WHO IRP 88/624 (solid lines).

Results are given in mU/L (top x-axis) and in μg/L (bottom x-axis). Calibration curves are plotted according to the GH content given on the kit calibrator vials (on the respective x-axes) and the measured counts per minute (on the y-axes). Calibration curves are plotted according to the manufacturers’ instructions, which account for the different scales between the assays.

![Fig. 2](https://academic.oup.com/clinchem/article-abstract/43/6/950/5640978) Four of the regression analyses used to produce the conversion factors given in Results for (a) p-IRMA-66 to p-IRMA-80; (b) m-IRMA-66 to m-IRMA-80; and in Table 2 for (c) p-IRMA-80 to m-IRMA-80 and (d) p-IRMA-80 to trIFMA-80.

The dotted line is the line of identity. The solid line is the line of regression. In b, both the linear and nonlinear lines of regression are shown.
profiles of the 10 girls measured by the m-IRMA and trIFMA with the same biosynthetic kit calibration curve calibrated against pituitary-derived WHO 80/505 (expressed as mU/L) were compared with the GH concentrations measured by the p-IRMA-80. GH values were ~40% and 22% lower when measured by the m-IRMA-80 and trIFMA-80, respectively (Table 2, Fig. 2c, d).

**GH isoforms.** Cross-reactivities for various GH isoforms are shown in Fig. 3. The GH isoforms were serially diluted with the p-IRMA or the trIFMA diluent. The biosynthetic and pituitary 22-kDa GH isoforms were completely detectable in both assays. For deaminated 22-kDa GH, cross-reactivity was 40% in the trIFMA and 100% in the p-IRMA, whereas for 22-kDa dimer GH, cross-reactivity was 80% in the trIFMA and 100% in the p-IRMA. Cross-reactivity of 20-kDa GH was <3% in the trIFMA and 100% in the p-IRMA. The 17-kDa GH did not cross-react in the trIFMA and its cross-reactivity was <6% in the p-IRMA. The 5-kDa GH did not cross-react in either the trIFMA or the p-IRMA.

**EFFECTS RELATED TO SAMPLES**

**Expected GH recovery.** Samples from two bank plasma pools with GH concentrations of ~30 and 24 mU/L in the p-IRMA and trIFMA, respectively, were used throughout the GH recovery studies. The pool samples were supplemented with GHBP or various GH isoforms to determine their impact on the respective measured GH concentrations.

**GHBP.** GHBP was diluted with human serum containing a known GH concentration and with a GHBP concentration of 306 pmol/L. When GHBP was present at concentrations >63 pmol/L (i.e., total GHBP 306 + 63 = 369 pmol/L), there was a gradual decrease in the measured serum GH concentrations. The total recovery of GH was ~75% in the p-IRMA and trIFMA assays when GHBP was added at 500 pmol/L (Fig. 4).

**GH isoforms.** Adding 20-kDa GH into the trIFMA confirmed that 20-kDa GH is not recognized by the trIFMA and showed that the presence of 20-kDa GH does not interfere with the measurement of GH forms detected by this assay. In contrast, 20-kDa GH is nearly fully detected in the p-IRMA and the presence of 20-kDa GH slightly decreases the expected measurement of GH forms detected by this assay when 20-kDa was present in concentrations >30 mU/L (Fig. 4). Supplementing samples with 17-kDa and 5-kDa GH showed that neither of these GH forms interfered with the expected recovery of GH in the two assays, with the exception of 17-kDa GH at very high concentrations in the trIFMA.

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**Table 2. Linear and nonlinear regression coefficient between assays for the p-IRMA, m-IRMA, and trIFMA when the same samples (n = 652) from 14 24-h profiles from 10 girls were analyzed for GH.**

<table>
<thead>
<tr>
<th>Conversion factors (n = 652)</th>
<th>linear</th>
<th>nonlinear</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B (CI) (regression coefficient)</td>
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<tr>
<td></td>
<td>(intercept)</td>
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<tr>
<td>p-IRMA-66 to m-IRMA-66</td>
<td>0.166</td>
<td>0.66 (0.64–0.68)</td>
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<tr>
<td>p-IRMA-80 to m-IRMA-80</td>
<td>−0.238</td>
<td>0.61 (0.59–0.62)</td>
</tr>
<tr>
<td>p-IRMA-66 to trIFMA-80</td>
<td>−0.817</td>
<td>1.22 (1.17–1.26)</td>
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<tr>
<td>p-IRMA-80 to trIFMA-80</td>
<td>−0.817</td>
<td>0.78 (0.76–0.81)</td>
</tr>
<tr>
<td>m-IRMA-66 to trIFMA-80</td>
<td>−0.127</td>
<td>1.71 (1.65–1.77)</td>
</tr>
<tr>
<td>m-IRMA-80 to trIFMA-80</td>
<td>0.458</td>
<td>1.19 (1.15–1.24)</td>
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Units for all values are mU/L.

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**Fig. 3.** The cross-reactivity of 22-kDa recombinant GH, 22-kDa dimer GH, 22-kDa deaminated GH, 22-kDa pituitary GH, and 20-kDa, 17-kDa, and 5-kDa GH assessed in the p-IRMA (left) and the trIFMA (right).
The two different WHO IRPs in the same assay gave very different inclusion of two kit calibration curves calibrated against and not to assume a linear relation. Nevertheless, the importance to calculate the relation between relative values concentrations were different in the two assays. It is crucially important to establish reliable conversion factors between calibrators for any particular method. Discrepancies in measurements of GH concentrations in any sample should then be attributable to the characteristics of the different assays.

The group of licensing authorities of the US, Europe, and Japan, and all major manufacturers of GH recently agreed to change from the pituitary-derived WHO IRP 80/505 GH standard to the first biosynthetic GH standard WHO IRP 88/624 [10]. Simultaneously, they decided to change the units from mU/L to µg/L, and that 3 mU/L = 1 µg/L. However, our results show that the conversion factors for both the polyclonal (4.17 mU/L = 1 µg/L) and monoclonal assays (4.31 mU/L = 1 µg/L) were significantly different from 3. We therefore recommend that each laboratory perform its own conversion study.

Inconsistency of standards that react variably with different antisera might be the most important cause of between-assay differences [1, 2]. In 1990 the first pituitary-derived WHO IRP 66/217 standard was replaced by the purified pituitary-derived WHO IRP 80/505. We found that this modification increased measured GH concentrations in both the p- and m-IRMA. However, both the magnitude and relation between the measured GH concentrations were different in the two assays. It is crucially important to calculate the relation between relative values and not to assume a linear relation. Nevertheless, the inclusion of two kit calibration curves calibrated against the two different WHO IRPs in the same assay gave very reliable conversion factors. Baumann [17] has suggested that the diverse antibody population present when polyclonal antisera are used results in antigen recognition spectra that are generally similar to each other. In contrast, monoclonal antibodies recognize specific epitopes on the GH molecule. Assays involving monoclonal antibodies may therefore vary considerably in their ability to detect various isoforms and may react differently with free GH and GH bound to GHBP. For example, a change from polyclonal to monoclonal antibodies in the IRMA in the present study decreased the measured GH concentration by 40%. Moreover, when using monoclonal antibodies in the trIFMA, measured GH concentrations were 22% lower than when measured with polyclonal antibodies in the IRMA. We also examined the cross-reactivity and interference of different GH isoforms in the GH assays. Except for deaminated and dimeric 22-kDa GH, which cross-reacted less in the monoclonal assay and were fully recognized by the polyclonal assay, all 22-kDa GH isoforms were 100% detectable in both assays. The 20-kDa isoform was not detected in the monoclonal assay, whereas it cross-reacted 100% in the polyclonal assay. GH 44–191 (17-kDa GH), which is present in the pituitary and in the circulation [7], showed no immunoreactivity in the monoclonal assay and very low immunoreactivity in the polyclonal assay, which is in agreement with the results reported by Sinha and Jacobsen [18], in which GH 44–191 was not appreciably measured in a GH RIA. In addition, GH 1–43 (5-kDa GH) was not detected by either the monoclonal or the polyclonal assay, and whether this peptide circulates in serum remains to be proved [19]. It is important, however, to mention that although we have tested the most prevalent circulating GH isoforms, several other variants and immunoreactive fragments of GH may be present in serum [8, 19] and could be potential sources of variability in GH measurements. The present study confirms earlier results [12] in which we showed the absence of a hook effect for GH in samples up to 10 000 mU/L for the trIFMA.

Jan et al. have suggested that GHBP does not contribute substantially to the variability of GH measurements between assays [9]. In this study, there was a similar decrease in both the polyclonal and monoclonal assay in recognizing GH in samples after the addition of GHBP. Our findings are not necessarily true for other assays that involve antibodies that recognize different epitopes of the GH molecule. When GHBP was added at 500 pmol/L, which is at the upper limit of the physiological range, the suppression was substantial.

When all the variables of diverse standards, presence of GH isoforms (monomers, oligomers, fragments) in serum, and variable binding of GH to GHBP are considered, there are obviously many potential sources of variability in GH estimates. These factors may account for the two- to fourfold variability in GH concentrations measured by different assays [2, 7, 20].

The ultimate aim is to develop a GH assay that reflects...
the biological activity of GH in the blood. It might well be that different isoforms of GH will account for different biological effects. Several assay systems have been developed in the search for this goal [21, 22, 23–25]. GH concentrations from different stimulation tests or serial sampling studies still form the basis for clinical decisions of whether to initiate GH therapy [26]. Therefore, standardizing the assays is important, whether they are monoclonal or polyclonal, competitive radioligand, or ultrasensitive fluorometric assays [11]. Excessively strict adherence to “cutoff values” is likely to be foolhardy [27, 28]. Specific values generated in the assay systems should not be the final determinants of a clinical decision, but should rather be used as confirmatory measurements [29].

It has been claimed that pituitary GH as standard is more suitable than a single highly purified form of GH because it approximates the normal mixture of GH forms [17]. On the contrary, from a theoretical point of view, it is always preferable to have a standard that is as pure and reproducible as possible, i.e., to use the biosynthetic GH standard and ideally the reference standard preparation by itself. Finally, with the use of biosynthetic standards, between-assay discrepancies may be explained by the existence of isoforms and binding proteins, resulting in different cross-reactivity patterns.

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References

