Problems with the Measurement of Human Choriongonadotropin (hCG) in External Quality Assessment Surveys

Probleme bei der Messung von humanem Choriongonadotropin (hCG) in Ringversuchen

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Summary: The Institute for Standardisation and Documentation in Medical Laboratories (INSTAND) carries out regular external quality assessment surveys in accordance with the Calibration Act of the Federal Republic of Germany. In this brief report, the state of the art of assays for human chorion gonadotropin (hCG) is discussed, taking results from surveys between October 1993 and January 1996 into account. Four serum samples were distributed several times over the observation period and a fifth serum, a recalcified plasma (blood group 0, Rh positive) was sent as a control for the reference range for men and nonpregnant women.The results showed a worsening of the comparison between methods, especially for assays measuring intact hCG. Whereas all methods could be compared in one group at the beginning of the observation period, with inter-laboratory coefficients of variation under 20%, this was no longer possible after October 1994, where differences between measured concentrations were almost a factor of two in the most extreme cases. The aim of this publication was to draw attention to this unacceptable state of affairs, which has most probably been brought about by the use of pairs of monoclonal antibodies recognising some, but not all, important epitopes of circulating hCG.

Keywords: Gonadotropins, Chorionic/analysis; Chorionic Gonadotropin, beta Subunit, Human; Sensitivity and Specificity; Reagent Kits, Diagnostic; Product Surveillance, Postmarketing; Quality Assurance, Health Care.

Zusammenfassung: Das Institut für Standardisierung und Dokumentation im medizinischen Laboratorium (INSTAND) führt regelmäßig Ringversuche in Übereinstimmung mit dem in der Bundesrepublik Deutschland gültigen Eichgesetz durch.

In der vorliegenden Arbeit wird eine Standortbestimmung der Assays für humanes Choriongonadotropin (hCG) vorgenommen, wobei die Ergebnisse von Ringversuchen zwischen Oktober 1993 und Januar 1996 betrachtet werden. Vier Serumproben wurden wiederholt während des Beobachtungszeitraums verteilt; zusätzlich wurde ein fünftes Serum (recalzifiziertes Plasma der Blutgruppe 0, Rh positiv) als Kontrollprobe im Referenzbereich für Männer und nichtschwangere Frauen versandt. Die Ergebnisse zeigten beim Vergleich zwischen den einzelnen Methoden. insbesondere bei solchen, die intaktes hCG messen, eine Verschlechterung. Während zu Beginn des Beobachtungszeitraumes bei einem maximalen Variationskoeffizienten von 20% alle Methoden in einer Gruppe verglichen werden konnten. war dies nach Oktober 1994 nicht weiter möglich, wobei sich in Extremfällen die gemessenen Konzentrationen nahezu um den Faktor 2 unterschieden. Zweck dieser Publikation ist es, auf diesen unhaltbaren Zustand hinzuweisen, der höchstwahrscheinlich durch die Verwendung von Paaren monoklonaler Antikörper entstanden ist, die nicht alle wichtigen Epitope von zirkulierendem hCG erkennen.

Schlüsselwörter: Choriongonadotropine/Analytik; Choriongonadotropin, beta- Kette, Humanes; Sensitivität und Spezifität; Reagenz-Kits, Diagnostische; Produktüberwachung nach Markteinführung; Qualitätssicherung, Gesundheitswesen.

As one of two recognised professional bodies in the Federal Republic of Germany, INSTAND carries out regular external quality assessment schemes for a multitude of laboratory analytes. One group of analytes for pituitary hormones includes the determination of human chorion gonadotropin (hCG). Until October 1994, all participants were able to be assessed as a single group; since this time, this procedure is no longer possible. This short communication describes the trends in hCG measurement between October 1993 and January 1996.

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The study includes kits declared to measure 'intact hCG' [hCG] and those declared as measuring 'intact hCG + beta-subunit' [hCG + β]. Although methodological differences and the use of different antibody-pairs have been seen as the cause for differing results in assays for lutropin (LH) [1-4], such problems for hCG have not been encountered, at least not sufficiently for cause of concern in inter-laboratory comparison.

Materials and Methods

The study includes four commercially prepared lyophilised human-based spiked sera which were distributed several times over the observation period. As a control in the normal/reference range for males and non-pregnant females a sample prepared from male and non-pregnant female blood donor plasma (blood group O, D positive) was used. The sample was first recalcified, then passed through a sterile filter and lyophilised without addition of stabilisers or anti-microbial agents.

Certain kits have been selected to show the worsening trend as far as comparability of results is concerned. These kits have been assigned codes to retain anonymity.

As the data were normally distributed within each group (*Kolmogorov-Smirnoff* test) the mean and coefficient of variation (CV) were used as points of central tendency and dispersion respectively.

Nomenclature

Problems arose as many participants did not know which forms of hCG were recognised by the kit used. This was further complicated as several manufacturers and suppliers of kits had methods for 'total hCG'/ 'intact hCG' as well as for 'total hCG + β-hCG'/ 'β-hCG'. Whereas the majority of kits for total/intact hCG were calibrated against the third international standard (NIBSC 75/537), those for measuring intact and β-subunit cannot be calibrated against a single standard, although this has been attempted by many producers.

The calibrator itself was given as 3rd IS, 3rd IS 75/537, 1st IRP, 1st IRP 75/537 and 3rd IRP 75/537, so that the user who has not informed himself about IRP/IS nomenclature is often confused as to how his kit has been calibrated!

Finally, problems arose from the instructions accompanying the kit – for example, a producer selling a kit for hCG (titled hCG), who gave excellent reasons for using the 3rd IS as calibrator, as it contained only intact hCG, described how the kit used two monoclonal antibodies specific for the β-subunit and declared that the kit measured intact hCG and free β-subunits.

Nicht standardisierte Abkürzungen: CV, coefficient of variation; hCG, humanes Choriongonadotropin; IRP, International Reference Preparation.

Groups used for Analysis

In the first period of observation (October 1993 – May 1994), all participants were assessed together. As it became apparent that the interlaboratory coefficient of variation was continually increasing, groups of kits measuring in the same range were constructed to allow a statistical evaluation. These groups were designated A1–C1 for kits measuring intact hCG, and A2–C2 for kits measuring intact plus β-subunit. The kits in each group remained constant. As this grouping was not optimal, single kits with sufficient participants for statistical analysis were selected and designated as above. Kit A1, for example, was from manufacturer A and was declared to measure intact hCG. Kit A2 was from the same manufacturer and was declared for measuring intact hCG + β-subunit. Kits not being able to be allotted to a group, or in insufficient numbers for seperate statistical analysis formed the rest group. The composition of this group was heterogeneous, the results being given in tables 1a–1c for completeness.

Results and Discussion

Tables 1a and 1b show the results from the study, arranged according to the samples and as to whether intact hCG (tab. 1a) or intact hCG + β-subunit (tab. 1b). Table 1c shows the results of the recalcified blood plasma sample, which was only analysed once during the observation period.

Intact hCG

It is clear to see that whereas from October 1993 until May 1994 all kits could be assessed together with an inter-laboratory coefficient of variation under 20%, from October 1994 on this was no longer possible.

The results from samples I and II show that changes in antibodies and/or standardisation occurred at or around the end of 1993. From mid-1994, Kit A1 was unable to be assessed with the rest of the kits and from the beginning of 1995, groups of kits giving similar results had to be formed, followed by single kit evaluation for kits with sufficient participants for statistical analysis.

From mid 1994 the results from each different kit/groups of kits was moreorless stable, if kit A1 was excluded, for each sample. The absolute measured concentrations however differed from kit to kit and group of kits to group of kits. For sample III, an unexplainable jump in the measured intact hCG concentration occured in Kit A1 between May 1995 and January 1996.

The trend for hCG, not seen for the other pituitary hormones of this family (lutotropin, follitropin, thyrotropin) in these four sera, can only be described as being counter-productive for inter-method comparison.

The same question must be raised as for lutropin, that is, whether the choice of monoclonal antibodies used in the hCG sandwich assays is perhaps too speci-

Table 1a Trends in human chorion gonadotropin measurement in four selected samples over a three year period. Results for 'intact hCG'

Date Sent	Mean (IU/l)	CV (%)	Participants (n)	Kit/ Group
Sample I				
October 1993	146	16.9	154	All
May 1994	144	18.3	130	All
October 1994	178	13.9	79	Kit A1
	128	17.8	103	Rest
May 1995	132	8.44	13	Kit A1
	131	11.5	37	Group A1
	113	8.53	18	Group B1
	122	12.3	5	Group C1
	142	1.08	4	Rest
Sample II				
October 1993	19.0	18.6	154	All
January 1994	35.4	16.1	87	All
October 1994	70.9	11.5	79	Kit A1
	36.4	17.2	103	Rest
January 1995	66.0	18.2	30	Kit A1
	36.8	16.8	4	Kit B1
	36.9	19.4	62	Rest
January 1996	77.8	7.39	23	Kit A1
	44.1	5.29	6	Kit C1
	28.5	11.5	11	Rest
Sample III				
May 1994	18.1	15.5	130	All
May 1995	14.9	10.7	13	Kit A1
	17.4	16.7	37	Group A1
	33.6	8.96	18	Group B1
	12.4	15.1	5	Group C1
	16.6	18.6	4	Rest
January 1996	70.8	9.92	23	Kit A1
	28.5	11.5	6	Kit C1
	11.8	12.4	11	Rest
Sample IV				
January 1994	625	19.3	87	All
January 1995	784	20.5	30	Kit A1
	600	7.1	4	Kit B1
	587	17.1	62	Rest
October 1995	821	17.3	40	Kit A1
	594	15.1	21	Group A1
	488	12.3	14	Group B1
	568	22.8	14	Rest

Table 1b Trends in human chorion gonadotropin measurement in four selected samples over a three year period. Results for 'intact hCG + β-subunit'

Date Sent	Mean (IU/l)	CV (%)	Participants (n)	Kit/ Group
Sample I				
May 1995	193	10.9	57	Kit A2
	174	16.4	9	Group A2
	123	13.6	9	Group B2
	168	18.7	5	Group C2
	177	21.8	13	Rest
Sample II				
January 1996	73.8	15.4	20	Kit A2
	36.8	8.01	11	Kit B2
	41.9	14.9	9	Group C2
Sample III				
May 1995	67.5	11.7	57	Kit A2
	67.1	19.6	9	Group A2
	38.1	23.5	9	Group B2
	57.9	5.09	5	Group C2
	55.8	18.4	13	Rest
January 1996	67.0	13.0	20	Kit A2
	36.8	8.01	11	Kit B2
	33.0	15.7	9	Group C2
Sample IV				
October 1995	911	11.6	34	Kit A2
	686	18.3	20	Group A2
	531	16.8	8	Group B2

Table 1c Sample in reference range for males and non-pregnant females (Recalcified blood donor serum sent October 1995)

	Mean (IU/l)	CV (%)	Participants (n)	Kit/ Group
Intact hCG				
	0.39	23.3	40	Kit A1
	0.97	25.2	21	Group A1
	1.42	24.7	14	Group B1
	1.34	15.9	14	Rest
HCG + β-subunit				
	0.47	28.1	34	Kit A2
	0.94	12.0	20	Group A1
	0.91	12.9	8	Group B2

fic, thus excluding some epitopes of the hormone molecule, which may be present, for example 'nicked-hCG'. Curiously enough, follitropin assays have not suffered problems during the change from competitive to immunometric assays and from the use of polyclonal antisera to pairs of monoclonal antibodies.

Intact hCG + β-subunit.

The sorting of kits into those measuring intact-hCG (holo-hCG) and those measuring both the intact molecule as well as the free β-subunit was first necessary in 1995 as more producers/suppliers had kits designed to measure both variations. This led to confusion amongst the user, who often did not know what his/her kit measured. The appearance of a bimodal distribution of the values for kit A1 in January 1995, reflected by the inter-laboratory coefficient of variation over 20% confirmed suspicions that both kits measured different analytes in sample IV. This was further supported by the results from sample I in May 1995, where the results for kit A2 were on average above 60 IU/l higher than for kit A1. In both cases, the inter assay coefficient of variation had been reduced by almost 50%.

Kit A1 and A2 are from the same producer and both are offered for use with the company's automatic analysers. It can be excluded that some participants were not aware of the difference between the declared specificity of Kit A1 and A2 and entered their results in the wrong column, as the interlaboratory precision was excellent (see table 1a, Kit A1, May 1995 and January 1996).

It is difficult to know whether free β-subunits alone can account for the difference in concentrations measured as kits designed to measure only the free β-subunit found similar concentrations in all four samples. As these kits were standardised against NIBSC 75/551, the 1st. IRP for the β-subunit of hCG, a comparison of results was not directly possible.

The presence of a wide range of hCG forms and metabolites in blood and urine is well known, as is the difference of these forms to react with monoclonal antibodies [5,6].

Although the within-kit inter-survey results are more stable for kits measuring intact hormone and free β-subunits, the absolute values vary enormously.

This short presentation is designed to highlight the problems occurring with the determination of human chorion gonadotropin in serum. In contrast to other hormones, for example thyrotropin and intact parathyrin, the situation with hCG has got worse instead of better. The producers of kits are encouraged to improve this situation by choosing pairs/groups of monoclonal antibodies which recognise the main forms of hCG occurring in serum. The problems of 'standardising' a mixture (holo-hCG and free β-subunits) should not be underestimated as this is theoretically impossible! Furthermore, the producers should use the correct terminology for what they offer. Despite the fact that a meeting of scientists and producers in Bonn in 1983 [7] who met to discuss standardising 'hCG-terminology', there are still many kits which are incorrectly labelled.

Finally, it should not be underestimated that incomparability between methods, especially in a decentralised health system, may lead to misclassification errors and errors in patient care [8]. In the days of full automation of laboratory methods, the question of adequate quality control is often falsely taken for granted [9].

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