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Differences in Recognition of the 1st WHO International Reference Reagents for hCG-Related Isoforms by Diagnostic Immunoassays for Human Chorionic Gonadotropin

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BACKGROUND: The 1st WHO International Reference Reagents (IRRs) for 6 human chorionic gonadotropin (hCG)-related molecular variants, highly purified and calibrated in substance concentrations by the IFCC Working Group for hCG, permit experimental elucidation of what commercially available hCG methods measure in molar terms and enable assessment of their fitness for clinical purposes.

METHODS: Pools containing known amounts of the IRRs spiked into normal human serum were issued to participants through the UK National External Quality Assessment Service for hCG for a period of 7 years. Among 16 assays used, 4 recognized only hCG, whereas 6 recognized hCG and its free β-subunit (hCGβ), and 6 recognized hCG, hCGβ, and the beta core fragment.

RESULTS: Differences in calibration of current hCG assays are moderate. Mean recovery of the current International Standard (IS), hCG IS 75/589, was 107% (range 93% to 126%), whereas that of the IRR 99/688 for hCG was 139% (range 109%–164%). Between-method variation for the latter (CV 12.3%) was also greater than for IS 75/589 (CV 8.8%). Recognition of hCGβ varied markedly (CV 37%). Most assays overestimated it, but 2 RIAs produced results that were slight underestimations. Recognition of the beta core fragment was even more variable (CV 57%) and was closest to equimolarity for the RIAs.

CONCLUSIONS: Assays for hCG show considerable variation in their recognition of various forms of hCG, and this variablility is the most important cause of method-related differences in hCG results in serum and an even more important cause of method-related differences in urine measurements. Equimolar recognition of the major hCG isoforms is essential if between-method comparability for hCG is to be improved.

A prerequisite for improved standardization of immunoassay methods is precise knowledge of what is being measured in clinical samples, which frequently contain heterogeneous mixtures of related molecules *(1, 2)*. To this end the IFCC Working Group for human chorionic gonadotropin (hCG)[8] established an unambiguous and user-friendly nomenclature that describes hCG and its 6 most important isoforms, and subsequently prepared the 1st WHO International Reference Reagents (IRRs) for these 6 isoforms *(1, 3)*. The 6 isoforms were prepared by use of previously developed purification methods, which included hydrophobic interaction chromatography and reversed-phase HPLC *(3)*. The high purity and homogeneity of the preparations—as confirmed by results of amino acid and sequence analyses, carbohydrate composition, electrophoretic patterns, and immunoassay studies—subsequently enabled their calibration in substance concentrations (i.e., molar units) (Table 1) *(4, 5)*.

The use of substance concentrations addresses a major limitation of earlier International Standards (IS) and International Reference Preparations (IRP) for hCG-related molecules, namely the 3rd and 4th hCG WHO IS (75/537 and 75/589) (which are essentially identical), hCGβ 1st IRP (75/551), and hCGα 1st IRP

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[8] Nonstandard abbreviations: hCG, human chorionic gonadotropin; IRR, International Reference Reagent; IS, International Standard; IRP, International Reference Preparation; hCGn, nicked hCG; cf, core fragment.

Table 1. IFCC nomenclature and WHO codes for the 1st WHO IRRs for 6 important isoforms of hCG.

hCG isoforms	IFCC nomenclature	WHO code[a]
Intact hCG	hCG	IRR 99/688
Nicked hCG	hCGn	IRR 99/642
hCG beta-subunit	hCGβ	IRR 99/650
Nicked hCG beta-subunit	hCGβn	IRR 99/692
hCG beta core fragment	hCGβcf	IRR 99/708
hCG alpha-subunit	hCGα	IRR 99/720

[a] Available from National Institute for Biological Standards and Control (http://www.nibsc.ac.uk/catalog/standards/preps/sub_endo.html).

(75/569) *(6)*. Although these preparations have served the scientific and clinical communities well, the hCG standard was originally intended for bioassay rather than immunoassay and was assigned units based on bioactivity, with 70 μg corresponding to 650 IU in the 3rd and 4th IS. The nonbioactive α and β subunits were assigned units based on mass, with 1 μg of each corresponding to 1 IU of the relevant IRP. Thus units for hCG and its subunits are not directly related, so it is difficult to compare results for the various forms of hCG to assess the extent to which they are recognized in different immunoassay systems *(7, 8)*. The presence of impurities and nicked forms of hCG in the 3rd and 4th IS was an additional reason to prepare new standards *(1)*.

The WHO Expert Committee on Biological Standards, when establishing these 1st IRRs, recommended that they should be used in the first instance to characterize the specificity of currently available immunoassays for hCG-related molecules. This recommendation is compatible with current thinking, in that the ideal metrological solution for heterogeneous analytes is to characterize and determine each of the components of the mixtures they comprise *(4, 9)*. In practice, although separate determination of the major forms has been shown to provide additional clinical information *(7, 10)*, most currently available hCG assays are designed to measure the main forms of hCG immunoreactivity together. Availability of highly purified IRRs calibrated in substance concentrations (mol) is therefore a major step forward toward improved characterization of hCG assays, which facilitates comparison of results for various forms of hCG and enables calibration of hCG assays in molar units. Universal adoption of the clear and unambiguous IFCC nomenclature (Table 1) is also a critically important prerequisite for improvement *(1)*, the adverse consequences of lack of clarity in descriptions of what hCG methods recognize having recently been clearly demonstrated *(11)*.

Here we report results of proficiency-testing studies, performed during a period of 7 years, to compare the immunoreactivity of the current 4th IS 75/589 and the more highly purified IRR 99/688 and to confirm in molar terms to the extent of recognition of the 6 newly established WHO IRRs in 14 commercially available assays and 2 in-house RIAs, all of which are currently calibrated against the essentially identical 3rd IS 75/537 or 4th IS 75/589.

Materials and Methods

Standards and reference preparations for hCG and its isoforms were obtained from the National Institute of Biological Standards and Controls. In addition to the 4th IS (75/589), these included the IRR for hCG (99/688), nicked hCG (hCGn, IRR 99/642), hCG β-subunit (hCGβ, IRR 99/650), nicked hCG β-subunit (hCGβn, IRR 99/692), and hCG β-core fragment (hCGβcf, IRR 99/708). The IRRs were assigned values in substance concentrations (mol) *(3, 4)*.

Specimens were prepared as previously described *(12, 13)*. Briefly, the contents of each ampoule of the IS or IRR were dissolved in 10 mL of 0.05 mol/L phosphate buffer containing 10 g/L BSA, pH 7.5, and were further diluted to the required concentrations (range 234–1680 pmol/L) with pooled normal human serum obtained from nonpregnant patients undergoing therapeutic venesection for hemochromatosis or polycythemia and kindly provided by the Scottish National Blood Transfusion Scheme. Serum pools were clarified by passage through a 0.2-μm filter. An antimicrobial agent (Kathon™; Rohm and Haas) was added to a final concentration of 0.5% vol/vol, before addition of known amounts of purified hCG-related preparations. Prepared pools were stored frozen in 1.0-mL aliquots at −30 °C before dispatch. Specimens were included in routine UK National External Quality Assessment Service distributions and, in accordance with usual practice, were sent by post at ambient temperature to participants in the UK National External Quality Assessment Service for hCG *(12, 13)*. The base pools were usually included as part of the specimen sets, with results confirming that hCG concentrations were undetectable (<5 U/L of IS 75/589). Details of the specimens issued and the dates of distribution are provided in Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue8. Specimens containing hCG IS 75/589 and IRR 99/688 were respectively distributed on 9 and 11 occasions during this period.

The participating laboratories used 16 different assays, i.e., 14 commercially available automated sandwich assays and 2 RIAs. Performance data for the

Table 2. Comparison of the mean response of the present WHO 4th IS for hCG (IS 75/589) and the new IRR for hCG (IRR 99/688).[a]

Method	Mean % recovery of IS 75/589 ± 95% CI	Mean % recovery (%) of IRR 99/688 ± 95% CI[b]
Assays for intact hCG		
Perkin-Elmer AutoDelfia	96 ± 4.7	122 ± 2.3
BioMérieux Vidas	107 ± 8.2	164 ± 3.4
Dade-Behring Dimension	94 ± 17.4	139 ± 11.6
Roche Elecsys Intact hCG	102 ± 6.3	141 ± 5.6
Assays for hCG and hCGβ		
Abbott Architect	104 ± 4.9	116 ± 1.7
Abbott AxSYM	121 ± 7.1	150 ± 4.3
Abbott IMx	126 ± 9.1	151 ± 8.6
Beckman Access	101 ± 4.0	109 ± 1.8
Siemens Centaur	98 ± 8.9	111 ± 6.8
Tosoh AIA Total	110 ± 10.8	146 ± 5.6
Assays for hCG, hCGβ and hCGβcf		
Roche Elecsys Total hCG	103 ± 2.7	124 ± 2.2
Siemens Immulite	113 ± 5.3	150 ± 6.2
Siemens Immulite 2000	118 ± 2.9	157 ± 4.6
Ortho Vitros ECi	112 ± 5.0	153 ± 2.3
RIA 1	109 ± 9.7	149 ± 14.0
RIA 2	93 ± 10.3	141 ± 9.0
Mean	106.6 ± 5.2	138.9 ± 9.2
SD	9.4	17.2
CV	8.8	12.3

[a] IS 75/589 issued on 9 occasions and IRR 99/688 on 11 occasions.
[b] Assuming a potency of 348 IU/nmol for IS 75/589.

former are available in the relevant kit inserts. The 2 RIAS show similar performance, with between-assay CVs approximately 12% at 7 U/L and 10% at 10–60 U/L and limits of detection approximately 5 U/L (at CV 15%). Using the results of this study, we classified the assays into 3 groups on the basis of their broad specificities, i.e., for hCG alone (4 assays), for hCG + hCGβ (6 assays), or for hCG + hCGβ + hCGβcf (6 assays) (Table 2). These classifications are in accord with manufacturers' data. (Changes in methods meant that not all methods were available during the entire 7-year time period of this study, as documented in online Supplemental Table 2.)

DATA ANALYSIS

All results reported by all users of each method examined were included, with the exception of any obvious outliers attributable to errors of transcription (incorrect data entry) or transposition (mislabeling of specimens) made by participants. Method mean results and variation were calculated for each specimen. For all assays, the response of each form of hCG (IRR) was compared with that of the 4th IS, which was included in the distributed specimen sets (Table 2). These specimen sets usually included the relevant base pool as one of the specimens. For this purpose the nominal relationship between units (IU and mol) for IS 75/589 was calculated to be 348 IU/nmol on the basis of its potency of 9286 IU/mg and its M_r of 37 500 *(3)*. Recognition in molar terms of the IRRs for each of the hCG-related isoforms was assessed relative to each individual method's recovery of hCG IRR 99/688 (Table 3).

Results

WITHIN-METHOD VARIATION

The CVs for results observed in various laboratories using the same method were in the range 3%–16%, and there were no significant differences between various methods. Whether assay calibration had changed dur-

Table 3. Recognition in molar terms of the various IRR preparations by each method.[a]

	hCGn 99/642	hCGβ 99/650	hCGβn 99/692	hCGβcf 99/708
Assays for intact hCG				
Perkin-Elmer AutoDelfia	121.8		—	—
BioMérieux Vidas	71.1	—	—	—
Dade-Behring Dimension	88.5	—	—	—
Roche Elecsys (intact) hCG	38.0	—	—	—
Assays for hCG and hCGβ				
Abbott Architect	87.9	115.1	77.4	—
Abbott AxSYM	92.7	140.9	88.1	—
Abbott IMx	85.9	144.3	88.7	—
Beckman Access	107.6	245.0	156.0	—
Siemens Centaur	97.9	115.0	68.9	—
Tosoh AIA Total	87.0	76.2	54.2	—
Assays for hCG, hCGβ and hCGβcf				
Roche Elecsys (total) hCG	96.5	130.3	92.2	33.6
Siemens Immulite	102.4	155.6	111.3	53.3
Siemens Immulite 2000	101.8	171.2	117.0	63.2
Ortho Vitros ECi	74.4	147.3	62.6	17.2
RIA 1	89.3	78.3	67.4	99.8
RIA 2	88.9	68.1	45.8	108.8

[a] Results are expressed as percentage ratios relative to the recognition of hCG IRR 99/688 for each method. Ratios of 100% ± 10% reflect equimolarity of recognition.

ing the study period was also evaluated by inspecting the recovery data for IS 75/589. The results of this evaluation confirmed for each individual assay that no significant changes had occurred, i.e., that the mean recovery for each method was within 10% of the method mean for the duration of the study.

RECOGNITION OF hCG

The mean recovery of hCG observed for specimens containing the 4th IS (IS 75/589) was 107% (range 93%–126%), with only 5 assays deviating from 100% by more than 10% (Table 2). These results confirm that all assays recognized fairly accurately the standard against which they were calibrated. As would be theoretically predicted *(2)*, results for the much purer new hCG preparation (IRR 99/688) were higher, with a mean recovery of 139% (range 109%–164%) (Table 2). Thus 1 nmol of hCG preparation 99/688 corresponds on average to 484 IU of IS 75/589 compared to the predicted 348 IU for IS 75/589. Also as predicted, there was more variation in the recovery of IRR 99/688 (CV 12.3%) than that of IS 75/589 (CV 8.8%), suggesting that the 2 standards are recognized slightly differently in these assays, although this difference did not reach statistical significance (F-test, $P = 0.24$).

RECOGNITION OF hCGn

The recognition of hCGn (IRR 99/642) was on average 11% lower than that of hCG IRR 99/688. Interestingly, in 2 assays, recognition of hCGn was somewhat higher than that for hCG 99/688, and 1 assay specific for intact hCG underestimated hCGn by 62% (Table 3). Between-method variation for hCGn was larger than for hCG, CVs being 20% vs 12%. These results suggest that nicking affects immunoreactivity variably and that the effect in some assays is quite significant.

RECOGNITION OF hCGβ AND hCGβn

Twelve of the assays also recognized hCGβ (IRR 99/650) and hCGβn (IRR 99/692); 9 of these overestimated hCGβ significantly compared to hCG (IRR 99/688). Mean recognition of hCGβ was 132% of the recognition of hCG itself, whereas that for hCGβn was 86%. The variation was large, the range for hCGβ being 68%–245% (CV 37%) and that for hCGβn 46%–156% (CV 36%). Interestingly, most sandwich assays overestimated hCGβ, whereas the 2 RIA methods underestimated it by 20%–30% (Table 3). The 2 forms of hCGβ were recognized somewhat differently by the various assays, such that the mean ratio of results for hCGβn and hCGβ was 62% with a range of 32%–77% (CV 19%). Nicking thus appears to affect immunoreactivity somewhat differently for hCG and hCGβ (Table 3).

RECOGNITION OF hCGβcf

The mean recognition of hCGβcf (IRR 99/708) compared to IRR 99/688 was 63% in 6 assays, but variation was large (CV 57%). The 4 sandwich assays all underestimated hCGβcf by 37%–83%. In contrast, recognition of hCGβcf by the 2 RIAs, which were both validated for quantitative measurement of hCG in urine, was close to equimolar (99.8% and 108.8%) compared to their recognition of IRR 99/688. Although these results were obtained in serum, data from the UK National External Quality Assessment Service for Pregnancy Testing, in which urine specimens containing added hCG (IS 75/589) are regularly issued, demonstrate that recognition mimicking that observed in serum is observed when urine specimens containing added hCG (IS 75/589) or hCGβcf are issued *(14)*.

RECOGNITION OF hCGα

Confirming results obtained on distribution of the 1st IRP for hCGα (IRP 75/569), this isoform was not recognized by any of the methods tested.

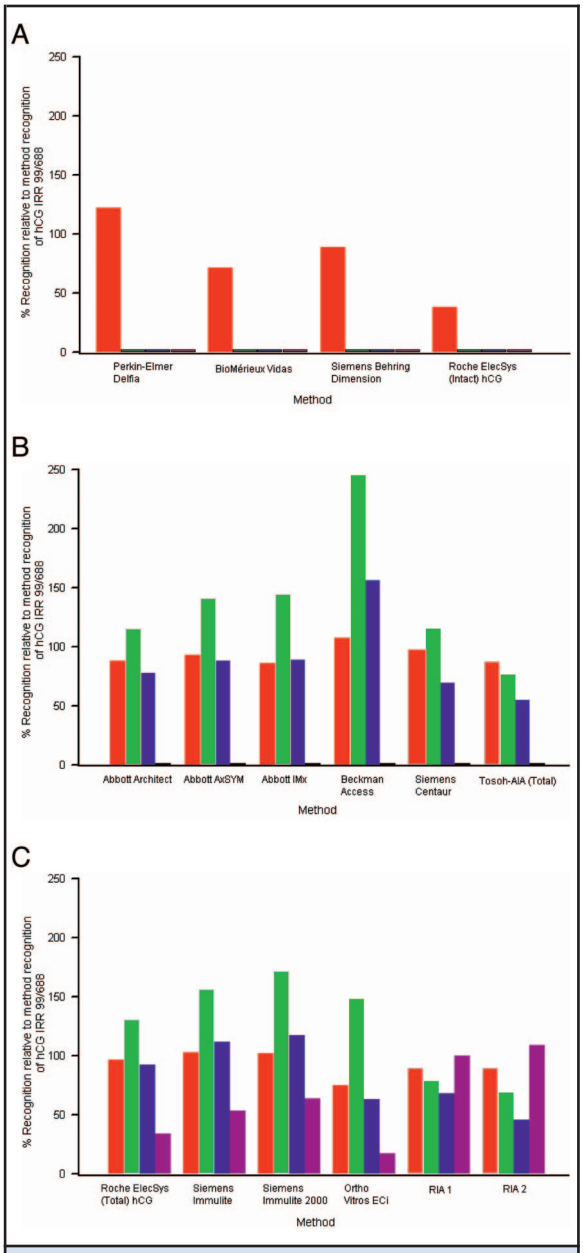

Fig. 1. Recognition in molar terms of the various IRR preparations by each method for (A), assays recognizing intact hCG; (B), assays recognizing hCG and hCGβ; and (C), assays recognizing hCG, hCGβ, and hCGβcf.

Results are expressed as percentage ratios relative to the recognition of hCG IRR 99/688 for each method. [hCGn; hCGβ; hCGβn; hCGβcf].

COMPARISON OF EQUIMOLARITY OF THE ASSAYS

The recognition of various forms of hCG (IRRs) on a molar basis is shown in Fig. 1. No assay provided equimolar recognition of all isoforms. Of the assays recognizing all forms, RIA 1 results underestimated hCGβ and hCGβn and recognized hCGβcf appropriately, whereas the sandwich assays tended to overestimate hCGβ and underestimate hCGβcf. The sandwich assays recognizing hCG and hCGβ tended to overestimate hCGβ and underestimate hCGβn and hCGβcf. Recognition of IRR 99/688 was close to expected for assays recognizing only hCG, but their recognition of hCGn was quite variable.

Discussion

Results demonstrate that most hCG assays represented are fairly well calibrated for assay of hCG in terms of IS 75/589, against which all assays are standardized. The importance of commutability has previously been highlighted *(15)*. The hCG variants used to prepare the specimens described in this study were purified from pooled human urine. Purification methods were selected with the aim of preserving the physiological characteristics of the variants, apparently successfully, as reflected in the high bioactivity of hCG IRR 99/688 *(3)*. The specimens used in the present study were prepared in normal human serum *(12)* and have been shown to behave similarly to specimens containing diluted patient sera *(14)*. Three assays demonstrated recovery of added IS 75/589 of 118%–126%, suggesting slightly different calibration. Based on the molar content of protein, recognition of hCG IRR 99/688 was on average 39% higher than that of the 4th IS. If the mean calibration error of the assays of +7% in relation to IS 75/589 was taken into account, the new hCG preparation IRR 99/688 had 32% higher immunoreactivity than the 4th IS. The higher results observed for IRR 99/688 could be predicted on the basis of its higher purity, i.e., more than 99% of the protein consists of intact hCG *(2, 3)*. This difference in immunoreactivity was also in accord with the higher bioactivity of IRR 99/688, which is reportedly 1.1–1.5-fold that of IS 75/589 *(3)*. The results for IRR 99/688 showed larger between-method variation than that for the 4th IS, with CVs of 12% and 9%, respectively. This finding probably reflects both the presence of impurities and nicked forms in IS 75/589 and differences in the specificities of the antibody combinations used by various manufacturers *(2, 16)*.

The presence of impurities and partially degraded forms of hCG (particularly hCGn) in IS 75/589 was a major reason for the preparation of new hCG standards *(1)*. The importance of pure standards is demonstrated by the differences in recognition of IS 75/589 and IRR 99/688 in various assays. Introduction of IRR 99/688 for standardization of hCG assays clearly would influence calibration, affecting both absolute values for

clinical specimens and the relationship between different assays (Table 2).

Calibration of the IRRs in substance concentrations (mol) facilitates comparison of the equimolarity of recognition of these isoforms in various methods. The results demonstrate that no assay is truly equimolar, with nonequimolarity most apparent for some sandwich assays recognizing all major forms of hCG. The variability observed almost certainly reflects differences in assay design and selected antibody specificities. Interestingly, results for the 2 RIAs obtained by using polyclonal antisera appear to be closest to equimolar.

All sandwich assays designed to detect hCGβ overestimated it to varying degrees. Results for the Beckman Access, Siemens Immulite, and Ortho Vitros methods were approximately 1.5–2.5 times higher than those for hCG IRR 99/688 (Table 3 and Fig. 1). In contrast, the RIAs underestimated hCGβ by 20%–30%. The large between-assay variation observed will inevitably lead to considerable discrepancies in hCG results for sera from patients with cancers producing hCGβ. This form of hCG is the only one expressed in 30%–50% of nontrophoblastic cancers, whereas many testicular cancers and choriocarcinomas express both hCG and hCGβ *(17–19)*. Recognition of hCGβn was on average 47% lower than that of hCGβ, a result that may reflect lower immunoreactivity of this isoform. However, some loss of immunoreactivity during sample delivery and handling may have occurred, because hCGβn is less stable than hCGβ, as observed during the purification procedure *(3)*. The differences between assays related to nicking were relatively small.

Variable recognition of nicked hCG (hCGn), in which peptide bonds in amino acids 44–48 of hCGβ are cut, has been suggested to contribute to discordant hCG results, particularly in sera from cancer patients *(20, 21)*. Although as expected most methods recognized hCGn *(22)*, results of the present study suggest that variability in hCGn recognition is not a major problem, because results for most methods were within 15% of equimolarity. One method specific for hCG clearly underestimated hCGn, suggesting the antibody used recognizes an epitope comprising parts of each subunit *(16, 23)*.

Only 4 of the sandwich assays [Roche Elecsys (total), Siemens Immulite and Immulite 2000, and Ortho Vitros] recognized hCGβcf, but recognition was not equimolar, varying from 17% to 63%, whereas recognition in the RIAs was close to 100%. hCGβcf concentrations are very low in plasma, but in urine this form is present as a major degradation product of hCG and hCGβ *(18)*. Two of the methods that recognize hCGβcf are currently validated for qualitative (but not quantitative) measurement of hCG in urine, either in Europe (Roche Elecsys) or worldwide (Siemens Immulite and Immulite 2000). Variable recognition of hCGβcf will clearly result in large method-related differences when hCG is measured in urine. Because hCGβcf is probably derived from hCGβ, assay of this metabolite in urine theoretically provides a possible alternative to measurement of hCGβ in serum of patients with nontrophoblastic tumors *(18)*. Assays specifically measuring hCGβcf have been established for scientific purposes *(16)*, but commercial assays for hCGβcf are not available, and use of assays that also detect hCG and hCGβ is undesirable because pituitary hCG may cause a background signal that reduces clinical sensitivity *(19)*. The clinical value of using such methods for monitoring cancer patients has not been studied in detail.

Major factors contributing to between-method differences in results for immunoassays include calibration errors and variations in antibody specificities, assay design, purity, and other characteristics of the standards and calibrators employed *(2, 16, 22, 23)*. Results of the present study suggest that all these factors contribute to the variation observed. Impurities in IS 75/589 may explain the differences between the results for this standard and the more highly purified IRR 99/688 as well as the larger between-method variation for the latter form. The more variable recognition of IRR 99/688 may seem surprising, but reflects the fact that currently available assays are calibrated against the 4th IS. Use of the purer standard IRR 99/688 as a primary calibrant is likely to reduce between-method variation when serum samples are assayed. A valid test for the purer standard IRR 99/688 as a primary calibrant will be to analyze clinical samples with assays calibrated with the new reference preparation for hCG, because these samples are likely to contain a mixture of hCG isoforms. Work relevant to this question is currently being undertaken by the IFCC Working Group.

Recommendations have been published regarding the most appropriate antibody combinations for various clinical applications *(23)*. However, without exact knowledge of the epitope specificity of the antibodies used, it is not possible to evaluate whether assays have been designed in accord with these recommendations. The present study shows that design of assays with equal recognition of the various isoforms of hCG is problematic. Some groups have solved this problem by using separate assays for the major forms of hCG, i.e., hCG, hCGβ, and hCGβcf *(7, 18)* but most manufacturers provide only 1 hCG assay. It remains to be established whether it is possible to design an assay that recognizes all clinically relevant forms of hCG in an equimolar fashion.

The extent to which differences in recognition of various isoforms of hCG are clinically problematic will

depend on the clinical application. In pregnant women, most circulating hCG consists of the intact form, and hCGβ comprises only a small percentage of hCG immunoreactivity in plasma. For monitoring pregnancy, problems caused by differences in isoform recognition and errors in calibration are relatively small and are likely to be relevant only if different assays are used to evaluate changes in hCG concentrations in plasma at relatively short time intervals (e.g., 1–2 days). In urine, however, much of the immunoreactivity consists of hCGβcf, and larger discrepancies in results are likely. Furthermore, considerable day-to-day changes in the proportions of various forms of hCG will also cause problems if urine samples are used to monitor pregnancy *(24)*. Measurement of urinary hCG with sandwich assays may also pose problems if hCGβcf is present at higher concentrations than hCG and reacts with only 1 of the antibodies used, potentially decreasing signal generation.

Variations in assay specificity are most critical when hCG is used to monitor cancer patients. Provided hCG and hCGβ are both measured, any of the assays studied can be used to monitor placental trophoblastic tumors, although differentiation between benign molar disease and choriocarcinoma may be improved by separate determination of hCGβ and hCG *(7)*. Separate determination of both hCG and hCGβ is also of value for diagnosis and monitoring of testicular cancer *(25)*. Recognition of hCGβ is useful in the diagnosis of nontrophoblastic tumors, 30%–60% of which produce hCGβ, but not hCG *(10)*. When these tumors are monitored, use of a separate assay for hCGβ is advantageous because the reference values for hCG + hCGβ are higher than those for hCGβ alone *(10, 18)*.

The IFCC hCG Working Group is considering how best to develop a reference material for hyperglycosylated hCG, a marker with particular relevance in gestational trophoblastic disease *(26)*. The highly heterogeneous carbohydrate structure of hyperglycosylated hCG presents considerable challenges, particularly in relation to selection of the most clinically appropriate starting material for such a standard. Optimal sources, which include early pregnancy urine, urine from patients with testicular cancer, and choriocarcinoma cell lines, are currently being investigated.

There is a need to replace the hCG 4th IS 75/589 within a few years. The present study clearly demonstrates that introduction of a new standard will affect assay calibration to varying extents in different assays and confirms the advantages of calibrating hCG assays in substance concentrations (mol). The same bulk material used to prepare the hCG IRR 99/688 has also been prepared as a potential candidate fifth IS and is currently undergoing evaluation in an international collaborative study. Values will be assigned to the new IS in terms of both molar units and IU, because this reference preparation will also be used in the assignment of potency (in IU) to therapeutic preparations of hCG. Introduction of a new standard would provide an excellent opportunity to start reporting hCG results in substance concentrations, one of the original aims of the IFCC hCG standardization project *(1)*. Expression of hCG results in molar units is especially beneficial for comparison of concentrations of various molecular forms, e.g., hCG and hCGβ *(1, 2)*. Furthermore, use of substance concentrations, whenever scientifically justified, is a major goal of the IFCC *(1, 9)*. Such a change would clearly require considerable educational effort and lobbying worldwide, but ultimately the potential benefits of expressing hCG results in molar units would be considerable.

Conclusions

The results of this study demonstrate large between-assay differences in recognition of isoforms of hCG. Because the highly purified 1st IRRs for the 6 major isoforms of hCG have been calibrated in substance concentrations, it was possible to characterize and assess objectively what is measured by current hCG methods. Differences in antibody specificity and assay design explain most of the differences in recognition, but impurities in the 4th IS also contribute to between-assay variation. It is evident that accurate calibration and equimolar recognition of various isoforms of hCG are essential for improved standardization. Achieving this goal will require introduction of the IRRs as standards, changes in assay design, and careful calibration.

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