Standardization of chorionic gonadotropin

- when heterogeneity is clinically relevant

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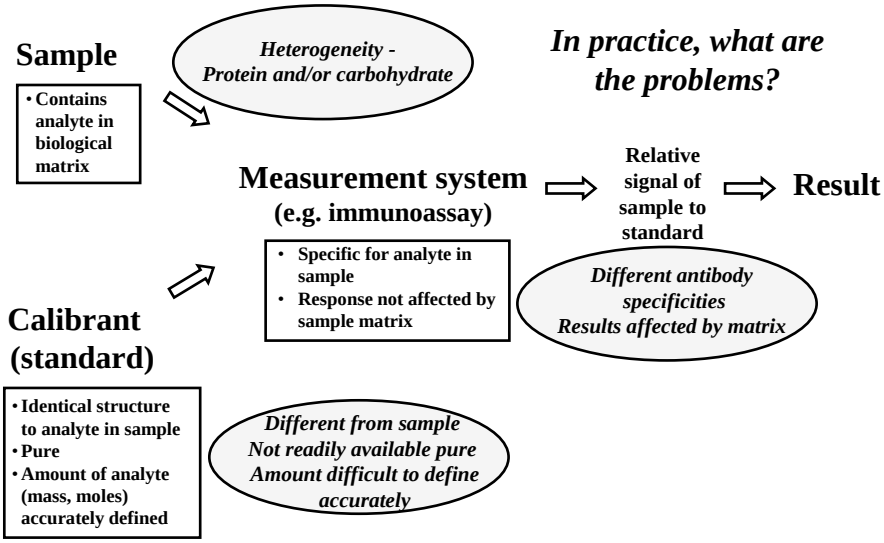
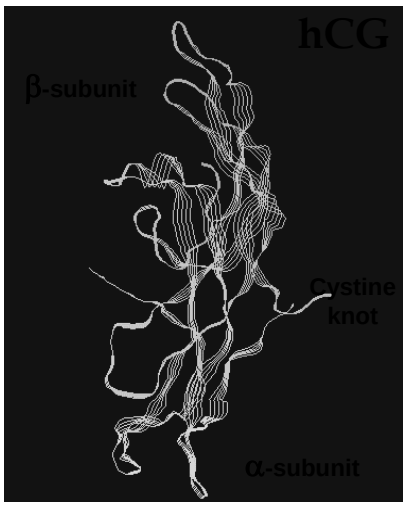
Fundamental components of the ideal assay system



Chorionic gonadotrophin (hCG)



Structure
Glycoprotein hormone, with linked α- and β-subunits.

Shares the same α-subunit with LH, FSH and TSH; hCG β-subunit most closely resembles that of LH.

Human serum contains:
- **Intact hCG**
- **Free beta subunit of hCG**
- **Other fragments and modified forms**

Why is standardisation of hCG assays a problem?

Multiple molecular forms in serum

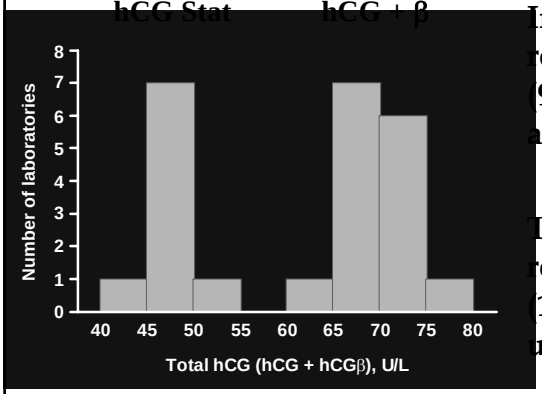
Previous lack of International Standards of defined composition and molarity

hCG assay methods measure different molecular forms – primarily three types of methods
- **"Intact" hCG**
- **"Total" hCG – measures intact hCG + free β-subunit**
- **"Free beta" hCG**

How do these fundamental problems affect laboratory and clinical practice?

Are laboratories reporting serum quantitative hCG results correctly?

Single method **Across all methods**



Intact hCG results reported by 22 / 235 labs (9.3%) registered as using a "total" method.

Total hCG results reported by 8 / 61 labs (13.1%) registered as using an "intact" method.

Cao and Rej, *Clin Chem* 2008; 54: 761-4

Contributory factors

- **Complexity of hCG molecule and confusion re nomenclature for various forms of hCG.**
- **Lack of clarity and uniformity in manufacturers' reagent labelling.**
- **Lab personnel's lack of awareness of hCG forms and specificity of method used.**
- **Lack of information relating to specificity to various forms of hCG in kit inserts.**

Cao and Rej, *Clin Chem* 2008; 54: 761-4

Stenman *et al*, *Scand J Clin Lab Invest* 1993; 53 (S216): 42-78

How are these problems being addressed?

IFCC hCG Working Group

Background
- **Established by the International Federation of Clinical Chemistry (IFCC) in 1994**

Remit
- **To investigate how best to "standardize" assays for complex analytes - taking hCG as a prototype for other molecules**

Working Group members

P Berger (Austria)	Antibody mapping
J-M Bidart (France)	Antibody mapping
S Birken (USA)	Protein chemist
R Norman (Australia)	Practicing gynaecologist
U-H Stenman (Finland)	Standardisation
C Sturgeon (UK)	External quality assessment

Supporters & collaborators

Abbott Diagnostics	Bayer Diagnostics
Beckman-Coulter	BioClone
BioMérieux	Chiron Diagnostics
CIS bio International	Dade Chemistry
DPC	Ortho Diagnostics
Perkin Elmer	Randox Labs
Roche Diagnostics	Unipath Ltd

A Bristow & C Burns (NIBSC)
J Sharratt (University of Cambridge)

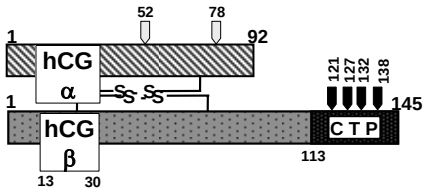
International Standards (IS) for hCG

Three IS available from early 1970s
- **hCG, hCGα and hCGβ.**
- **Arbitrary units assigned to all three**
  → comparing method recognition difficult.

IFCC Working Group approach
- **Prepare highly purified International Standards for six clinically relevant forms of hCG.**
- **Assign values in molar units to these**
  → comparing method recognition straightforward.

Structure and IFCC nomenclature

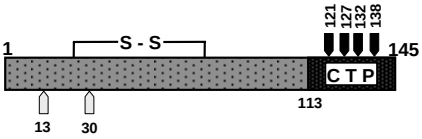
hCG



Intact hCG

Biologically active. In plasma, serum and urine in pregnancy and cancer.

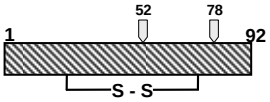
hCGβ



Free beta-subunit

No biological activity. In plasma, serum and urine in pregnancy and cancer.

hCGα

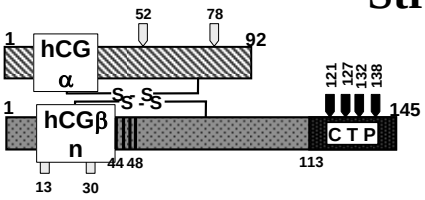


Free alpha-subunit

No biological activity. In plasma and serum, especially in cancer (infrequently).

Structure and IFCC nomenclature

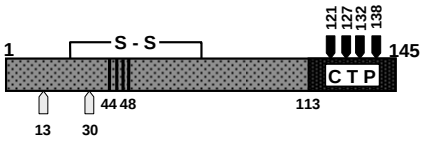
hCGn



Nicked hCG

Absent or diminished biological activity. May be present in plasma and serum.

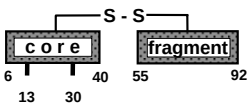
hCGβn



Nicked free beta-subunit

Unstable. Common form in urine; may occur in plasma.

hCGβcf



Beta core fragment

Major form in urine. Plasma concentrations very low.

International Collaborative Study

Participants

- **Two laboratories using four different procedures for amino acid analysis**
- **Ten laboratories using different immunoassay systems → estimates of recovery (and indication of reactivity)**

Results

- **Values corrected for loss on reconstitution generally in good accord with predicted**
- **Values assigned in substance concentrations – i.e. moles**

Bristow *et al. Clin Chem* 2005; 51: 177-182

New International Standards for hCG

Symbol	Species	WHO code
hCG	Intact chorionic gonadotropin	99/688
hCGn	Nicked hCG	99/642
hCGβ	Free beta-subunit of hCG	99/650
hCGβn	Nicked free beta-subunit	99/692
hCGβcf	Core fragment of hCG	99/708
hCGα	Free alpha-subunit of hCG	99/720

In November 2001, the preparations were officially established as the first WHO Reference Reagents for Immunoassay for these hCG-related molecules. Calibrated in molar units, WHO recommended that they be used primarily to enable better characterization of the specificities of current hCG assays.

Benefits of new reagents

- **Calibration in molar units permits ready comparison of the extent to which different hCG-related molecules are recognised in different methods.**

- **Availability of these highly purified International Reference Reagents should ultimately improve between-method comparability.**

“Standardisation” of antibody specificities

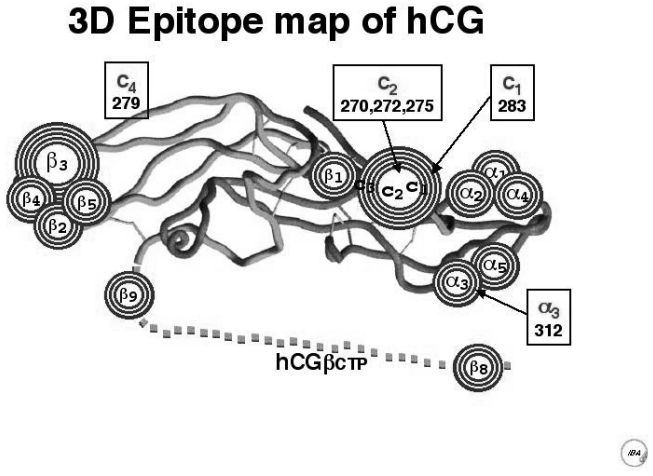
ISOBM Antibody Workshops

- **Panel of antibodies coded, aliquoted and circulated to participants undertaking experimental work.**
- **Characterisation studies reported, discussed and published in the journal *Tumor Biology*.**
- **Results may inform choice of antibodies for the next generation of tumour marker assays.**

Workshops have been held on
AFP, CEA, hCG, PSA
CA125, CA15.3, CA19.9
Cytokeratins, SCC, S100, Alkaline phosphatase

ISOBM, International Society for Oncology and Biomarkers

First ISOBM Workshop on hCG



ISOBM, International Society for Oncology and Biomarkers

Berger *et al.*
***Tumor Biol* 2002; 23: 1-38**

Key points for assay construction

Assay specificity	Recommended MAb combinations	Appropriate clinical use
hCG+ hCGβ	β_1 MAb with β_2 or β_4 detection MAbs	Oncology Early pregnancy
hCG	$c_{1/2}$ MAbs with β_2 or β_4 detection MAbs	Early pregnancy Prenatal (Downs)
hCGβcf only	β_{11} MAbs with β_2 or β_4 detection MAbs	In urine only Clinical utility to be established

Berger et al.
***Tumor Biol* 2002; 23: 1-38**

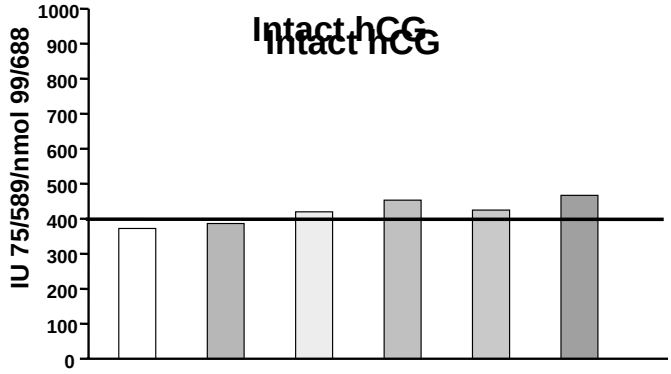
Characterization of current methods

Assessment of specificity

- **Pools containing known amounts of hCG Reference Reagent prepared in normal human serum.**
- **Issued to all participants without identification as part of routine distributions in the UK NEQAS for hCG.**
- **Pools issued periodically from 2001 to 2007.**
- **Results analyzed by difference or by linear regression → Units of IS 75/589 per nanomole of preparation.**
- **Excellent reproducibility of results demonstrated.**

UK NEQAS, UK National External Quality Assessment Service

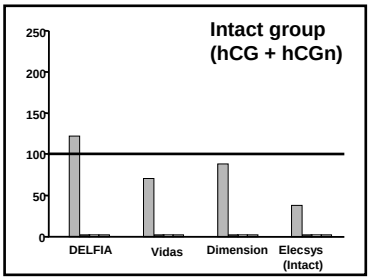
Intact hCG

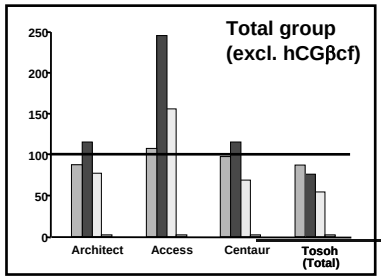


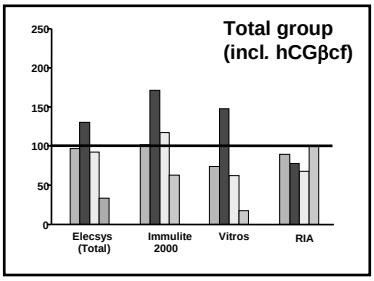
- **Results in very good accord with expected.**
- **If recognition is ~equimolar, results should be ~400 U/L for other isoforms.**

Detection of hCG species by method group

(Data show % molar response relative to hCG)







- **hCGn, nicked hCG**
- **hCGβ, hCG beta-subunit**
- **hCGβn, nicked beta-subunit**
- **hCGβcf, hCG beta-core fragment**

Sturgeon *et al. Clin Chem* 2009; 55: 1484-91.

Clinical implication

For a patient sample containing only free hCG beta-subunit, the result reported could differ by more than two-fold depending on the method used.

Achievements & work in progress

IFCC hCG Standards

- **Enable experimental assessment of recognition of hCG isoforms in different methods.**
- **Together with IFCC nomenclature permit unambiguous description of what methods are measuring, e.g. hCG+hCGβ+ hCGβcf.**
- **Provide a sounder analytical basis for improved assay design and calibration.**
- **Are a pre-requisite for a clear understanding of the effects of disease on circulating hCG and other related species.**

hCG – An exemplary IFCC approach

- **Clinically relevant forms of hCG identified, purified and made available as calibrators.**
- **Systematic nomenclature for above agreed, enabling clear description of what is measured.**
- **With diagnostic manufacturers, broad MAb specificities defined → standardization of reagents.**
- **Influence of calibrator on agreement → In progress**
- **Audit of results in practice → In progress**

Summarizing…a pragmatic approach to improving comparability for clinical practice involves

- **Establishing what should be measured, i.e. the most clinically relevant form(s) of the analyte.**
- **Preparing those forms of the analyte in sufficient quantity and purity for use as primary calibrant.**
- **Defining calibrant quantity in mass or molar units.**
- **Identifying antibodies with high specificity for the analyte form(s).**
- **Validating each assay system within stated ranges of isoform distribution - including specificity, accuracy, reference intervals.**

with continued close collaboration among clinical and laboratory users, diagnostic manufacturers and proficiency testing providers to determine the effect on outcome.

Acknowledgements

The IFCC hCG WG would like to thank all those who have contributed to this project in different ways, and in particular

- **The IFCC Scientific Division**
- **Supporting diagnostic companies**
- **Participants in the UK NEQAS for hCG**
- **John Seth and Andy Ellis**