Measurand characteristics & reference materials for hCG

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on behalf of the IFCC Working Group for hCG
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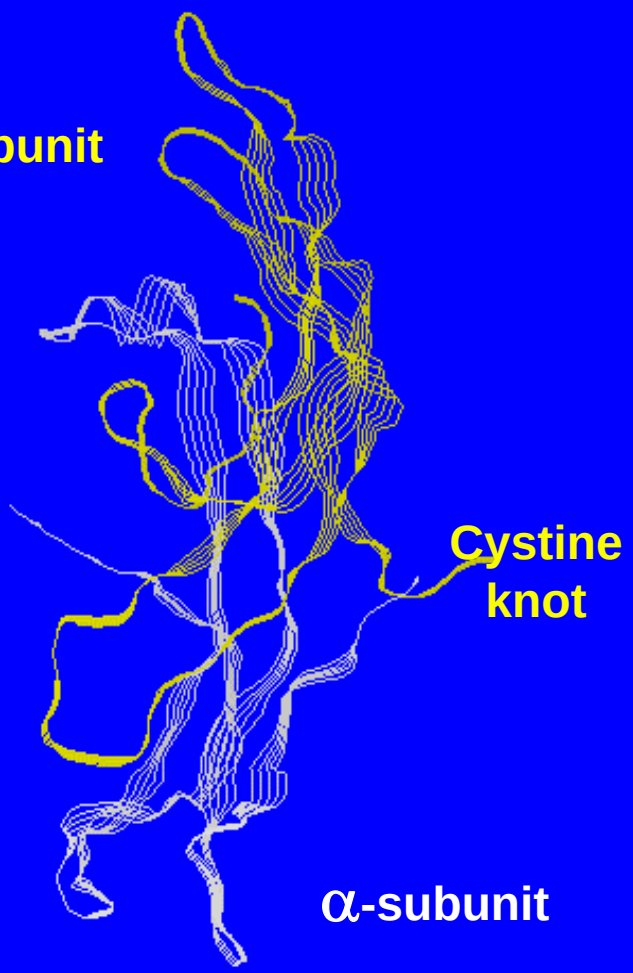
hCG as a measurand

- **hCG isoforms in biological specimens**
  - **Which isoforms are of clinical relevance?**
  - **Which isoforms should methods recognise?**
- **Achievements of international initiatives**
  - **Clear descriptive nomenclature – isoforms, methods**
  - **Well-characterised International Standards**
  - **Recommendations re desirable antibody specificities**
- **Impact of pure reference materials on hCG measurements in biological specimens**
  - **Elucidation of what methods are measuring**
  - **Effect on between-method agreement**
  - **Relevance to calibration and assay design**

Chorionic gonadotrophin (hCG)



Structure of hCG

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

Shares the same α-subunit with LH, FSH and TSH

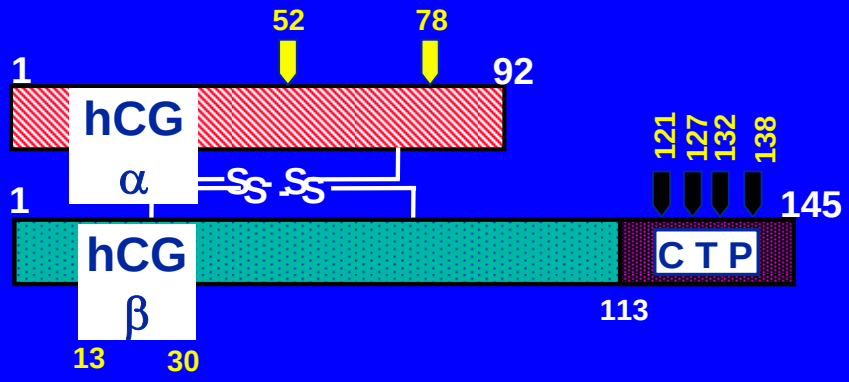
β-Subunits confer functional & immunological specificity.

Shares considerable homology with LH.

Crystal structure (1994) similar to that of some protein growth factors.

Structure and IFCC nomenclature

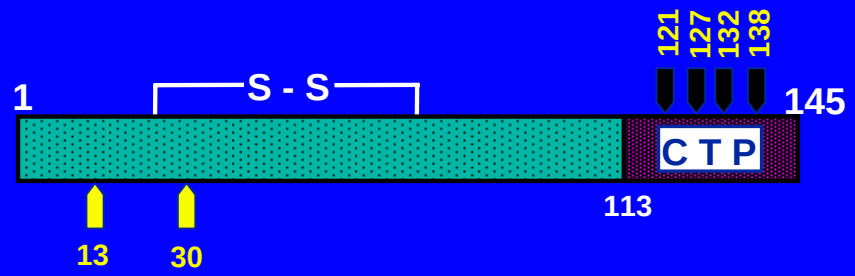
hCG



Intact hCG

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

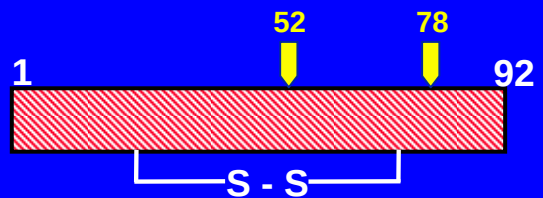
hCGβ



Free beta-subunit

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

hCGα

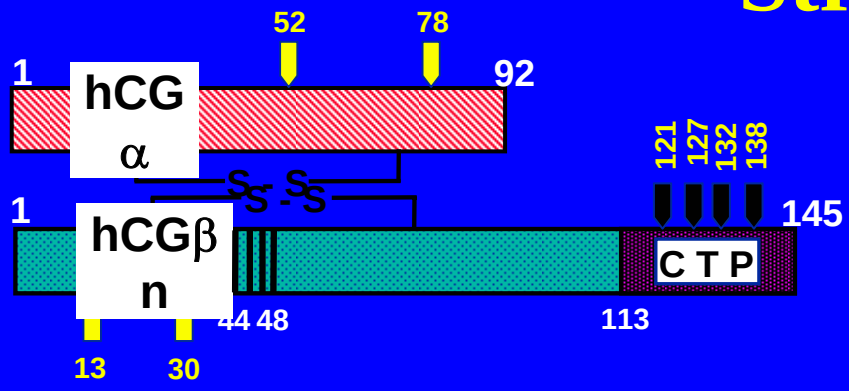


Free alpha-subunit

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

Structure and IFCC nomenclature

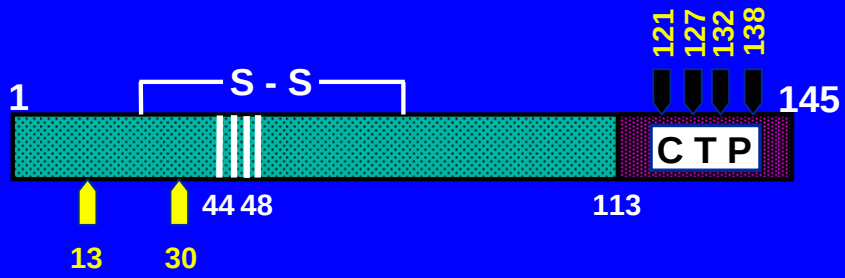
hCGn



Nicked hCG

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

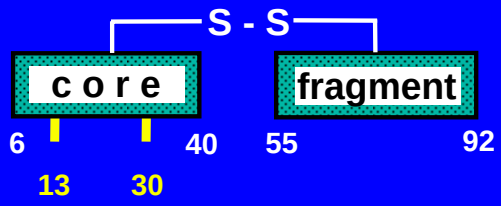
hCGβn



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.

Recognition of hCG-related species

Species	Recognized by	Range* (U/L)
hCG	All hCG & "hCG+hCGβ" assays	172-268
hCGn	All hCG & "hCG+hCGβ" assays	80-213
hCGβ	All "hCG+hCGβ" assays	266-1082
hCGβn	All "hCG+hCGβ" assays	148-457
hCGβcf	9 of 16 "hCG+hCGβ" assays	47-543
hCGα	None of the hCG or "hCG+hCGβ" assays	-

*U/L of hCG IS 75/589. NB Concentrations issued differ.

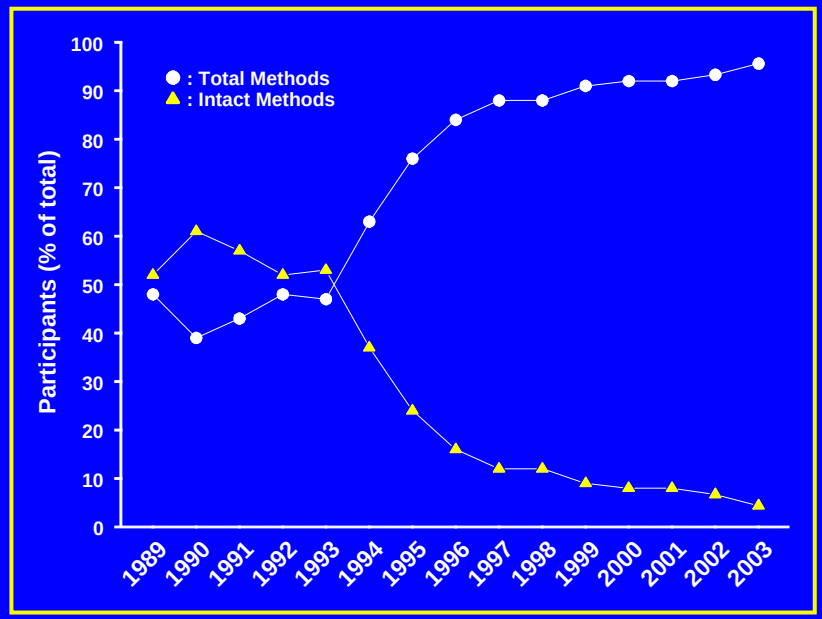
Preparations very kindly gifted by Dr S Birken, Columbia University, New York

UK NEQAS data

hCG assays for oncology

"…the NACB and EGTM guidelines emphasise that both intact hCG and its free β-subunit should be recognised by hCG assays used in oncology…"

Clin Chem 2002;48: 1151-1159



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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 (NIBSC)
J Sharratt (University of Cambridge)

hCG WG project time-line

1992

Bergmeyer conference.

IFCC hCG WG established.

1996

2000

2004

2008

Review* published (1993)

- **Current state-of-the-art for hCG methods in the field.**

Two major areas of concern identified

- **Lack of clear nomenclature**
- **Difficulty of comparing extent of recognition of different hCG species**

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

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- Difficulty in comparing extent of recognition of different hCG-related molecules

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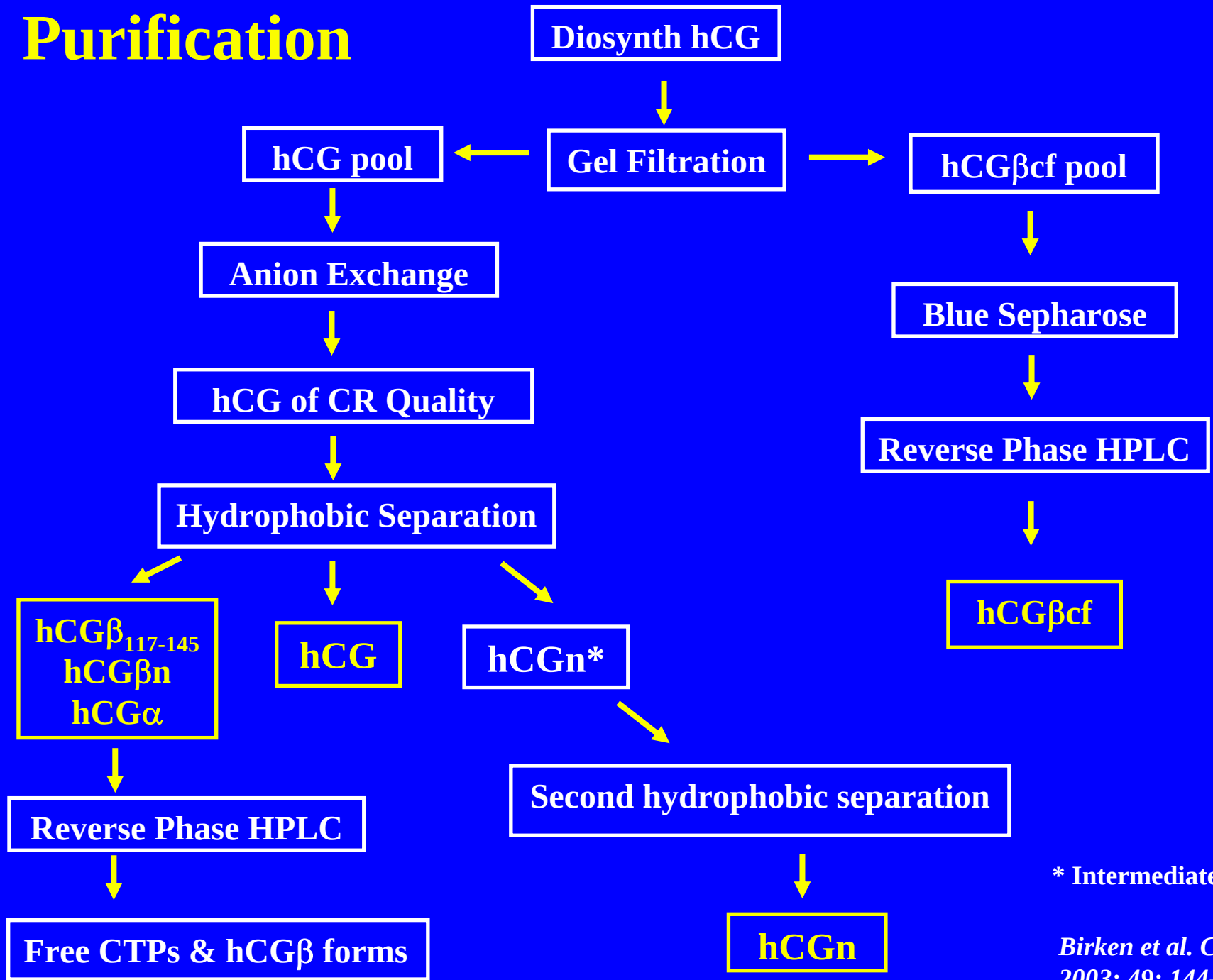
- Lack of clear nomenclature
- Difficulty in comparing extent of recognition of different hCG-related molecules

Limitations of the existing International Standards (IS)

- IS only for hCG, hCGα and hCGβ.
- Arbitrary units. Difficult to relate results for different isoforms.
  → 1 U/L of hCG ≠ 1 U/L of hCGβ
- IS for hCG contaminated with hCGn (~10% cross-reaction)

Purification



* Intermediate purity

Birken et al. Clin Chem 2003; 49; 144-154

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Value assignment

"hCG"
weighed bulk material

Analytical methods

Excipient-free concentrate (FC, frozen concentrate) → Amino acid Analysis (AAA) (nmol/ml) FC → Immunoassay (FC)

Dilution into excipient & distribution into ampoules (FZB, frozen baseline) → Immunoassay (FZB) → FZB/FC Loss on dilution

Lyophilisation (FD, freeze-dried) → Immunoassay (FC) → FD/FC x AAA (i) Loss on FD (ii) nmol/ampoule

1st WHO RR for hCG, hCGn, hCGβ, hCGα, hCGβcf, hCGβn [nmol/amp]

Bristow et al. Clin Chem 2005. In press.

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 nominal ("expected") values
- Closest for hCGβcf (102%)–most discrepant for hCG βn

Bristow et al. Clin Chem 2005. In press.

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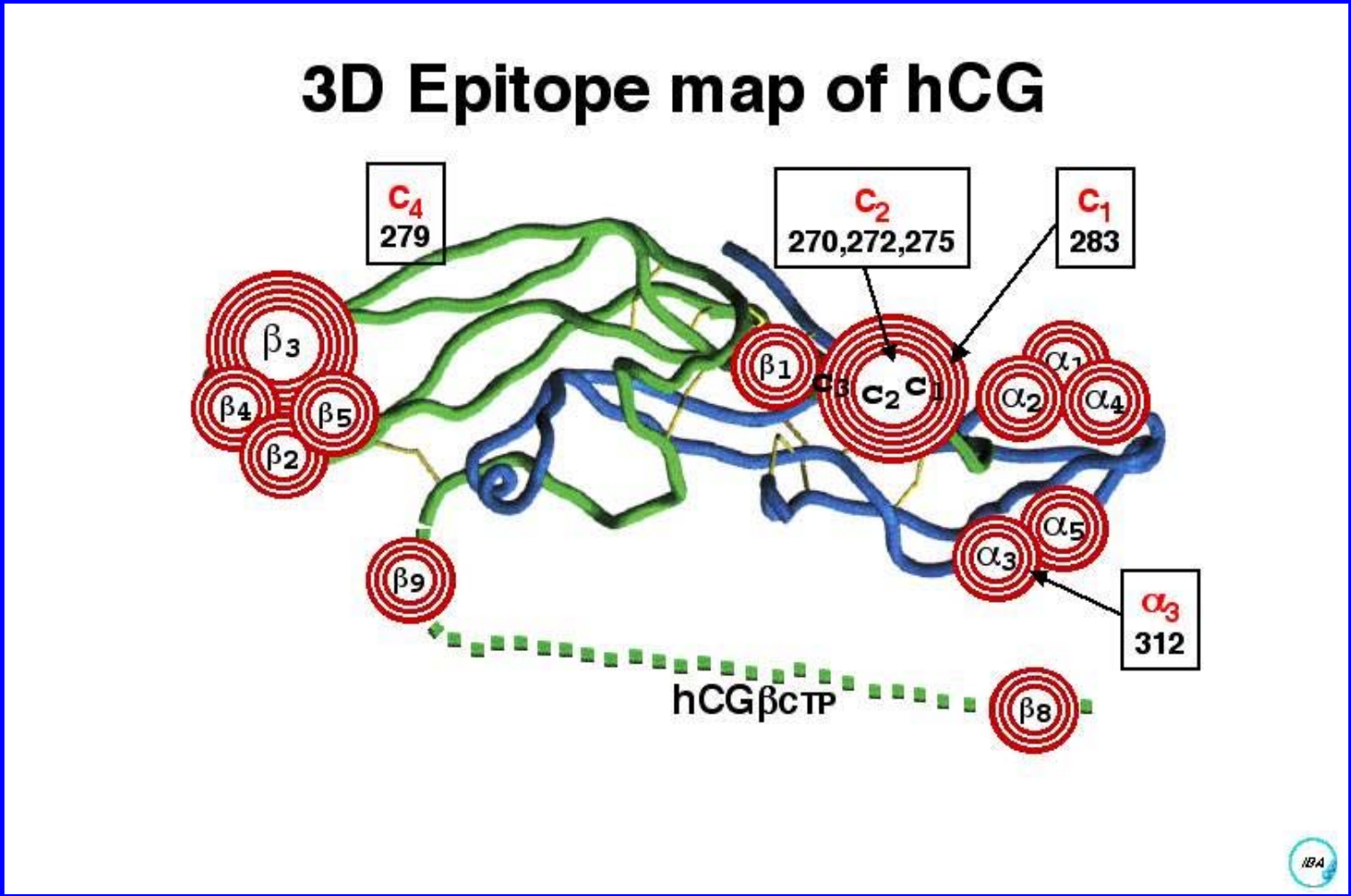
2004 — Data submitted to WHO Expert Committee on Biological Standards (ECBS)

2008

ISOBM study of fine specificities of a panel of 27 MAbs to hCG and related molecules, 2000-2002.

ISOBM Workshop on hCG antibodies



ISOBM, International Society for Oncodevelopmental Biology & Medicine

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

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IFCC Working Group 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, these preparations were officially established as the first WHO Reference Reagents for Immunoassay for these hCG-related molecules. Calibrated in molar units, their primary purpose initially is to enable better characterization of the specificities of current hCG immunoassays.

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New reference reagents

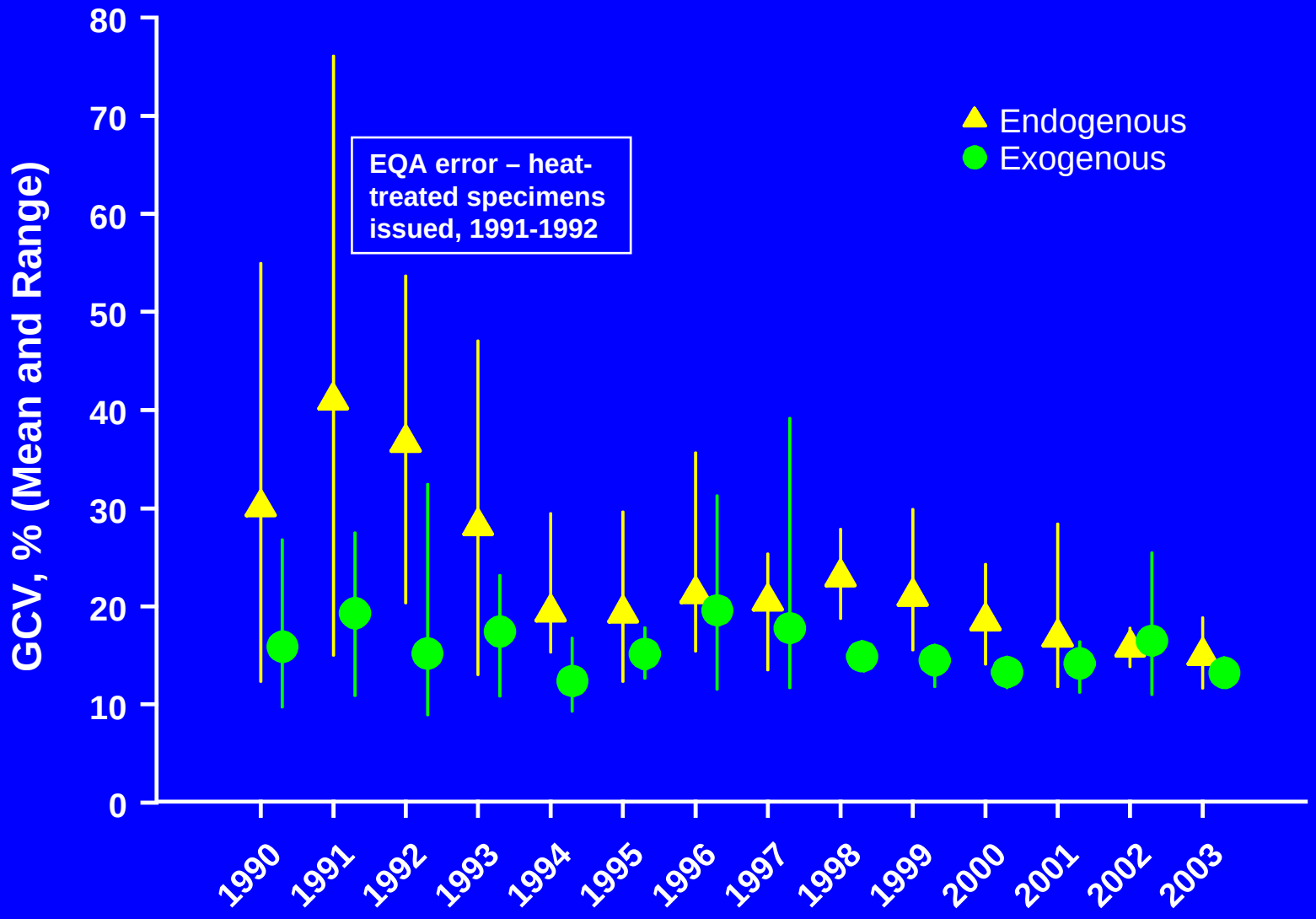
Potential benefits

- Calibration in molar units permits ready comparison of the extent to which different hCG –related molecules are recognised by different immunoprocedures.
- Availability of these highly purified International Reference Reagents should ultimately improve between-method comparability.

Recognised omission

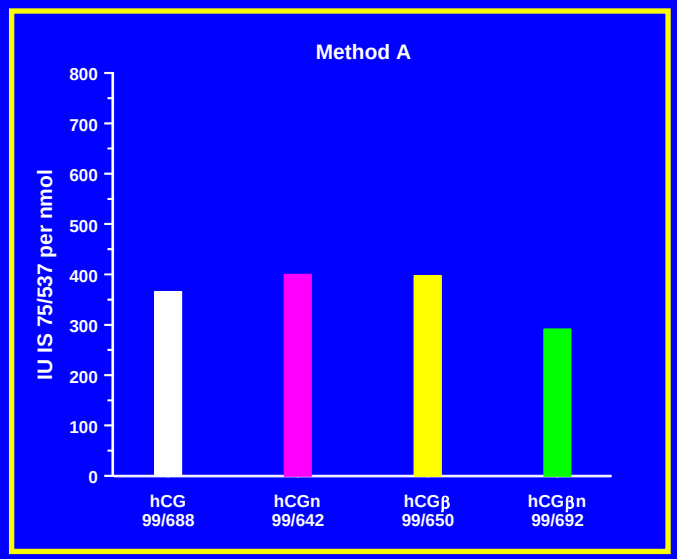
- International Reference Reagent for hyperglycosylated hCG required to elucidate its potential clinical importance.

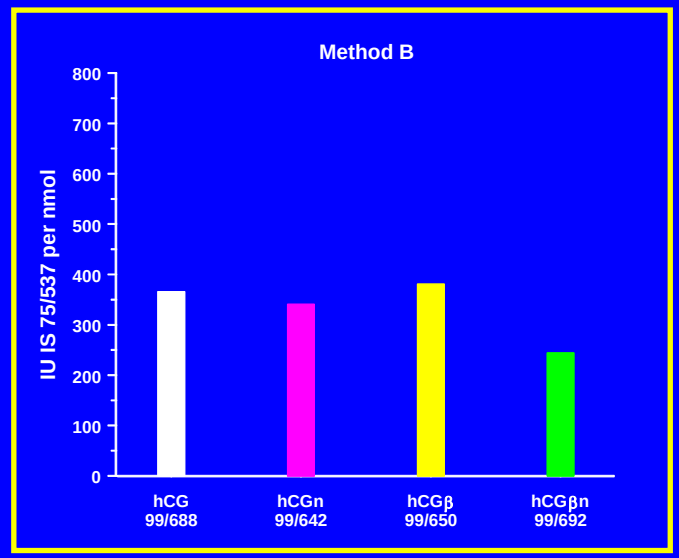
Between-lab agreement - hCG

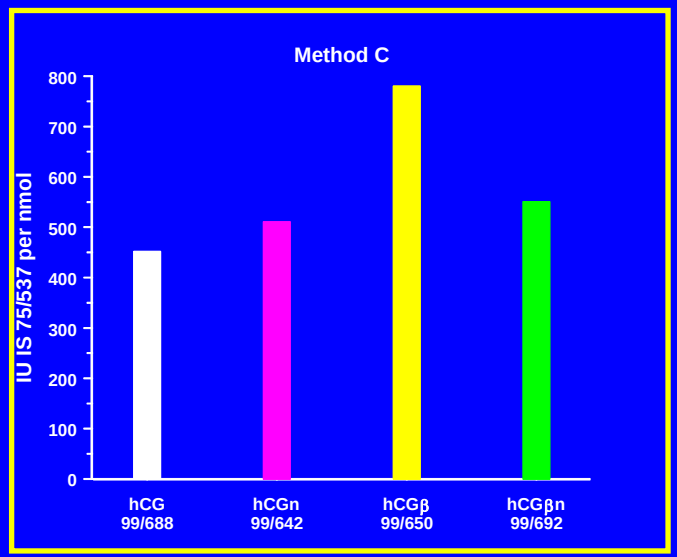


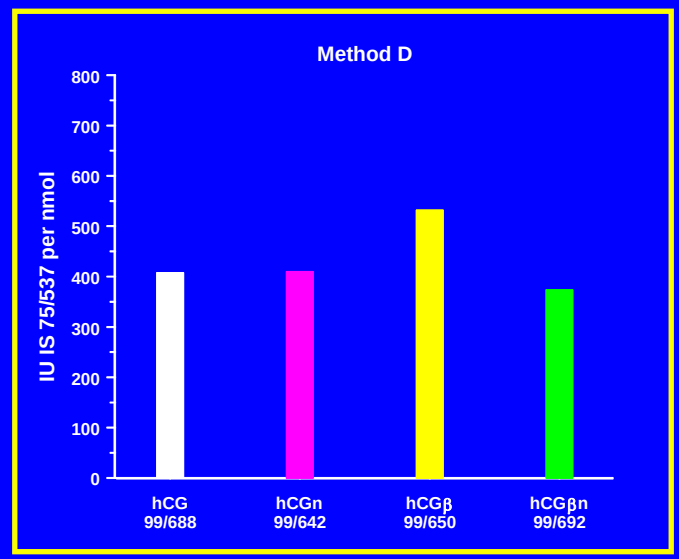
UK NEQAS data

Method characterisation









hCG

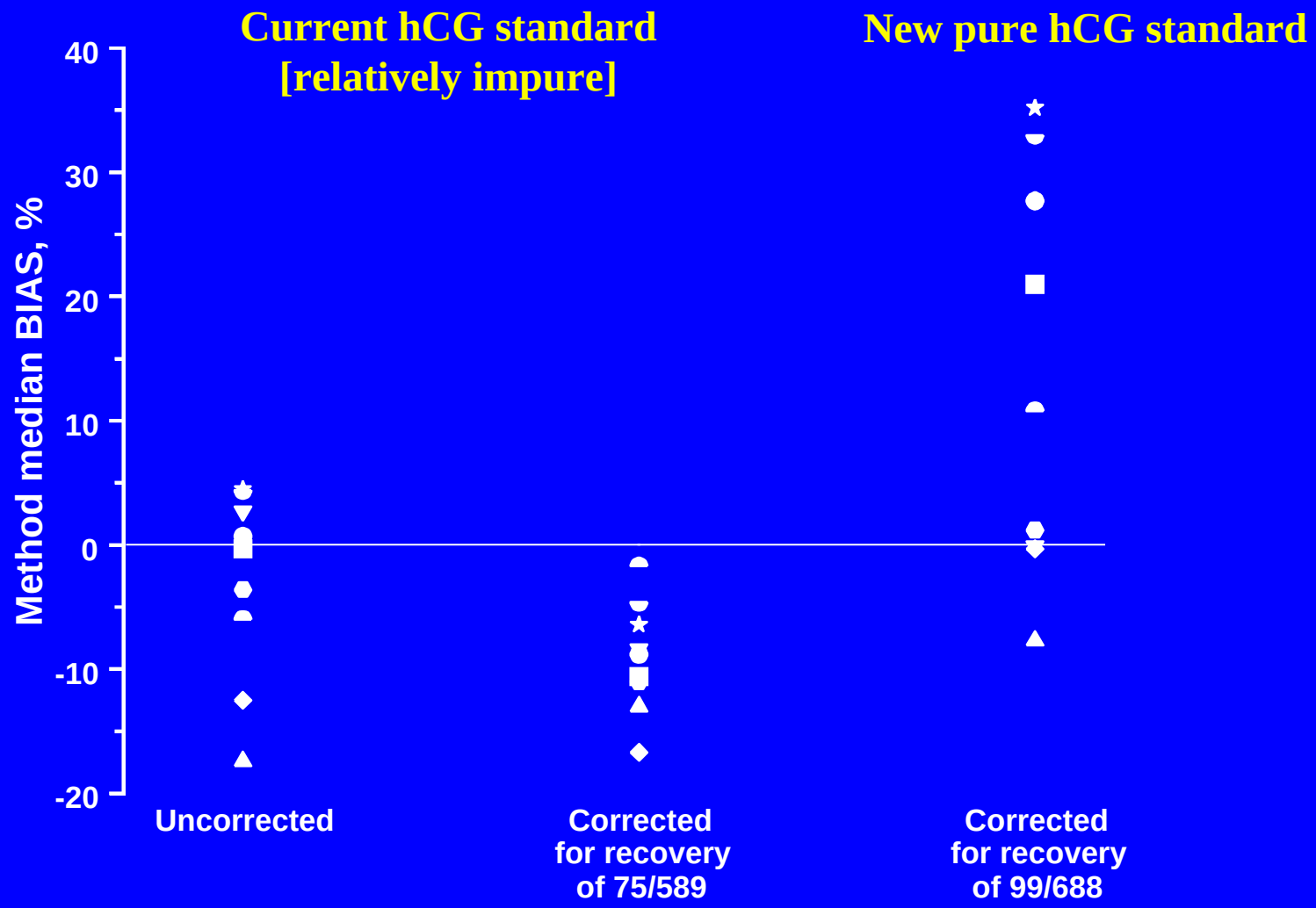
hCGn

hCGβ

hCGβn

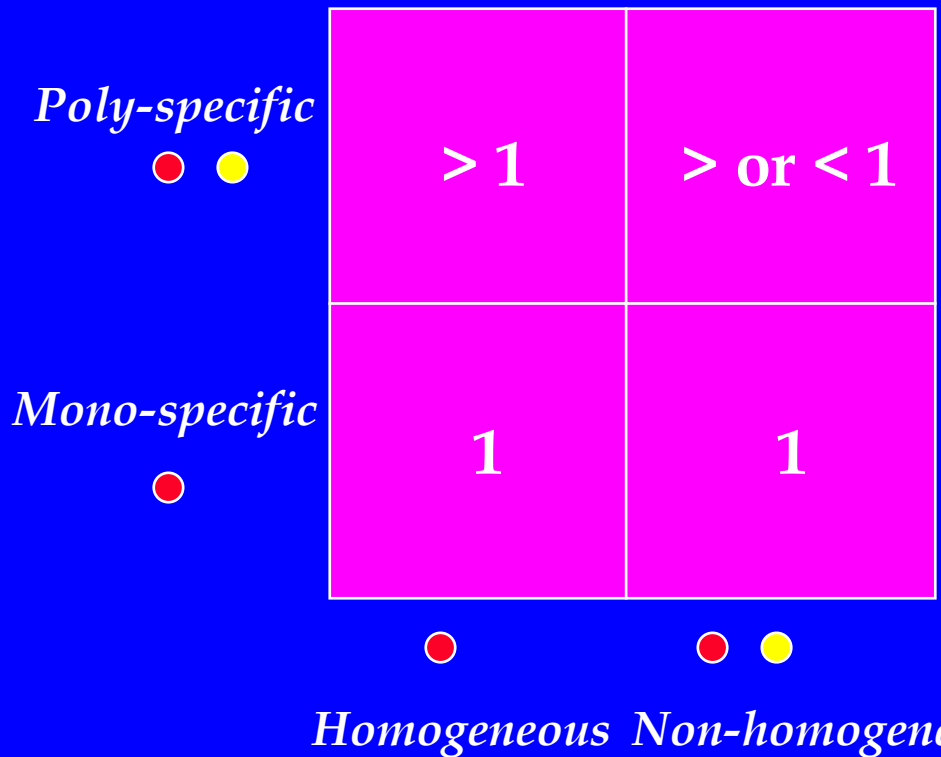
UK NEQAS & IFCC hCG WG data

Relative recognition



UK NEQAS and IFCC hCG WG data

Immunoassay standardization



For any given analyte, the results depend on:

- Assay specificity
- Calibrant homogeneity

Slide kindly provided by J Seth

How does the use of the new reference materials impact on measurement of hCG in practice?

The pragmatic approach to standardisation of measuring “like vs like” has served well, but does not provide a basis for understanding the pathological significance of specific hCG species.

How does the use of the new reference materials impact on measurement of hCG in practice?

Initial data indicate that use of purified hCG standards increases divergence among methods, but these standards

- 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.

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Current plans

- Continue to evaluate new standards – and influence of pure calibrators.
- Prepare International Standard for hyperglycosylated hCG.

2008

- Evaluate feasibility of developing a reference method for hCG, investigating whether LC-Tandem MS techniques can contribute.