The Analytical & Clinical Complexities of Measuring hCG

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Disclosures

- Ann M. Gronowski has served as a consultant & expert witness to Church and Dwight Co., Inc.
Objectives

1) Identify the numerous hCG variants and the current problems with standardization.

2) Describe the causes of persistently low hCG results and explain the investigations that can be done to investigate them.

3) Explain the causes of false positive and false negative hCG results.
hCG Variants
Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family

- hCG
- LH
- FSH
- TSH
Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family

hCG Structure

- **Dimer is ~38,000 daltons**
  - 30% of weight due to carbohydrate

- **Alpha subunit**
  - 92 amino acids
  - 2 N-linked carbohydrate chains

- **Beta subunit**
  - 145 amino acids
  - 2 N-linked & 4 O-linked carbohydrate chains

- **Half-life ~48 hours**

http://www.chem.gla.ac.uk/protein/glyco/hyper/hcg_act.html
hCG Concentrations

- Serum concentrations increase progressively in early pregnancy
  - Peak 7–9 wks
- Decrease until ~24 weeks then plateau
hCG Heterogeneity

- Numerous molecular forms of hCG present in pregnancy serum
  - Dissociated or degraded molecules with no biological activity

- Key β-containing variants
  - Intact hCG
  - Nicked hCG
  - Free β subunit
  - Nicked free β subunit
  - β-core fragment (urine)

Modified from: Cole, LA. *Clin Chem* 1997;43:2233-2243
Hyperglycosylated hCG

- Structure

Mid-pregnancy

Early-pregnancy & Testicular Cancer

Valmu et al. Glycobiology 2006;16:1207-18
hCG Immunoassays
hCG Immunoassays

Qualitative

- Home (Urine)
  - Analytical Specificity: Most are “designed” to detect intact hCG
  - Analytical Sensitivity: ~20 IU/L urine, ~10 IU/L serum

- Lab (Serum & Urine)
  - Analytical Specificity: Most are “designed” to detect intact hCG

Quantitative

- Serum (Immulite FDA-approved for Urine-QUALITATIVE)
  - Analytical Specificity: Most total hCGβ, Intact hCG, free hCGβ
  - Analytical Sensitivity: ~2 IU/L serum, Cutoff = 5 IU/L
hCG Immunoassay Questions

- Analytical Specificity?
- Analytical Sensitivity?
- Clinical Sensitivity?
- Clinical Specificity/Limitations?
Analytical Specificity
Lack of Assay Standardization

1) Different antibody pairs used in different assays
   - Recognize different epitopes of different variants
## Assay Antibodies

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Code</th>
<th>MAb Specificities</th>
<th>hCG</th>
<th>hCGβ</th>
<th>hCGβcf</th>
<th>hCGn</th>
<th>hCGβn</th>
<th>–CTP hCG</th>
<th>–CTP hCGβ</th>
<th>hLH</th>
<th>hLHβ</th>
<th>hFSH</th>
<th>hTSH</th>
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</table>

Filled squares = strong reactivity; white squares = no reactivity; gray squares = minor reactivity. GPHα = glycoprotein hormone α–subunit; n.t = not tested.

Lack of Assay Standardization

1) Different antibody pairs used in different assays
   - Recognize different epitopes of different variants

2) Secondary standards (calibrators) used by manufacturers differ in purity

Wide variation in:
- measured hCG concentrations
- and detection of hCG variants
Variation in Measured hCG Concentrations

CAP Ligand Survey A, 2008
### WHO International Reference Reagents

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

<table>
<thead>
<tr>
<th>hCG isoforms</th>
<th>IFCC nomenclature</th>
<th>WHO code&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>hCG</td>
<td>IRR 99/688</td>
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<td>Nicked hCG</td>
<td>hCGn</td>
<td>IRR 99/642</td>
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<td>hCG beta-subunit</td>
<td>hCGβ</td>
<td>IRR 99/650</td>
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<td>Nicked hCG beta-subunit</td>
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<td>IRR 99/692</td>
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<td>hCG beta core fragment</td>
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<td>hCG alpha-subunit</td>
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<td>IRR 99/720</td>
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<sup>a</sup> Available from National Institute for Biological Standards and Control (http://www.nibsc.ac.uk/catalog/standards/preps/sub_endo.html).
Differential recognition of hCG WHO standards, by different assays

Are Laboratories Reporting Serum Quantitative hCG Results Correctly?

Zhimin (Tim) Cao, 1* Robert Rej 1,2

Prepared:
- Intact hCG
- Free hCGβ
- Intact hCG + free hCGβ

Sent to 266 laboratories

"Many laboratories using the hCG STAT procedure reported total β hCG"

Roche Elecsys

hCG STAT
Intact hCG
47 IU/L

hCG + β
Intact hCG + β
67 IU/L
Are Laboratories Reporting Serum Quantitative hCG Results Correctly?

Zhimin (Tim) Cao, 1* Robert Rej 2

Prepared:
- Intact hCG
- Free hCGβ
- Intact hCG + free hCGβ

Sent to 296 laboratories
15 different methods

61 labs report “intact hCG” → 8 (13.1%) actually measured “Total β hCG”

235 labs report “Total β hCG” → 22 (9.3%) actually measured “Intact hCG”
## Variation in hCG Variant Detection

### POC hCG Tests

<table>
<thead>
<tr>
<th>Sure-Vue</th>
<th>Clinitest</th>
<th>QuickVue+</th>
<th>Osom</th>
<th>hCG Combo</th>
<th>ICON II</th>
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</thead>
<tbody>
<tr>
<td>Anti-α (u)</td>
<td>Anti-CG dimer (m)</td>
<td>Anti-β (m)</td>
<td>Anti-α (m)</td>
<td>Anti-β (m)</td>
<td>Anti-α (m)</td>
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<td>Anti-β (m)</td>
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<td>Anti-β (m)</td>
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</table>

**Elecys**, IU/L, pmol/L:

- Anti-β (m) Anti-β (m)
- 1220 NA
- 2263
- 7800
- 2336
- 8800
- 630
- 3300
- 815
- 10 200
- <2.0
- 8400

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## Variation in hCG Variant Detection OTC hCG Tests

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<thead>
<tr>
<th>Over-the-counter device&lt;sup&gt;a&lt;/sup&gt;</th>
<th>First Response</th>
<th>EPT</th>
<th>Clearblue Easy</th>
<th>Target Early Result</th>
<th>Answer</th>
<th>Wal-Mart Equate</th>
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<td>5/5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10/10</td>
<td>10/10</td>
<td>6/6&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>9/9&lt;sup&gt;c&lt;/sup&gt;</td>
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<th>Roche, Elecsys, IU/l&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup> Over-the-counter clevice a quantitative device

<sup>b</sup> First EP Clearblue Target Answer Wal-Mart Roche, Elecsys, IU/l

Why do we care which hCG variants are recognized by a qualitative device?
False Negative Qualitative hCG

- 18 yoF with vaginal spotting and cramping
- States 3 months pregnant
- ED POC hCG negative
- Serum hCG = 419,680 IU/L
- Ultrasound - live intrauterine pregnancy
- Urine hCG = 176,498 IU/L

False Negative Qualitative hCG

- Urine re-tested in laboratory
  - Result negative
  - Positive following 1:5 dilution
- Clearly a false-negative
- Why?

Normal Lateral Flow

\( \alpha \beta \) hCG

Measured urine hCG in expected, normal physiological range

Patient urine

Anti- \( \alpha \)hCG

Sample well
Normal Lateral Flow

Sample well

Anti- αhCG
Normal Lateral Flow

Sample well

Anti-βhCG

Visible Band
Hook Effect

Measured urine hCG in **great excess** of normal physiological range
Hook Effect

Anti-βhCG

Sample well
Hook Effect

- Sample well
- Anti-βhCG
- No Band

Graph showing Measured Concentration vs. Actual Concentration.
Not a “Hook Effect”

- **Hook effect**
  - 1.6 - 1.9 million IU/L
- **Patient = 176,498 IU/L**

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<th>Dilution</th>
<th>Total beta hCG Concentration (IU/L)</th>
<th>Osom Result</th>
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<td>80%</td>
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<td>70%</td>
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<td>60%</td>
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<td>50%</td>
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<td>20%</td>
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<td>10%</td>
<td>320,000</td>
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## Analytical Specificity: POC hCG Tests

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<th>Sure-Vue</th>
<th>Clinitest</th>
<th>QuickVue+</th>
<th>Osom</th>
<th>hCG Combo</th>
<th>ICON II</th>
<th>Elecsys&lt;sup&gt;a&lt;/sup&gt;, IU/L, pmol/L&lt;sup&gt;b&lt;/sup&gt;</th>
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Notes:
- <sup>a</sup> Elecsys<sup>b</sup> values are given in IU/L, pmol/L.
- <sup>b</sup> pmol/L values are given for hCGβ (u) and hCGα (u).

NA indicates not available.
Could an excess of an hCG variant bind only one of the assay antibodies, negating the formation of a “sandwich”? 
Effect of hCGβcf on 3 POC Devices

Hook Effect with hCG Variant

Measured urine hCG in expected, normal physiological range, but [variant] > [intact hCG]
Hook Effect with hCG Variant

Anti-βhCG

Sample well
Hook Effect with hCG Variant

Sample well

Anti-hCG

No Band
Hook Effect

Traditional Hook Effect

- hCG concentrations pathologically high
- Antigen recognized by both antibodies
- Only need one form of hCG to detect

Variant Hook Effect

- hCG concentrations normal
- Antigen recognized by both antibodies
- Need two forms of hCG to detect
hCG Variant Content of False Negative Urine

- Patient urine contained an excess of hCGβcf

GA 7 wks

Clinical Implications

- hCG$\beta$cf is major hCG$\beta$ subunit-related molecule in urine after ~5-8 wks of pregnancy. Accounts for up to 90% of immunoreactive urine hCG from mid pregnancy.

- **CAUTION** should be used when testing women who are beyond 5-8 wks gestation, as false neg results may occur

- Take Home Message
  - Positive = good evidence patient is pregnant
  - Negative does not mean patient is not pregnant
Points for Discussion

- Manufacturers don’t currently characterize what hCG variants their devices recognize—should they?
- What hCG variants should POC devices recognize?
- Should results of hCG testing include a description of which variants are detected?
- Should POC devices be designed to recognize early and late pregnancy?
What about Quantitative Assays?
hCG Variant Effect on Quantitative Tests

- Advia Centaur® Total hCG (Siemens)
- AIA-1800 ST Total β-hCG (Tosoh Bioscience)
- Architect® Total β-hCG (Abbott Laboratories)
- Cobas® e411 hCG Stat (Roche Diagnostics)
- Dimension® RxL® hCG (Siemens)
- DxI® Total βhCG (Beckman Coulter)
- Modular Analytics e170 hCG+β (Roche Diagnostics)
- Immulite 2000 hCG
- Vitros® ECi Total β-hCG II (Ortho Clinical Diagnostics)
hCG Variant Effect on Quantitative Tests

hCGβcf (pmol/L)

By day 35
hcgβcf
65,000 pmol/L

Grenache and Gronowski Clin Chem 2010;56:1839-44
Analytical Sensitivity
## Analytical Sensitivity Serum Quantitative

<table>
<thead>
<tr>
<th>Instrument</th>
<th>IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche emodule</td>
<td>0.1</td>
</tr>
<tr>
<td>Immulite 2000</td>
<td>0.4</td>
</tr>
<tr>
<td>Beckman DxI</td>
<td>0.5</td>
</tr>
<tr>
<td>Abbott Architect</td>
<td>1.2</td>
</tr>
<tr>
<td>Siemens Centaur</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitros ECi</td>
<td>2.39</td>
</tr>
</tbody>
</table>

**Interpretation:**

- $<5$ Non-pregnant
- $\geq 5$ Pregnant

OR

- $<5$ Non-pregnant
- $5-25$ Indeterminate
- $>25$ Pregnant
Analytical Sensitivity **Urine** Quantitative

**Immulite 1000:**

- **LOQ:** 2 IU/L
- **CV:** <11%

The take home message:
1) Analytical sensitivity varies with brand
2) Cutoffs often not in agreement with manufacturer’s claimed cutoffs.

## Analytical Sensitivity

### POC Devices

<table>
<thead>
<tr>
<th>Device</th>
<th>Read at, min</th>
<th>Regular hCG, IU/L</th>
<th>Manufacturer's hCG limit, IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Blue Easy</td>
<td>1</td>
<td>+</td>
<td>50</td>
</tr>
<tr>
<td>Target Early Pregnancy Test</td>
<td>5</td>
<td>±</td>
<td>100</td>
</tr>
<tr>
<td>American Fare Easy to Read</td>
<td>3</td>
<td>–</td>
<td>~50</td>
</tr>
<tr>
<td>First Response Early Result</td>
<td>3</td>
<td>–</td>
<td>40</td>
</tr>
<tr>
<td>E.P.T.</td>
<td>3</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Answer</td>
<td>2</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Fact Plus Pro</td>
<td>3</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Fact Plus Select</td>
<td>3</td>
<td>±</td>
<td>100</td>
</tr>
<tr>
<td>Equate°</td>
<td>1</td>
<td>–</td>
<td>25</td>
</tr>
<tr>
<td>Walgreens E.P.T.</td>
<td>5</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Walgreens One Step</td>
<td>3</td>
<td>–</td>
<td>50</td>
</tr>
<tr>
<td>Inverness Medical E.P.T.</td>
<td>3</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Longs Pregnancy Test</td>
<td>3</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Rite Aid One Step</td>
<td>3</td>
<td>–</td>
<td>50</td>
</tr>
<tr>
<td>Confirm</td>
<td>2</td>
<td>–</td>
<td>25</td>
</tr>
</tbody>
</table>

Butler Clin Chem 2001;47:2131-6
Defining Cutoffs

Specimens showing positive result

Concentration at which: 50% test positive & 50% test negative

Concentration at which: 100% test positive

Concentration at which: 95% test positive

hCG Concentration IU/L

5 10 15 20 25 30 35 40
Analytical Sensitivity

POC & OTC Devices

Median Concentration (IU/L)
3/3 devices test positive (n=11 patients)

POC Device
- Clinitest: 12.5
- Osom: 18.8
- Quick-Vue: 25
- hCG Combo: 25
- ICON II: 25
- SureVue: 25

OTC Device
- First Response: 2.4
- Answer: 3.1
- Target Early Result: 6.3
- EPT Certainty: 6.3
- Clearblue Easy: 12.5
- Wal-Mart Equate: 12.5

Debate—how low should we go?

Clinical Sensitivity
How early can urine hCG detect pregnancy?

- Method used to determine day of menses influences timing of pregnancy detection
- As reference point, LH surge showed less variability for pregnancy detection

How early can **urine** hCG tests detect pregnancy?

<table>
<thead>
<tr>
<th>Day relative to missed period</th>
<th>Achieved pregnancy, %</th>
<th>hCG median, mIU/mL</th>
<th>First Response manual, %</th>
<th>First Response Gold digital, %</th>
<th>EPT manual, %</th>
<th>EPT Certainty digital, %</th>
<th>ClearBlue Easy manual, %</th>
<th>ClearBlue Easy digital, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>41</td>
<td>100</td>
<td>98</td>
<td>55</td>
<td>65</td>
<td>58</td>
<td>60</td>
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<tr>
<td>+3</td>
<td>100</td>
<td>154</td>
<td>100</td>
<td>95</td>
<td>80</td>
<td>80</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

Expected day of menses calculated from average length of the 3-5 previous menstrual cycles for each woman

How early can **urine** hCG tests detect pregnancy?

<table>
<thead>
<tr>
<th>Day relative to missed period</th>
<th>Achieved pregnancy, %</th>
<th>hCG median, mIU/mL</th>
<th>First Response manual, %</th>
<th>First Response Gold digital, %</th>
<th>EPT manual, %</th>
<th>EPT Certainty digital, %</th>
<th>ClearBlue Easy manual, %</th>
<th>ClearBlue Easy digital, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study 5. Pregnant woman, 40 total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>41</td>
<td>100</td>
<td>98</td>
<td>55</td>
<td>65</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>+3</td>
<td>100</td>
<td>154</td>
<td>100</td>
<td>95</td>
<td>80</td>
<td>80</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td><strong>Study 6. Pregnant woman, 80 total</strong></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>−6</td>
<td>29</td>
<td>2.1</td>
<td>25</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>−5</td>
<td>40</td>
<td>2.9</td>
<td>33</td>
<td>25</td>
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<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
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<tr>
<td>−4</td>
<td>76</td>
<td>5.2</td>
<td>58</td>
<td>42</td>
<td>6.3</td>
<td>6.3</td>
<td>8.8</td>
<td>3.8</td>
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<tr>
<td>−3</td>
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<td>77</td>
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<td>302</td>
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<td>100</td>
<td>80</td>
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<td>87</td>
<td>84</td>
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<tr>
<td>+4</td>
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<td>534</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Expected day of menses calculated from average length of the 3-5 previous menstrual cycles for each women

How early can serum hCG detect pregnancy?

### Table II. Mean and 95% confidence intervals, median, maximum and minimum for β-hCG concentrations on day 11.

<table>
<thead>
<tr>
<th>Pregnancy outcome</th>
<th>Miscarriage</th>
<th>Term delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pregnancies</td>
<td>33</td>
<td>186</td>
</tr>
<tr>
<td>Minimum/maximum β-hCG (mIU/mL)</td>
<td>2–42</td>
<td>4–235</td>
</tr>
<tr>
<td>Mean β-hCG (mIU/mL)</td>
<td>16.5</td>
<td>27.7</td>
</tr>
<tr>
<td>Median (mIU/mL)</td>
<td>16.9</td>
<td>25.1</td>
</tr>
<tr>
<td>95% confidence interval (mIU/mL)</td>
<td>12.9–20.2</td>
<td>24.7–30.6</td>
</tr>
</tbody>
</table>

### Table III. Mean and 95% confidence intervals, median, maximum and minimum for β-hCG concentrations on day 14.

<table>
<thead>
<tr>
<th>Pregnancy outcome</th>
<th>Miscarriage</th>
<th>Term delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pregnancies</td>
<td>33</td>
<td>173</td>
</tr>
<tr>
<td>Minimum/maximum β-hCG (mIU/mL)</td>
<td>11–184</td>
<td>32–365</td>
</tr>
<tr>
<td>Mean β-hCG (mIU/mL)</td>
<td>73.1</td>
<td>141.7</td>
</tr>
<tr>
<td>Median (mIU/mL)</td>
<td>61.2</td>
<td>129.0</td>
</tr>
<tr>
<td>95% confidence interval (mIU/mL)</td>
<td>58.0–88.2</td>
<td>131.3–152.1</td>
</tr>
</tbody>
</table>

Clinical Specificity/Limitations
Persistently Low hCG

- Standardized protocols
- Low concentrations of hCG that persist for months to years
  - hCG <50-200 IU/L
- Create clinical confusion, may delay needed therapies, or result in unnecessary therapy
- Uncommon event attributed to
  1) Interfering antibodies
  2) Pituitary hCG
  3) Exogenous hCG
1) Interfering Antibodies

- Interfering antibodies can be:
  - **Endogenous anti-analyte antibodies**
    - Analytical interference (false + and false -)
    - Physiological- extend analyte half life
  - **Human anti-animal immunoglobulin antibodies**
    - anti-Fc - most common (False +)
    - anti-idiotype (false + or -)
The Abbott HCG Story

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Number of pregnancies</th>
<th>Parity</th>
<th>Reason for hCG test</th>
<th>Surgical treatment</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>1</td>
<td>0</td>
<td>Incidental</td>
<td>D&amp;C, laparoscopy, TAH</td>
<td>Methotrexate, EMAC, vincristine</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>1</td>
<td>0</td>
<td>Menstrual irregularity</td>
<td>D&amp;C, laparoscopy, TAH, thoracotomy</td>
<td>Methotrexate, actinomycin D, EMAC, vincristine</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>1</td>
<td>0</td>
<td>Vaginal bleeding</td>
<td>D&amp;C, laparoscopy, TAH</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>3</td>
<td>0</td>
<td>Abdominal pain</td>
<td>D&amp;C, laparoscopy, TAH, BSO</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>1</td>
<td>3</td>
<td>Abdominal pain</td>
<td>D&amp;C, laparoscopy, TAH</td>
<td>Methotrexate, actinomycin D</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>2</td>
<td>2</td>
<td>Menstrual irregularity</td>
<td>D&amp;C, laparoscopy</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>2</td>
<td>2</td>
<td>Incidental</td>
<td>D&amp;C, laparoscopy</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>4</td>
<td>4</td>
<td>Incidental</td>
<td>D&amp;C, laparoscopy</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>2</td>
<td>1</td>
<td>Incidental</td>
<td>D&amp;C, laparoscopy</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>10</td>
<td>42</td>
<td>2</td>
<td>2</td>
<td>Incidental</td>
<td>D&amp;C, laparoscopy</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>3</td>
<td>1</td>
<td>Incidental</td>
<td>D&amp;C, laparoscopy</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>Not known</td>
<td></td>
<td></td>
<td>D&amp;C</td>
<td>Methotrexate</td>
</tr>
</tbody>
</table>

D&C=dilatation & curettage
TAH=total abdominal hysterectomy
BSO= bilateral salpingo-oophorectomy
EMAC= etoposide, methotrexate, actinomycin D, cyclophosphamide

Jury awards $15.5 million to woman misdiagnosed with cancer. UW and drug company share blame

Seattle Post-Intelligencer
Saturday, June 30, 2001
Approaches for Identifying Interfering Antibodies

- **Dilution Studies**
  Usually do not show linear dilution pattern

- **Blocking antibodies**
  Purified non-specific animal immunoglobulins

- Perform using different assay
- Measure urine hCG
2) Pituitary hCG

- First reported 30 years ago

- Gonadotrope cells of pituitary gland produce small amounts of hCG
2) Pituitary hCG

- First reported 30 years ago
- Gonadotrope cells of pituitary gland produce small amounts of hCG
hCG is Correlated with Age


r = 0.034, p = 0.60
r = 0.156, p = 0.02
r = 0.038, p = 0.55
r = 0.333, p < 0.0001
hCG is Correlated with Age

Serum in Nonpregnant Women and Men

**FEMALES**

**hCG (pmol/L)**

**hCGβ (pmol/L)**

Age (years):

<30 30-39 40-49 50-59 60-69 >70

Urine in Nonpregnant Women and Men

Normal Production of Human Chorionic Gonadotropin in Menopause

Cole, L. A., Sasaki, Y., Muller, C. Y.


Hormone Replacement Therapy for 2 weeks
**Table 1. hCG concentration ranges and the 97.5 percentile values for the nonpregnant cohorts in the study.**

<table>
<thead>
<tr>
<th>Nonpregnant cohort</th>
<th>n</th>
<th>hCG range, IU/L</th>
<th>97.5 percentile, IU/L</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal, 18–40 years</td>
<td>240</td>
<td>&lt;2.0 to 4.6</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Perimenopausal, 41–55 years</td>
<td>240</td>
<td>&lt;2.0 to 7.7</td>
<td>8</td>
<td>4.8</td>
</tr>
<tr>
<td>Postmenopausal, &gt;55 years</td>
<td>240</td>
<td>&lt;2.0 to 13.1</td>
<td>14</td>
<td>7.7</td>
</tr>
</tbody>
</table>

- Compared with the nonpregnant premenopausal cohort.
- Compared with the nonpregnant premenopausal and nonpregnant perimenopausal cohorts.

Pituitary hCG vs pregnancy

- hCG 5-14 IU/L
  - Age 18-40
    - Possible pregnancy
  - Age 41-55
    - Measure FSH
      - FSH <45 IU/L
        - Possible pregnancy
      - FSH ≥45 IU/L
        - Pregnancy unlikely
  - Age >55
    - Pregnancy unlikely

3) Exogenous hCG

Why would a male athlete like Manny Ramirez take a chemical used as a female fertility drug?

May 8, 2009 02:00 PM in Basic Science | 8 comments

The news broke yesterday that Los Angeles Dodgers slugger Manny Ramirez was being suspended for 50 games for violating Major League Baseball's performance-enhancing drug policy. Ramirez, 36, was suspended after baseball officials discovered he had been prescribed human chorionic gonadotropin (HCG), according to the New York Times. HCG is a hormone used as a fertility drug in women—so what would a male athlete stand to gain by using it?

In a statement released by the players' union, Ramirez provided few details, saying only that the suspension, which he is not appealing, stemmed from "a medication, not a steroid" that his doctor prescribed "for a personal health issue."

We checked in with Andrew Kicman, head of R&D at the King's College London Drug Control Center and lead author of the 1991 study "Human chorionic gonadotrophin and sport," published in the British Journal of Sports Medicine.

AP Source: Cushing Tested Positive for HCG

Person familiar with Cushing's case: Texans LB tested positive for HCG, a fertility drug

By BARRY WILNER AP Football Writer

NEW YORK May 11, 2010 (AP)

Houston Texans linebacker Brian Cushing tested positive for HCG, a fertility drug that is on the NFL's banned substance list.

A person familiar with Cushing's case told The Associated Press on Tuesday that Cushing had one positive test last September, then subsequently tested negative several times. The person spoke on condition of anonymity because the test results were supposed to remain confidential.

"He had one low-level positive test for HCG in September, and then every test after that was negative," the person said. "He has said he has no idea where the positive test came from."

The NFL has suspended Cushing for the first four games of the season. He won the AP's NFL Defensive Rookie of the Year honor in January for outstanding on-field performance. Now, the AP is taking a revote for the award, as well as All-Pro outside linebacker because Cushing made the second team.

ESPN first reported the banned substance was human chorionic gonadotropin, which is widely taken by steroid users to help restart natural testosterone production. HCG can mitigate the side effects of ending a cycle of drugs. It's also used to induce ovulation and treat ovarian disorders.
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Lose 1-2 Pounds Per Day
Easy to Make Recipes
No Hunger on the HCG Diet
HCG is A Natural Hormone

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HCG Diet

You can purchase your HCG drops from our collection of HCG diet supplies. We have everything you need for your HCG diet. Browse through our HCG diet supplies today to find just the HCG drops that you are looking for. You can't go wrong with high quality products online.
HCG weight-loss products are fraudulent, FDA says

By Nanci Hellmich, USA TODAY

A popular type of weight-loss products, heavily promoted on the Internet, is fraudulent and illegal, Food and Drug Administration officials say.

HCG weight-loss products that promise dramatic results and claim to be homeopathic are sold as drops, pellets and sprays on the Web, in drugstores and at General Nutrition Centers. They are supposed to be used in combination with a very low-calorie diet of 500 calories a day.

Many of the labels indicate the products contain HCG, or human chorionic gonadotropin, a hormone made by the placenta during pregnancy. The hormone itself is approved as a prescription treatment for infertility and other conditions.

There is no evidence the oral over-the-counter products are effective for weight loss, says Elizabeth Miller, FDA’s leader for the Internet and health fraud team. While they may not be dangerous, they’re at least “economic fraud,” she says.

Because the products do not seem to be “a serious direct health hazard or a serious indirect health hazard,” they have been a lower priority for FDA action than other products. Still, Miller says, “they could be subject to enforcement at any time.”

One of the issues is the homeopathic label. Homeopathy is an alternative medicine practice of using very small or diluted preparations of medicines or remedies to treat a condition. Miller says, “We are aware of HCG products that claim to be homeopathic, but it is not recognized in the Homeopathic Pharmacopoeia.” Therefore, these products “are not recognized by the FDA as homeopathic drugs, so they are unapproved drugs and are illegal,” she says.

Miller says HCG began being used for weight loss in the 1950s when a British physician had a theory that it could help people on a near-starvation diet not feel hungry. “Since then, a lot of research and clinical trials debunked that theory.”

Samuel Klein of Washington University School of Medicine in St. Louis agrees: “Data from most randomized controlled trials show that HCG is no better than placebo in achieving weight loss or reducing hunger.”

Stephen Barrett, a retired psychiatrist who operates quackwatch.org, says, “The bottom line is there is no reason to think the products work.”
Summary

- hCG is a heterogeneous molecule

- Variability in the detection of hCG variants for quant & qual assays

- hCG variants can interfere with qual & quant hCG assays

- hCG assays are not approved for use in cancer patients, but if your test is being used for this purpose, it must recognize hCGβ

- Persistent low hCG may be due to interfering antibodies, pituitary hCG, or exogenous hCG

- Laboratory professionals should know the analytical specificity of their hCG assays
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