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Editorial

Characterization of the hCG Variants Recognized by Different hCG Immunoassays: An Important Step Toward Standardization of hCG Measurements

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Human chorionic gonadotropin (hCG)[4] is a dimeric glycoprotein hormone consisting of noncovalently associated α and β subunits. It is produced principally by the trophoblastic cells of the placenta but may also be produced by nontrophoblastic tissues including normal pituitary and neoplastic cells. Therefore, qualitative and quantitative measurement of hCG is clinically useful in the diagnosis of normal and abnormal pregnancy and the management of patients with gestational trophoblastic disease (GTD) and other hCG-secreting malignancies.

The measurement of hCG is complicated by its molecular heterogeneity in both structure and carbohydrate content. Multiple molecular variants of hCG are present in serum and urine and include intact hCG, nicked hCG (hCGn), free β-subunit hCG (hCGβ), nicked free β-subunit hCG (hCGβn), free α-subunit hCG (hCGα), and the β-core fragment hCG (hCGβcf). In addition, hCG is differentially glycosylated in various tissues, resulting in a range of molecular forms from hypoglycosylated to hyperglycosylated. So-called hyperglycosylated hCG (hCG-h) is probably the best known of these glycosylated variants.

This molecular heterogeneity has led to numerous problems, including use of a nonstandard nomenclature for hCG variants, absence of purified standards to achieve accurate calibration, and discrepant characterizations of hCG variants that are recognized by different hCG immunoassays. This, in turn, has led to misunderstandings about which hCG immunoassays are appropriately used in different clinical scenarios. For example, although intact hCG is the principal variant in serum throughout most of pregnancy *(1)*, hCG-h accounts for a relatively higher proportion of total hCG in the first several weeks of gestation *(2)*, and hCGβ is present in much lower abundance *(3)*. Although most trophoblastic tumors produce intact hCG, unusually increased concentrations of the other hCG variants (particularly hCGβ) can also be present *(4)*. Finally, among germ cell tumors that produce hCG, 20%–40% produce hCGβ alone *(5)*.

In 1994, in an effort to address some of these problems, the IFCC established a Working Group for the Standardization of hCG *(6)*. The group was able to prepare and characterize new standards for 6 of the hCG variants described above *(7)*. Additionally, they assigned nomenclature and quantified the standards using substance (molar) concentration *(8)*. In 2001, the WHO Expert Committee on Biological Standardization approved the 6 standards as the 1st WHO International Reference Reagents (IRRs).

Currently, hCG immunoassays are calibrated against the 3rd or 4th WHO International Standards (IS 75/537 or IS 75/589), which are impure mixtures of hCG that were assigned units based on bioactivity (70 μg, corresponding to 650 IU). In contrast, the IRRs are highly purified with only IRR 99/642 (hCGn) containing $<$1.4% of hCG, hCGα, and fragments of hCGβ *(7)*. Clinically, the most important IRR is 99/688 (hCG), and it contains no hCGn and only negligible amounts of the other variants *(7)*. It was recommended that the IRRs should be used to investigate and characterize the analytical specificity of hCG immunoassays *(9)*.

In this issue of *Clinical Chemistry*, Sturgeon et al., on behalf of the IFCC Working Group for Standardization of hCG, report their use of the new IRRs to do exactly that *(10)*. As part of the UK National External Quality Assessment Service, Sturgeon et al. sent specimens containing the IRRs to 150–240 different laboratories over a 6-year period (2001–2007) and determined the analytical specificity of 14 immunometric assays and 2 RIAs for hCG.

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[4] Nonstandard abbreviations: hCG, human chorionic gonadotropin; GTD, gestational trophoblastic disease; hCGn, nicked hCG; hCGβ, free β-subunit hCG; hCGβn, nicked free β-subunit hCG; hCGα, free α-subunit hCG; hCGβcf, β-core fragment hCG; hCG-h, hyperglycosylated hCG; IRR, International Reference Reagent; IS, International Standard.

They report their results as mean recoveries for each IRR (IRR 99/688 against IS 75/589 and 4 other IRRs against 99/688) by method. As one would hope, the mean method recovery of IS 75/589 was 107%, indicating that hCG immunoassays recognize well the material on which they are currently calibrated. By comparison, the mean method recovery of IRR 99/688 was 39% higher, which is likely due to its substantially greater purity. The larger between-method CV (12%) observed for IRR 99/688 than for IS 75/589 (9%) is a function of the impurity of IS 75/589, and between-method variation should decrease with the use of a more pure standard. Indeed, when IRR 99/688 was used to calibrate 9 hCG assays, between-method variation improved significantly *(11)*. Thus, if IRR 99/688 is used for hCG assay standardization, it will clearly influence not only the measured hCG concentrations but also the relationship between hCG immunoassays.

Sturgeon et al. also report wide variability in hCG variant detection among hCG immunoassays, even among those assays that detect a specific hCG variant *(10)*. Whereas all 16 assays detected 99/642 (hCGn), the mean recovery was 89%. Twelve assays detected hCGβ and hCGβn, with mean recoveries of 132% and 86%, respectively, and only 6 assays detected hCGβcf, with a mean recovery of 63%.

Similar studies have been published but have not used the IRRs *(11, 12)*, although the latter study did use 99/688. Some notable differences between these studies can be found. Sturgeon et al. report that the Roche Elecsys (total) hCG and Ortho Vitros ECi methods detected all 4 hCG variants, whereas Cole et al. *(11)* reported that these methods failed to detect hCGβcf. In addition, the ability of the Dade-Behring Dimension to detect only hCG and hCGn is described by Sturgeon et al., whereas Cole et al. *(11)* reported that this method also recognized hCGβ. The reason for these differences is unclear. Material differences between the hCG variants and/or their concentrations as used in these studies is 1 possible explanation. Alternatively, the regulation of in vitro diagnostic tests varies between the US and the European Union, and so the hCG assays used in these studies may not be equivalent. Also, the practice of referring to a manufacturer's instrument rather than identifying the name of the hCG assay (a common source of confusion), may result in inappropriate comparisons.

The impact that hCG standardization could have on the clinical community is particularly evident by the wide variability seen in the detection of hCGβ. None of the hCG immunoassays demonstrated equimolarity (defined as a recovery of 90%–110%) of recognition of this variant, and instead showed a wide variation in recovery (68%–245%). As discussed earlier, hCG has clinical utility as a tumor marker, and in some malignancies hCGβ is the principal variant. Patients who are being monitored by following hCG concentrations are at risk of mismanagement if different assays are used over time or the assay used for measuring hCG fails to detect hCGβ. Likewise, assay standardization will be exceptionally important as evidenced-based guidelines are developed for the use of tumor markers such as hCG.

Only 6 of the hCG immunoassays were able to detect hCGβcf and did so with high variation (CV 57%). hCGβcf is the most abundant hCG variant present in urine after 5 weeks of gestation *(13)*, and although the clinical importance of quantitative hCG measurements from urine is unclear, laboratories often use them when investigating discrepant results between qualitative urine and quantitative serum hCG tests. Assays that fail to recognize this variant should not be used for this purpose.

As good investigations should, this one raises additional questions. (*a*) When it comes to hCG immunoassays, what is actually being measured? Clearly, use of the available IRRs provides some insight. Yet we know there are hCG variants (e.g., hCG-h) for which purified material does not exist. In addition, there are likely hCG variants that we do not yet know about. (*b*) Which variants should we be measuring? It is clear that a "one size fits all" approach is likely insufficient for all clinical situations, and there are those who advocate for the development and utilization of hCGβ-specific assays *(14)*. (*c*) Might there also be a need for assays specific for hCG-h and hCGβcf? (*d*) How should hCG results be reported? When asked to report results as either "intact hCG" or "total β hCG," 9% of laboratories that used hCG assays that are unable to detect hCGβ inappropriately reported their results as "total β hCG," and 13% that used assays that could detect hCGβ reported results as "intact hCG" *(15)*. (*e*) Should results of hCG tests include a description of exactly which variants are detected? At the very least, laboratories should be familiar with what hCG variants are detected by their hCG immunoassays. (*f*) Should we abandon the reporting of hCG results in activity units and use substance concentration instead? The use of substance concentration would allow the direct comparison of hCG results between methods and between variants but would present a considerable educational hurdle.

Clearly, this type of characterization is an important step in establishing standardization of hCG measurements. Continued progress in this area will result in less confusion regarding the interpretation of hCG results and will ultimately improve patient care.

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