

# The Analytical & Clinical Complexities of Measuring hCG

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Obstetrics & Gynecology





# Disclosures

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



# Objectives

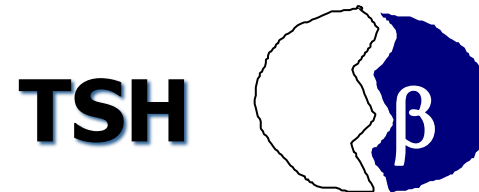
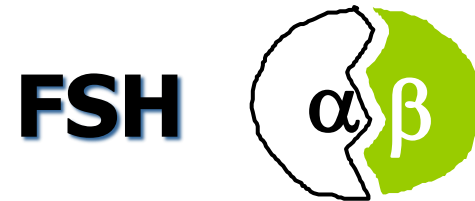
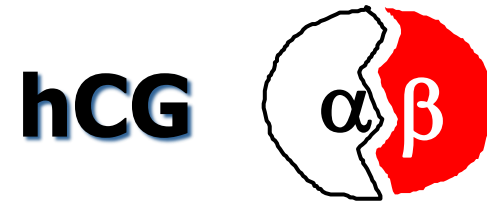
- 1) Identify the numerous hCG variants and the current problems with standardization.
- 2) Describe the causes of persistently low hCG results and explain the investigations that can be done to investigate them.
- 3) Explain the causes of false positive and false negative hCG results.



# hCG Variants

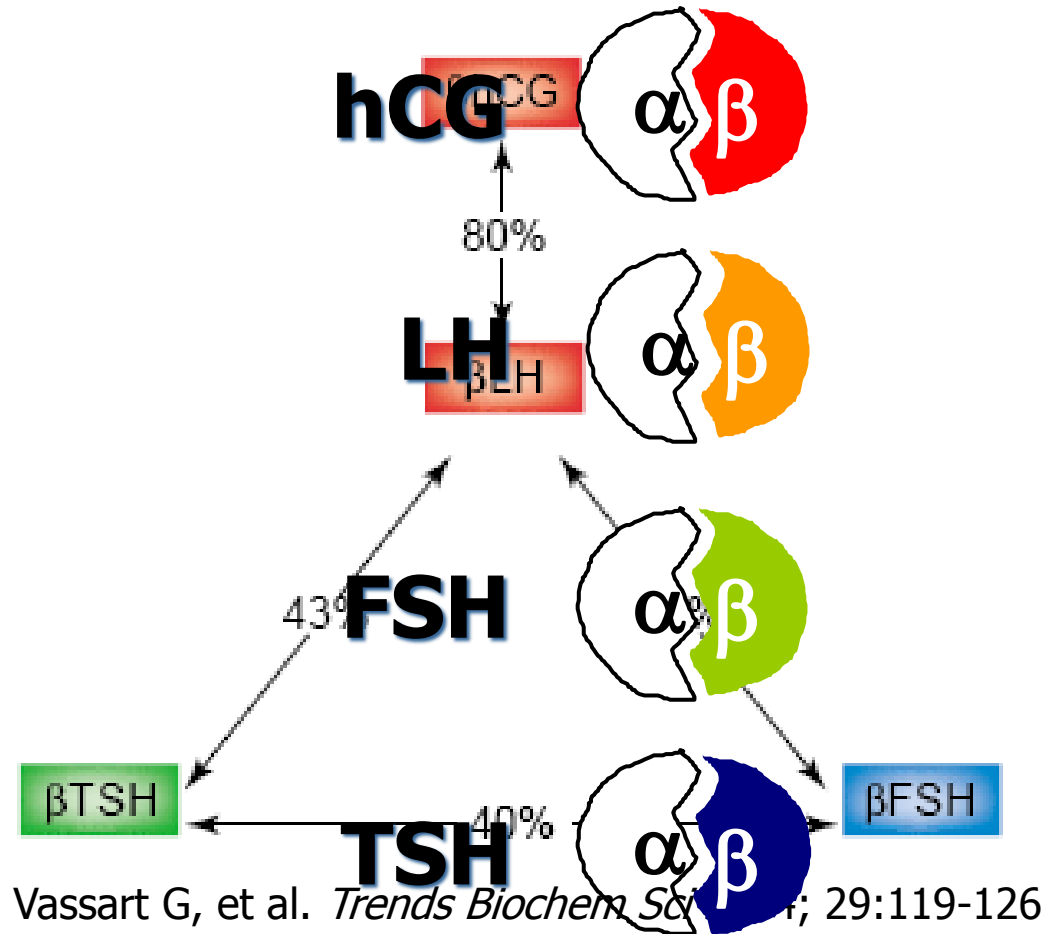
# Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family



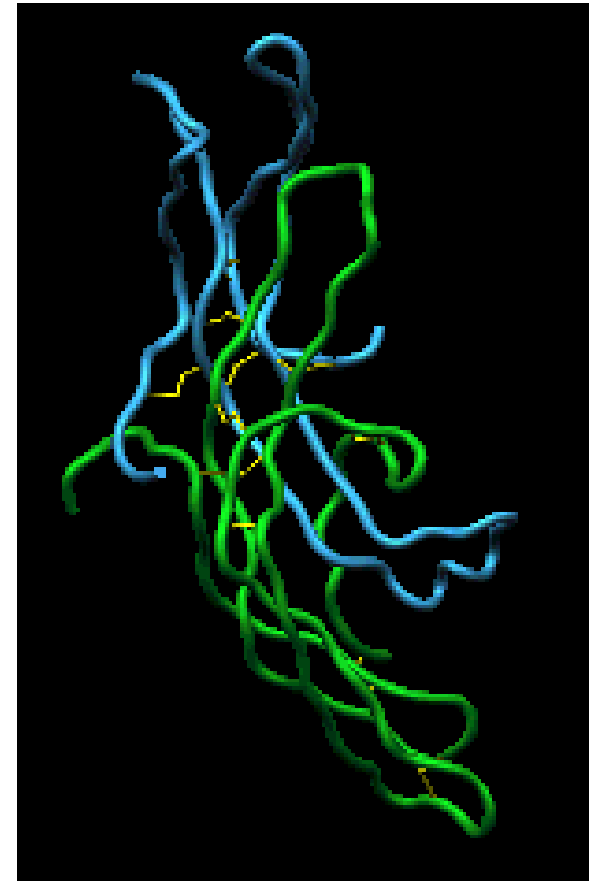
# Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family



# hCG Structure

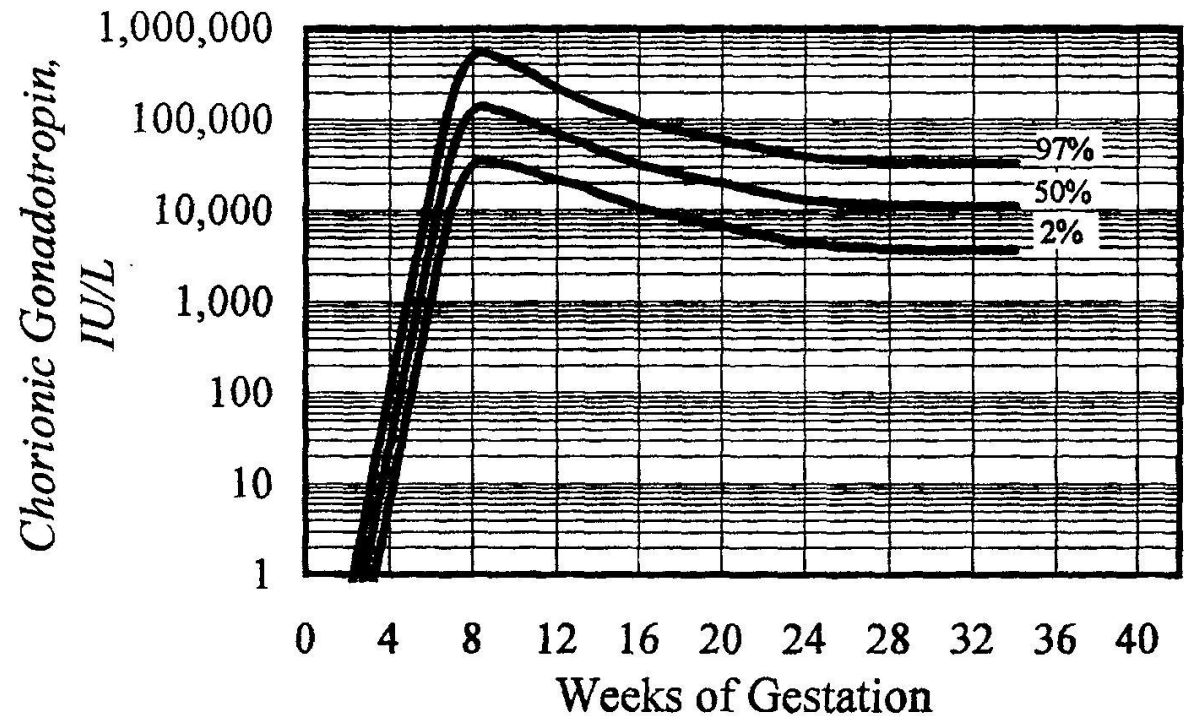
- Dimer is ~38,000 daltons
  - 30% of weight due to carbohydrate
- Alpha subunit
  - 92 amino acids
  - 2 N-linked carbohydrate chains
- Beta subunit
  - 145 amino acids
  - 2 N-linked & 4 O-linked carbohydrate chains
- Half-life ~48 hours



[http://www.chem.gla.ac.uk/protein/glyco/hyper/hcg\\_act.html](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



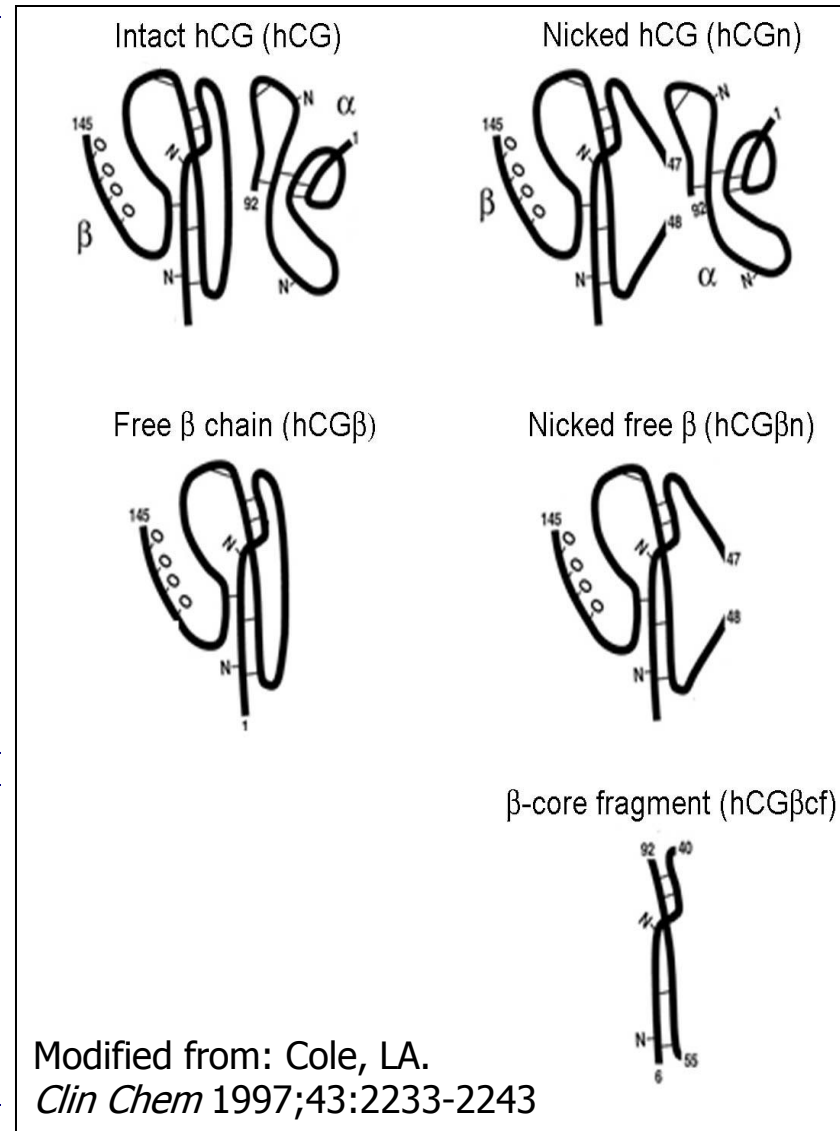


# hCG Heterogeneity

- Numerous molecular forms of hCG present in pregnancy serum
  - Dissociated or degraded molecules with no biological activity
- Key  $\beta$ -containing variants
  - Intact hCG
  - Nicked hCG
  - Free  $\beta$  subunit
  - Nicked free  $\beta$  subunit
  - $\beta$ -core fragment (urine)

Serum  
and  
Urine

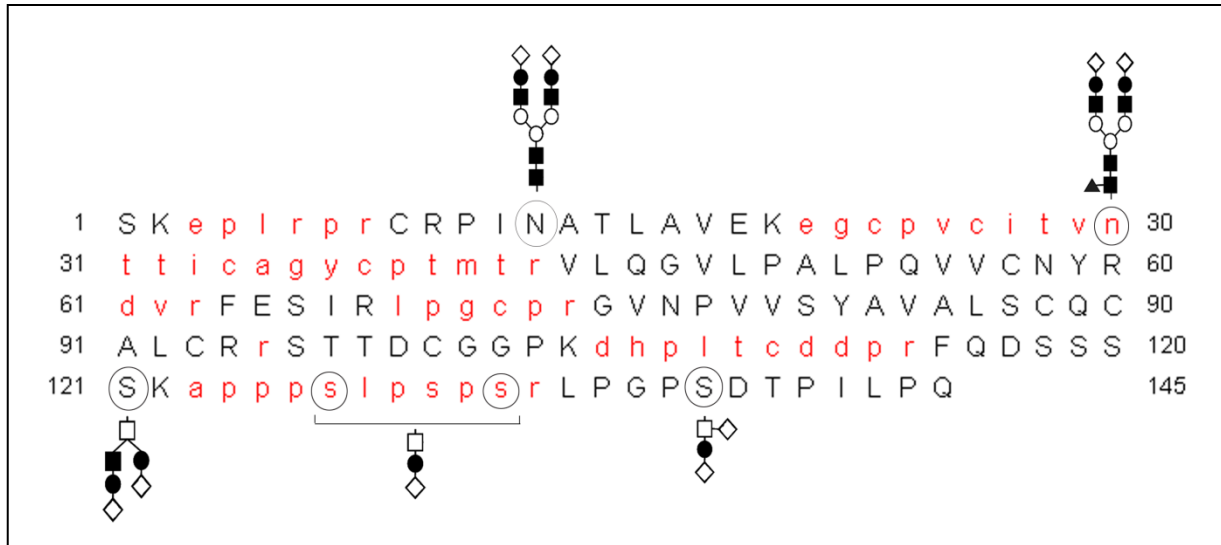
Urine



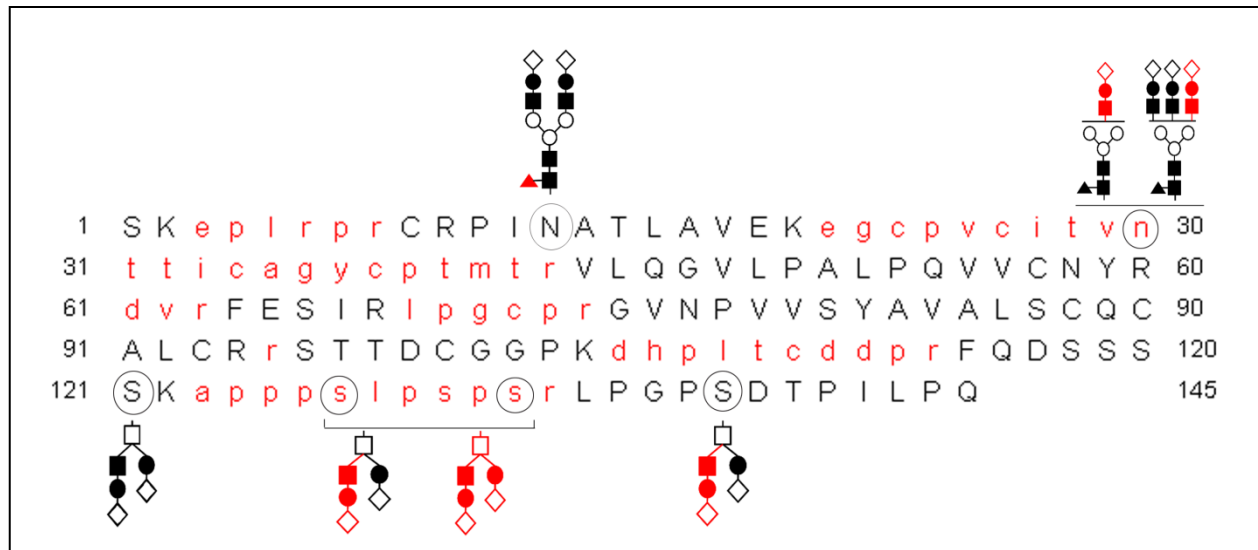
# Hyperglycosylated hCG

## Structure

Mid-pregnancy



Early-pregnancy & Testicular Cancer



▲ Fuc  
 ■ GlcNAc  
 □ GalNAc  
 ○ Man  
 ● Gal  
 ◇ NeuAc



# hCG Immunoassays

# hCG Immunoassays

## Qualitative

Home  
(Urine)

Lab  
(Serum & Urine)

Analytical Specificity  
Most are “designed”  
to detect intact hCG

Analytical Sensitivity  
~20 IU/L urine  
~10 IU/L serum

## Quantitative

Serum  
(Immulite FDA-approved for  
Urine-QUALITATIVE)

Analytical Specificity  
Most total hCG $\beta$   
Intact hCG, free hCG $\beta$

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

Epitope	Code	MAb Specificities											Ref.
		hCG	hCG $\beta$	hCG $\beta$ cf	hCGn	hCG $\beta$ n	-CTP hCG	-CTP hCG $\beta$	hLH	hLH $\beta$	hFSH hTSH	GPH $\alpha$	
$\beta_1$	INN-hCG-2												4, 18, 27
$\beta_2$	INN-hCG-22												4, 18, 27
$\beta_3$	INN-bLH-1												4, 18, 27
$\beta_4$	INN-hCG-24												4, 18, 27
$\beta_5$	INN-hFSH-58												4, 18, 27
$\beta_6$	INN-hCG-64					?		?					4, 18, 27
$\beta_7$	INN-hCG-68					?		?					4, 18, 27
$\beta_8$	h54												4, 18, 27
$\beta_9$	FB-12												4, 18, 27
$\alpha_1$	INN-hFSH-73												26, 34
$\alpha_2$	INN-hFSH-98												26, 34
$\alpha_2$	INN-hFSH-100												26, 34
$\alpha_3$	INN-hFSH-179												26, 34
$\alpha_4$	INN-hFSH-132												26, 34
$\alpha_5$	INN-hFSH-158												26, 34
$\alpha_6$	INN-hCG-72,-80												26, 34
$\alpha_6$	AHT-20												3, 34, 35
$c_1$	INN-hCG-10						?						25, 51
$c_2$	INN-hCG-40,-53						?						25, 51
$c_3$	INN-hCG-45						n.t.						25, 51
$c_4$	INN-hCG-26						?						25, 51

Filled squares = strong reactivity; white squares = no reactivity; gray squares = minor reactivity. GPH $\alpha$  = glycoprotein hormone  $\alpha$ -subunit; n.t = not tested.



# Lack of Assay Standardization

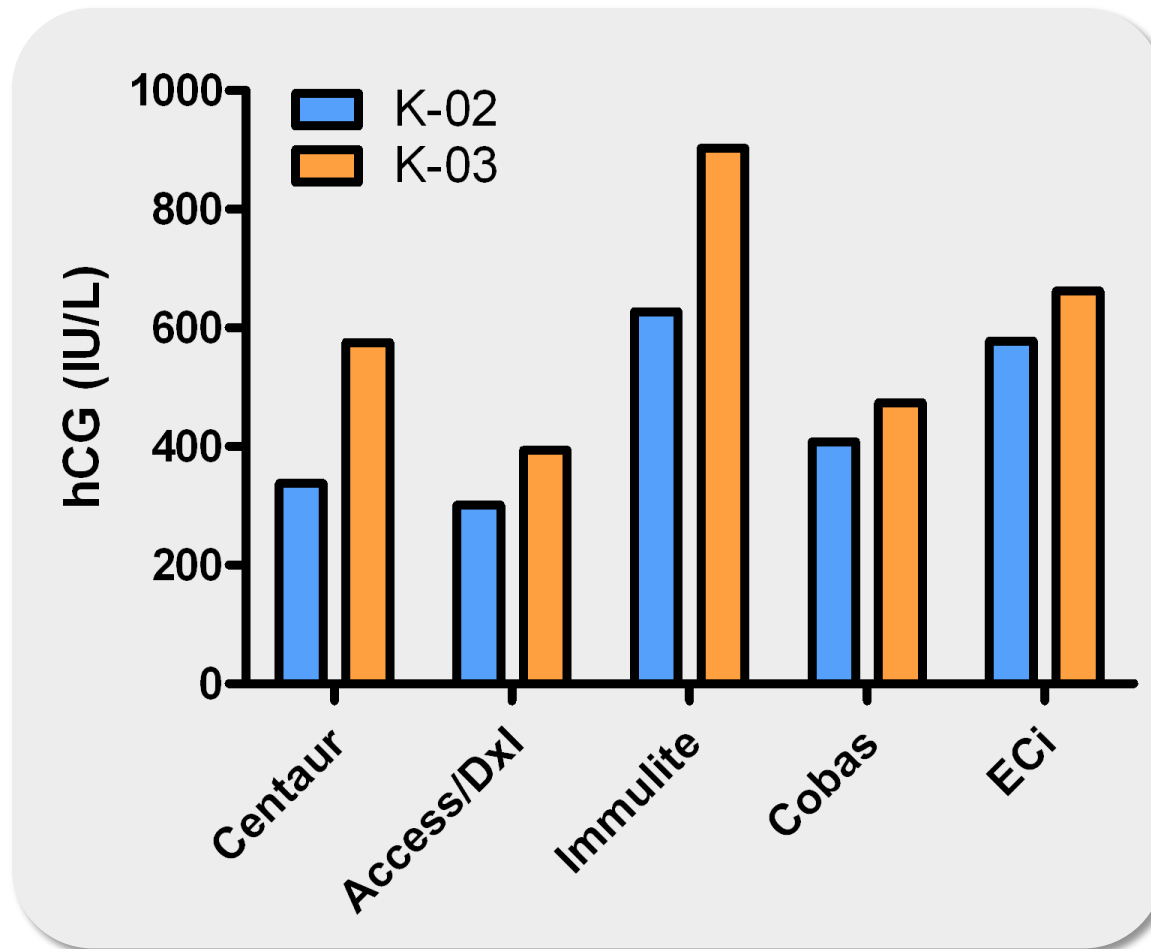
- 1) Different antibody pairs used in different assays
  - Recognize different epitopes of different variants
- 2) Secondary standards (calibrators) used by manufacturers differ in purity

*Wide variation in:*

*measured hCG concentrations*

*and detection of hCG variants*

# Variation in Measured hCG Concentrations



# WHO International Reference Reagents

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

hCG isoforms	IFCC nomenclature	WHO code <sup>a</sup>
Intact hCG	hCG	IRR 99/688
Nicked hCG	hCG <sub>n</sub>	IRR 99/642
hCG beta-subunit	hCG $\beta$	IRR 99/650
Nicked hCG beta-subunit	hCG $\beta$ <sub>n</sub>	IRR 99/692
hCG beta core fragment	hCG $\beta$ cf	IRR 99/708
hCG alpha-subunit	hCG $\alpha$	IRR 99/720

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

# Differential recognition of hCG WHO standards, by different assays

Intact hCG

hCG & hCG $\beta$

hCG, hCG $\beta$  & hCG $\beta$ cf

Table 3. Recognition in molar terms of the various IRR preparations by each method.<sup>a</sup>

	hCGn 99/642	hCG $\beta$ 99/650	hCG $\beta$ n 99/692	hCG $\beta$ cf 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 hCG $\beta$				
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, hCG $\beta$ and hCG $\beta$ cf				
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

Sturgeon CM, et al.

*Clin Chem* 2009;55:1484-91-38

## Are Laboratories Reporting Serum Quantitative hCG Results Correctly?

Zhimin (Tim) Cao,<sup>1\*</sup> Robert Rej<sup>1,2</sup>

Prepared:

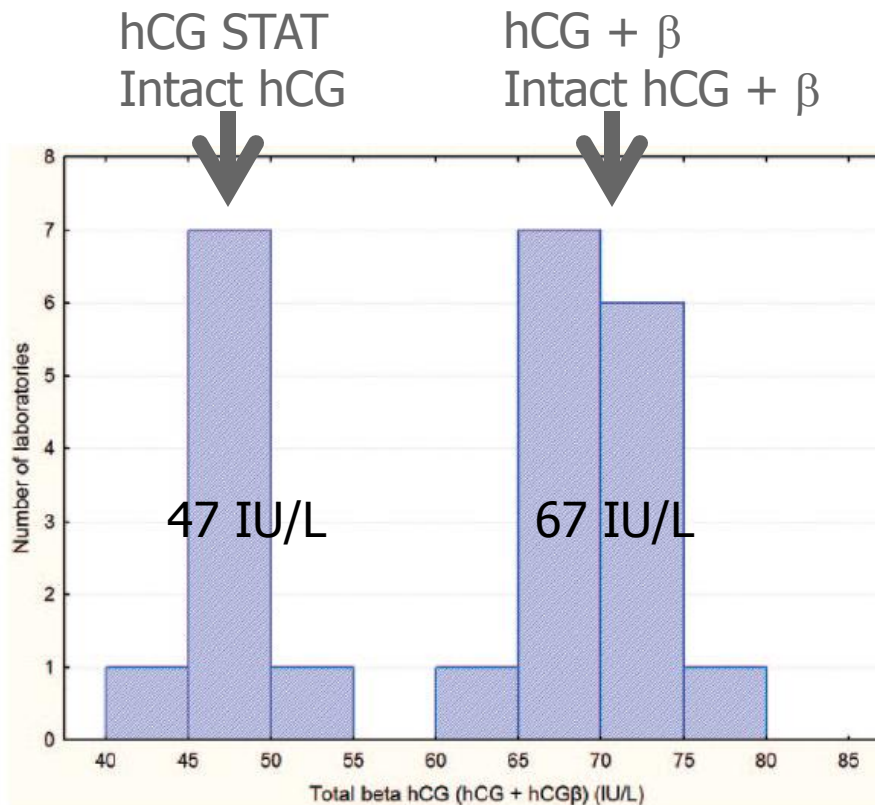
Intact hCG

Free hCG $\beta$

Intact hCG + free hCG $\beta$

Sent to 266 laboratories

Roche Elecsys



*“Many laboratories using the hCG STAT procedure reported total  $\beta$  hCG”*

## Are Laboratories Reporting Serum Quantitative hCG Results Correctly?

Zhimin (Tim) Cao,<sup>1\*</sup> Robert Rej<sup>1,2</sup>

Prepared:

Intact hCG

Free hCG $\beta$

Intact hCG + free hCG $\beta$

Sent to 296 laboratories

15 different methods

61 labs report  
“intact hCG”



8 (13.1%) actually measured  
“Total  $\beta$  hCG”

235 labs report  
“Total  $\beta$  hCG”



22 (9.3%) actually measured  
“Intact hCG”

# Variation in hCG Variant Detection POC hCG Tests

	Sure-Vue Anti- $\alpha$ (u) Anti-CG dimer (u)	Clinitest Anti -CG dimer (m) Anti- $\beta$ (m)	QuickVue+ Proprietary (p) Anti- $\beta$ (m)	Osom Anti- $\alpha$ (m) Anti- $\beta$ (m)	hCG Combo Anti- $\alpha$ (m) Anti- $\beta$ (m)	ICON II Anti- $\alpha$ (m) Anti- $\beta$ (m)	Elecsys <sup>a</sup> , IU/L, pmol/L <sup>b</sup> Anti- $\beta$ (m) Anti- $\beta$ (m)
<b>hCG</b>	10/10	10/10	10/10	5/5	10/10	10/10	1220 NA <sup>d</sup>
<b>hCGn</b>	10/10	10/10	10/10	10/10	10/10	10/10	2263
<b>hCG<math>\beta</math></b>	10/10	10/10	10/10	0/10	10/10	10/10	7800 2336
<b>hCG<math>\beta</math>n</b>	10/10	10/10	10/10	0/10	10/10	10/10	8800 630
<b>hCG<math>\beta</math>cf</b>	0/10	10/10	6/10	0/10	10/10	0/10	3300 815
<b>hCG<math>\alpha</math></b>	0/10	0/10	0/10	0/5	0/10	0/10	10 200 <2.0 8400

# Variation in hCG Variant Detection OTC hCG Tests

	Over-the-counter device <sup>a</sup>						Quantitative device
	First Response	EPT	Clearblue Easy	Target Early Result	Answer	Wal-Mart Equate	
Claimed analytical sensitivity (IU/l)	25	25	25	25	25	25	Roche, Elecsys, IU/l <sup>b</sup>
hCG	10/10	5/5 <sup>c</sup>	10/10	10/10	6/6 <sup>d</sup>	10/10	8882
hCGn	10/10	9/9 <sup>c</sup>	10/10	10/10	10/10	10/10	3628
hCGβ <sup>e</sup>	10/10	8/8 <sup>c</sup>	10/10	10/10	6/6 <sup>f</sup>	10/10	6129
hCGβn	10/10	2/5 <sup>c</sup>	0/10	10/10	7/7 <sup>g</sup>	10/10	1328
hCGβcf	0/10	0/9 <sup>c</sup>	0/10	0/10	1/6 <sup>d</sup>	0/10	2341



# Why do we care which hCG variants are recognized by a qualitative device?

Clinical Chemistry 55:7  
1389–1394 (2009)

Endocrinology and Metabolism

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## False-Negative Results in Point-of-Care Qualitative Human Chorionic Gonadotropin (hCG) Devices Due to Excess hCG $\beta$ Core Fragment

Ann M. Gronowski,<sup>1\*</sup> Mark Cervinski,<sup>1</sup> Ulf-Håkan Stenman,<sup>2</sup> Alison Woodworth,<sup>3</sup> Lori Ashby,<sup>4</sup> and Mitchell G. Scott<sup>1</sup>

# False Negative Qualitative hCG

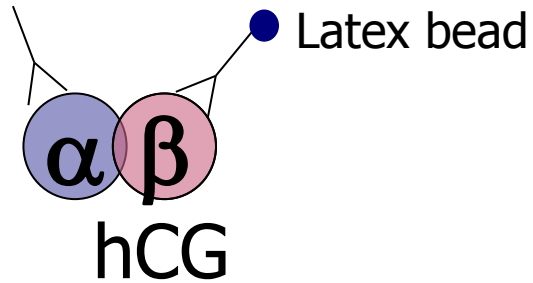
- 18 yoF with vaginal spotting and cramping
- States 3 months pregnant
- ED POC hCG negative
- Serum hCG = 419,680 IU/L
- Ultrasound - live intrauterine pregnancy
- Urine hCG = 176,498 IU/L



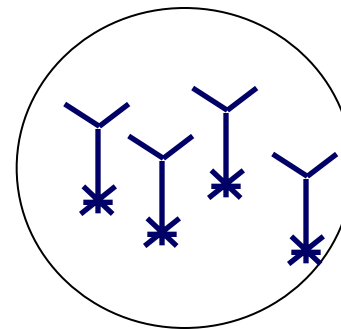
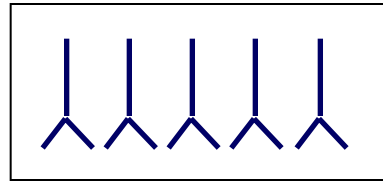
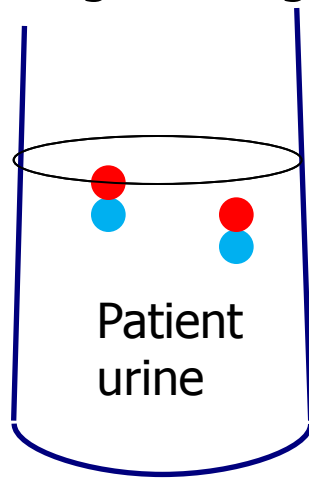
# False Negative Qualitative hCG

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

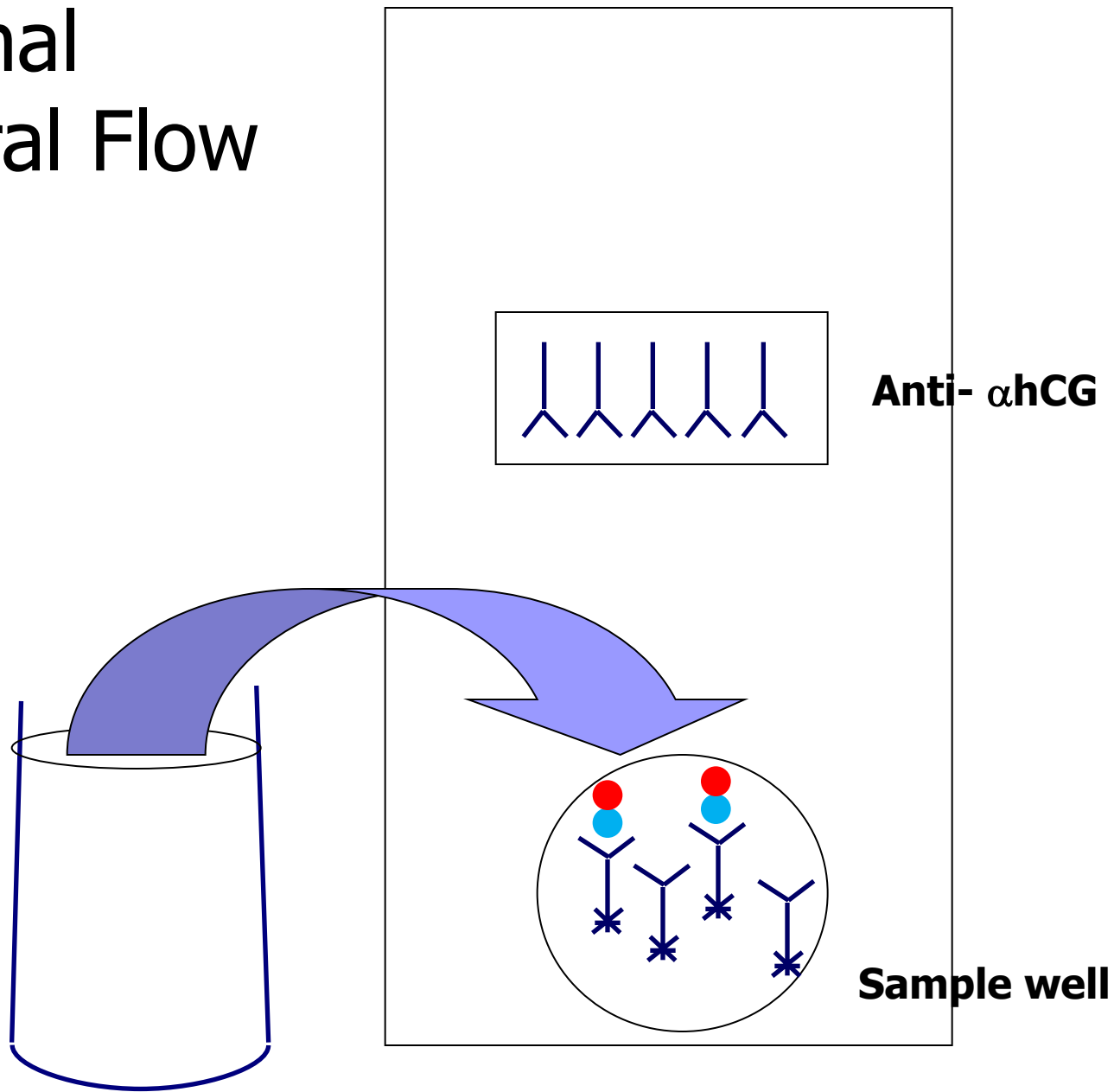
# Normal Lateral Flow



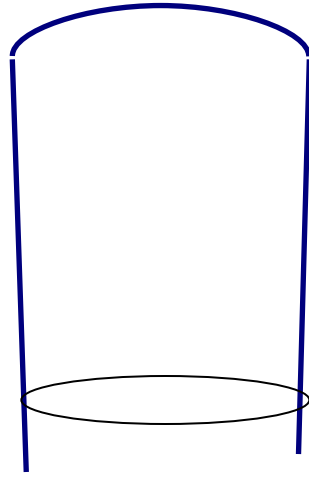
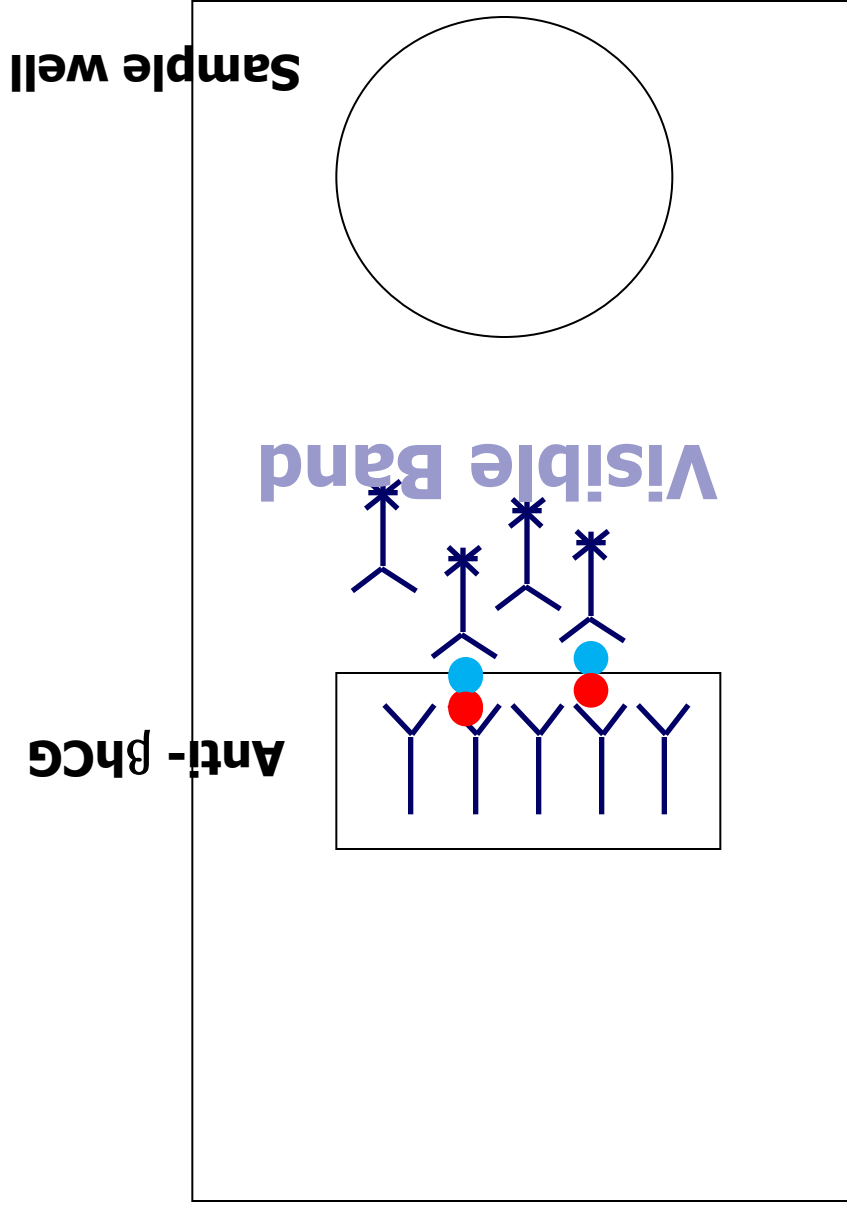
Measured urine hCG  
in expected, normal  
physiological range



# Normal Lateral Flow

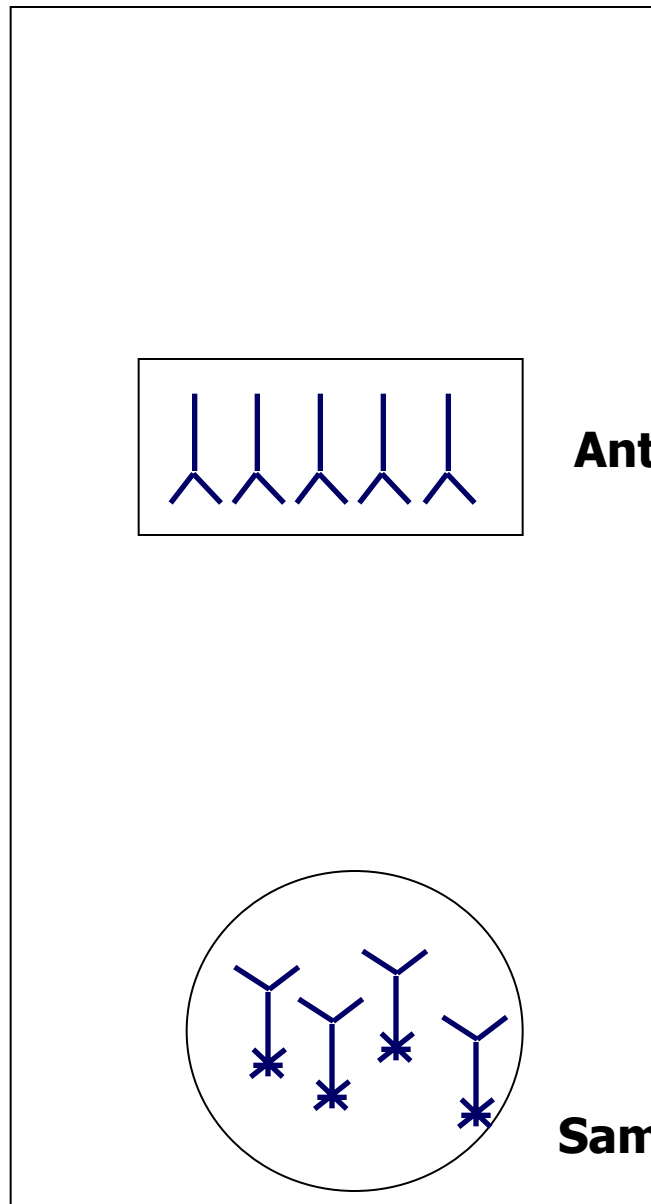
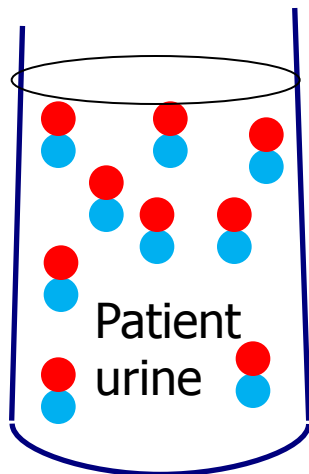


# Normal Lateral Flow



# Hook Effect

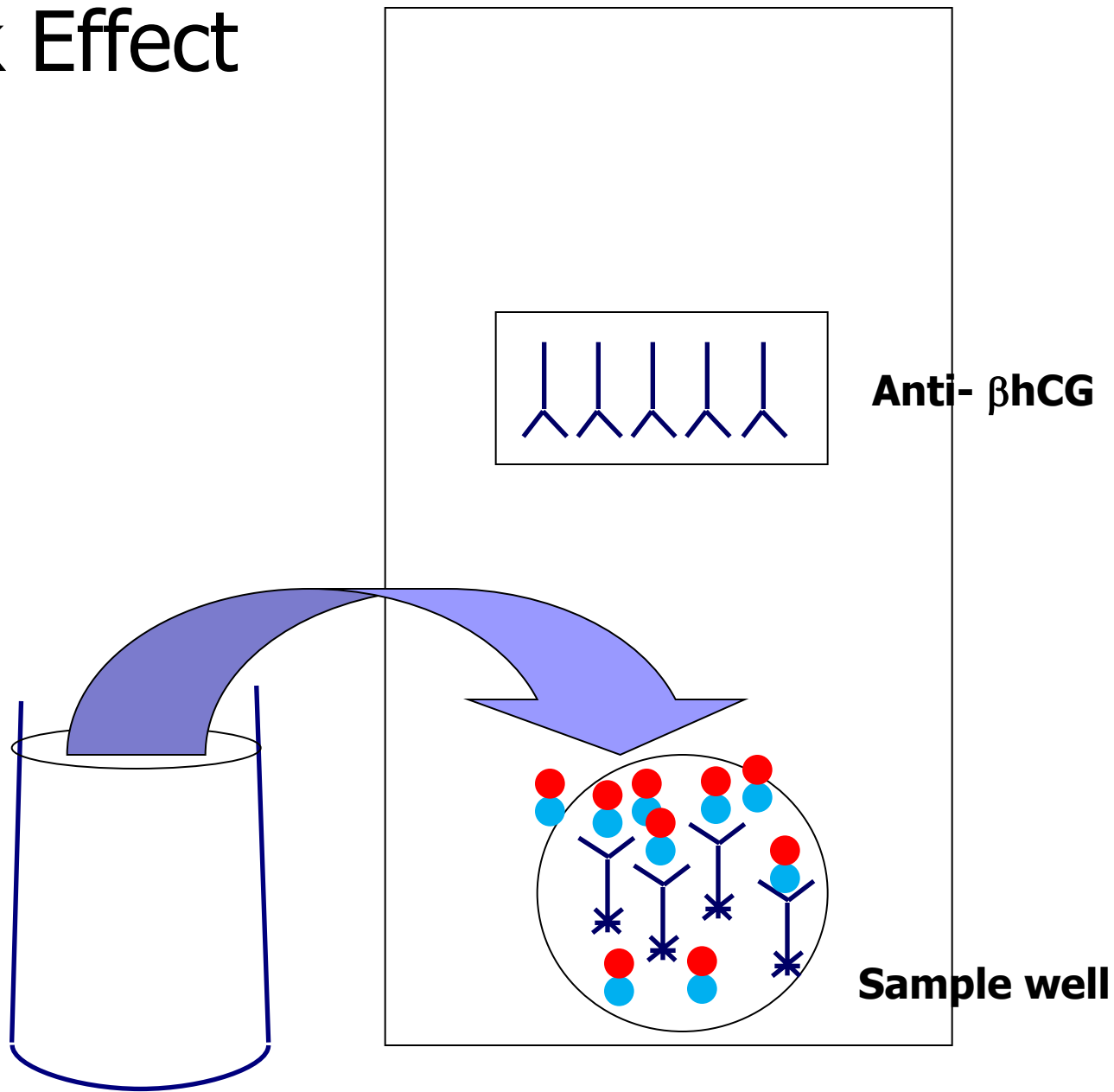
Measured urine hCG  
in **great excess**  
of normal  
physiological range



**Anti- $\beta$ hCG**

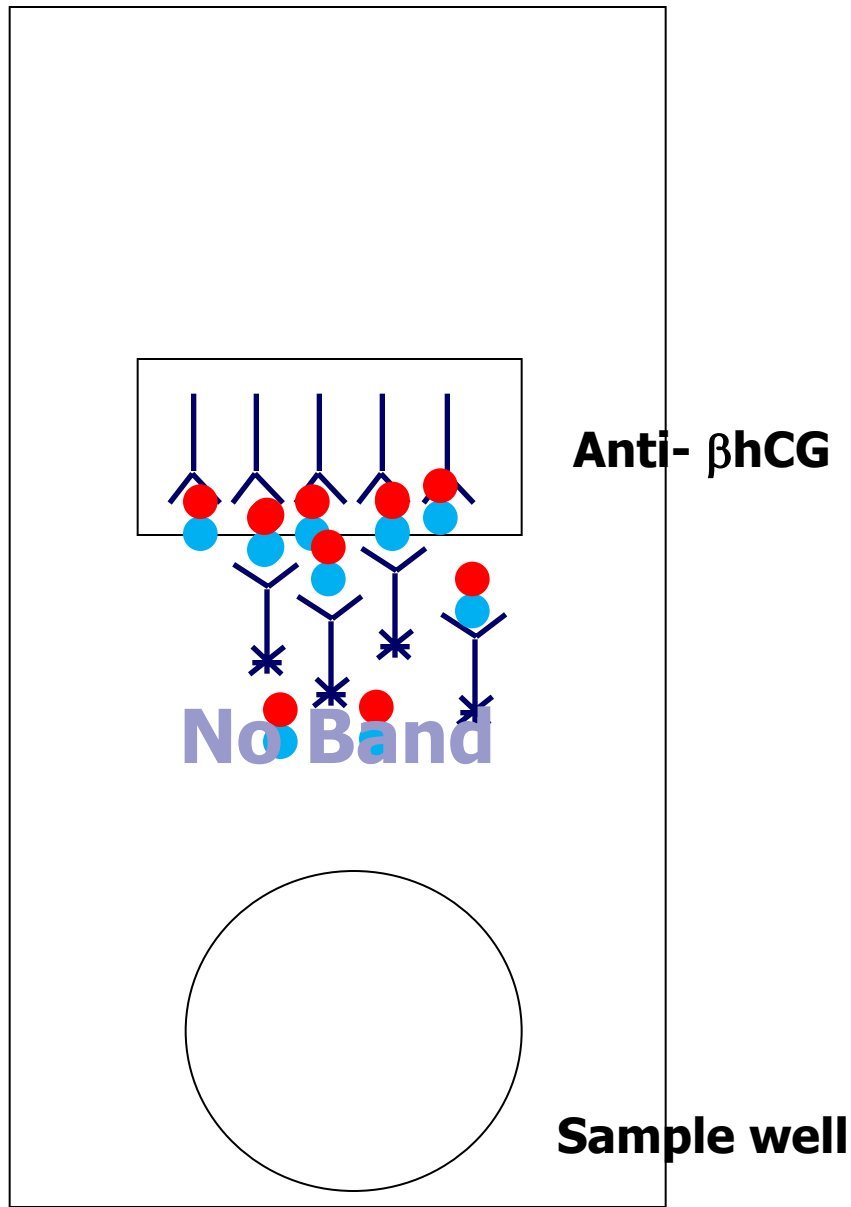
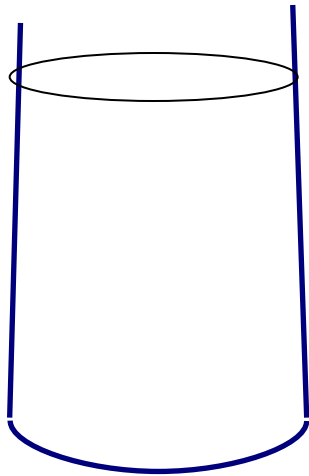
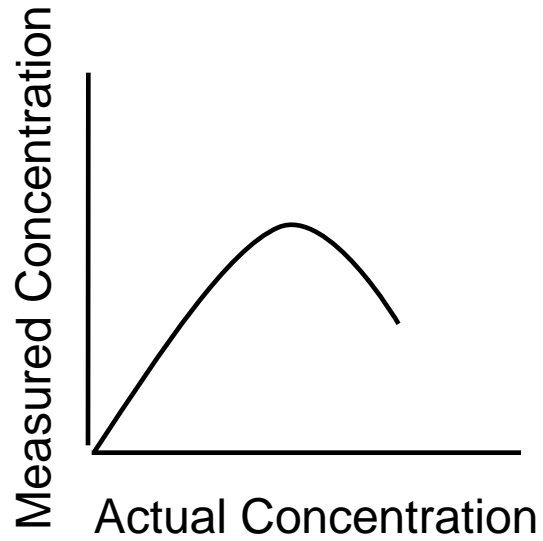
**Sample well**

# Hook Effect





# Hook Effect




# Not a “Hook Effect”

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

Dilution	Total beta hCG Concentration (IU/L)	Osom Result
90%	2,880,000	Negative
80%	2,560,000	Negative
70%	2,240,000	Negative
60%	1,920,000	Negative
50%	1,600,000	Positive
40%	1,280,000	Positive
30%	960,000	Positive
20%	640,000	Positive
10%	320,000	Positive

# Analytical Specificity: POC hCG Tests

	Sure-Vue	Clinitest	QuickVue+	Osom	hCG Combo	ICON II	Elecsys <sup>a</sup> , IU/L, pmol/L <sup>b</sup>
	Anti- $\alpha$ (u)	Anti -CG dimer (m)	Proprietary (p)	Anti- $\alpha$ (m)	Anti- $\alpha$ (m)	Anti- $\alpha$ (m)	Anti- $\beta$ (m)
	Anti-CG dimer (u)	Anti- $\beta$ (m)	Anti- $\beta$ (m)	Anti- $\beta$ (m)	Anti- $\beta$ (m)	Anti- $\beta$ (m)	Anti- $\beta$ (m)
<b>hCG</b>	10/10	10/10	10/10	5/5	10/10	10/10	1220 NA <sup>d</sup>
<b>hCG<sub>n</sub></b>	10/10	10/10	10/10	10/10	10/10	10/10	2263 7800
<b>hCG<math>\beta</math></b>	10/10	10/10	10/10	0/10	10/10	10/10	2336 8800
<b>hCG<math>\beta</math><sub>n</sub></b>	10/10	10/10	10/10	0/10	10/10	10/10	630 3300
<b>hCG<math>\beta</math>cf</b>	0/10	10/10	6/10	0/10	10/10	0/10	815 10 200
<b>hCG<math>\alpha</math></b>	0/10	0/10	0/10	0/5	0/10	0/10	<2.0 8400



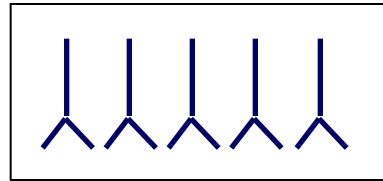
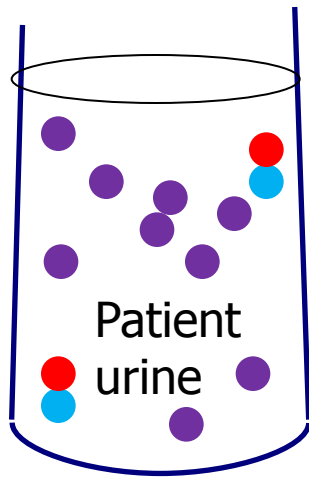
Could an excess of an hCG variant bind only one of the assay antibodies, negating the formation of a “sandwich”?

# Effect of hCG $\beta$ cf on 3 POC Devices

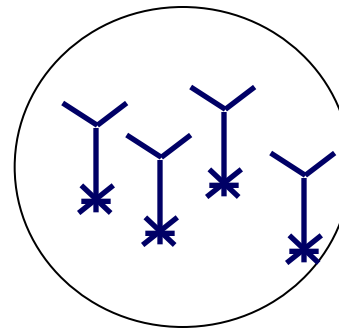


# Hook Effect with hCG Variant

Measured urine hCG in expected, normal physiological range, but [variant] > [intact hCG]

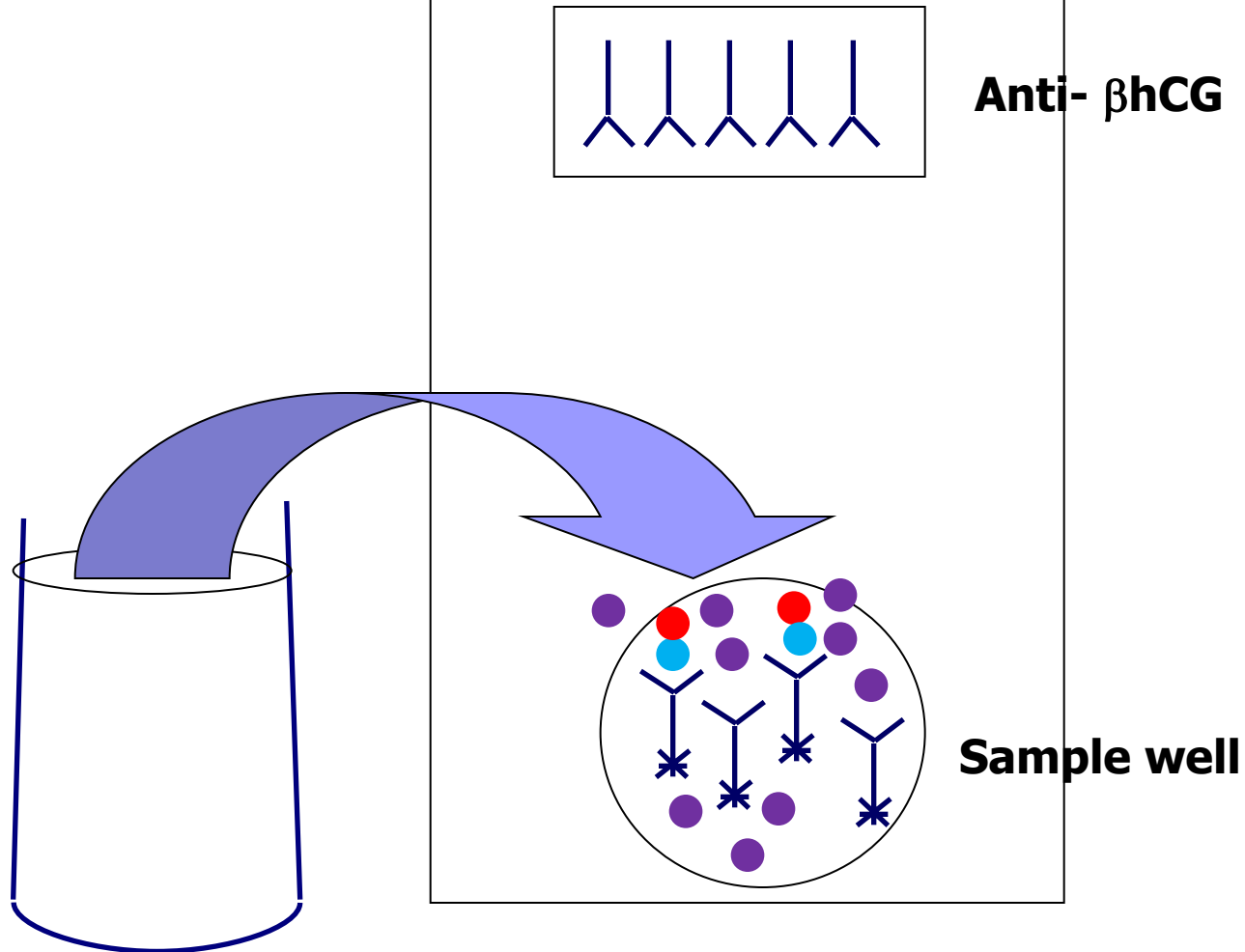


**Anti-βhCG**

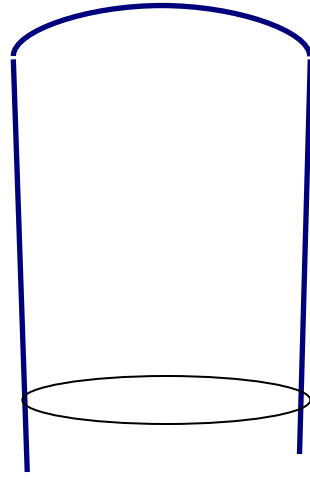
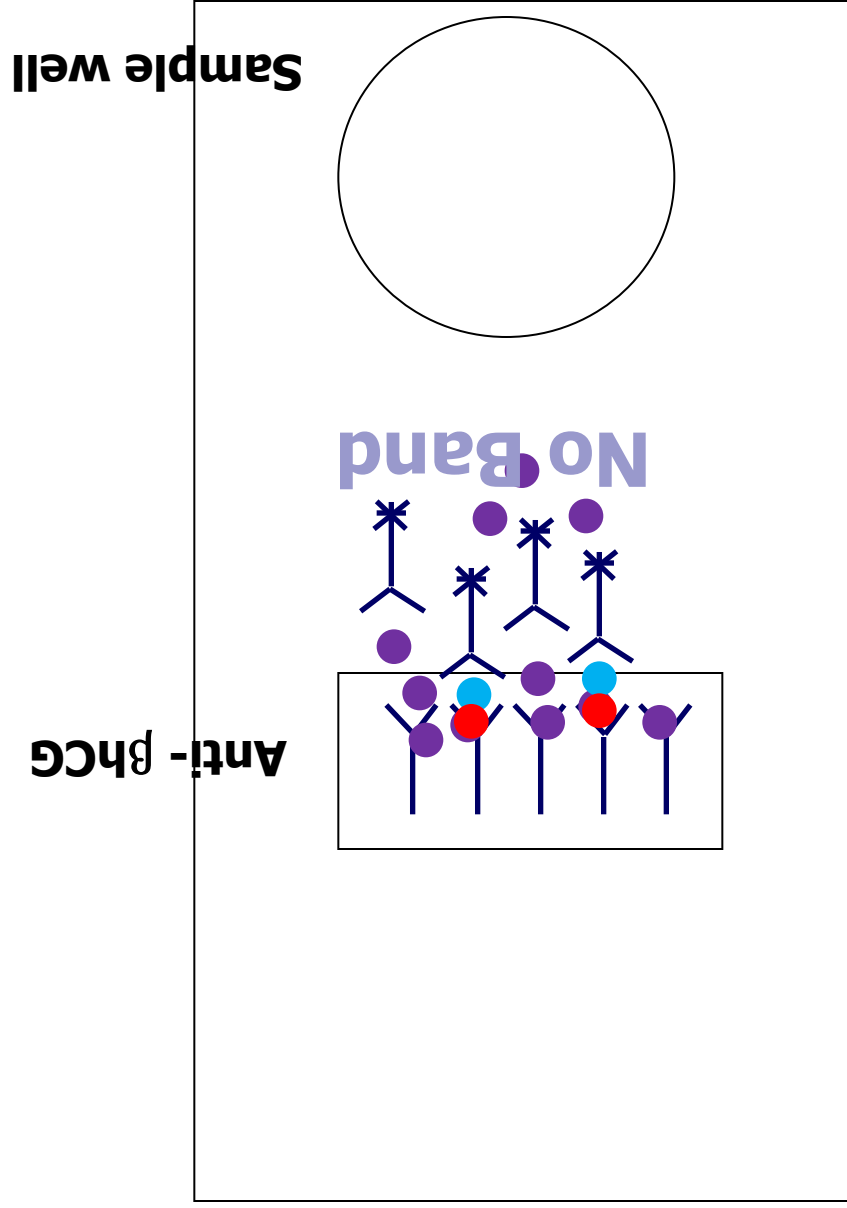


**Sample well**

# Hook Effect with hCG Variant



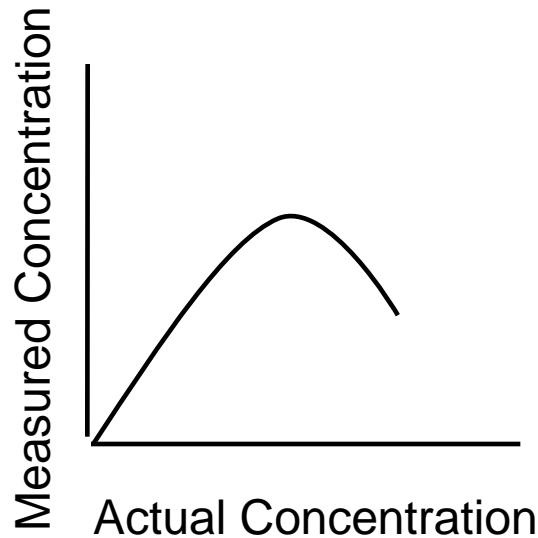
# Hook Effect with hCG Variant





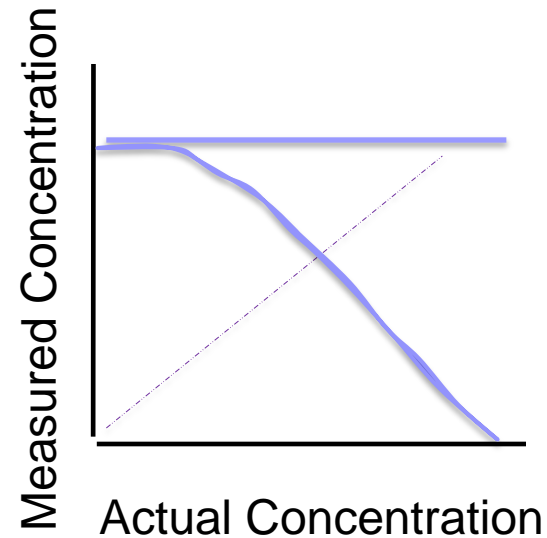
# Hook Effect

Traditional Hook Effect



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

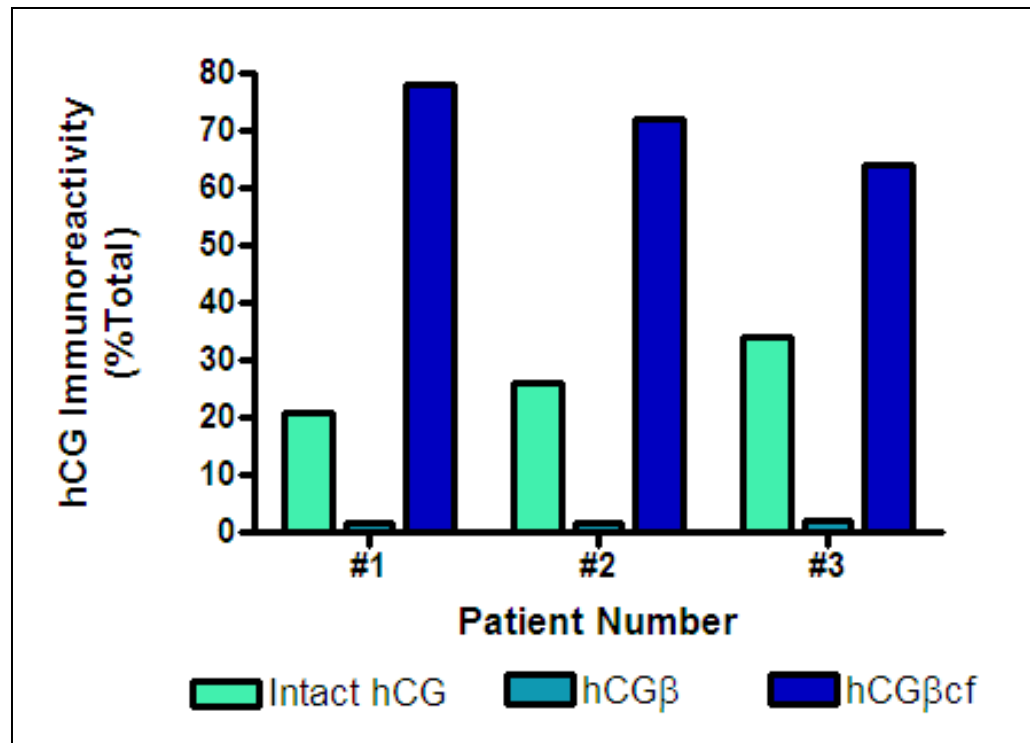
Variant Hook Effect

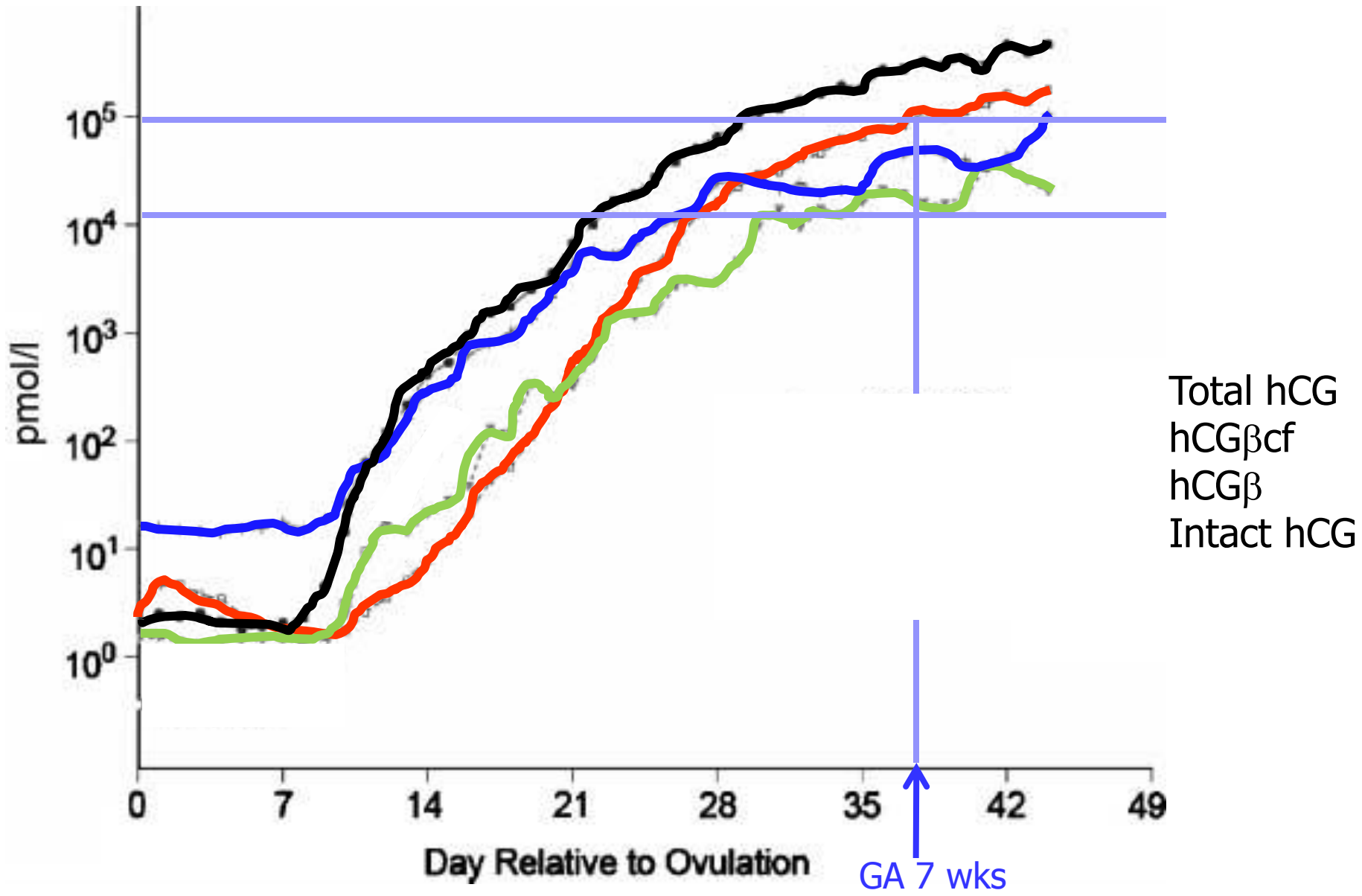


- hCG concentrations normal
- Antigen recognized by both antibodies
- Need two forms of hCG to detect

# hCG Variant Content of False Negative Urine

- Patient urine contained an excess of hCG $\beta$ cf






# 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?

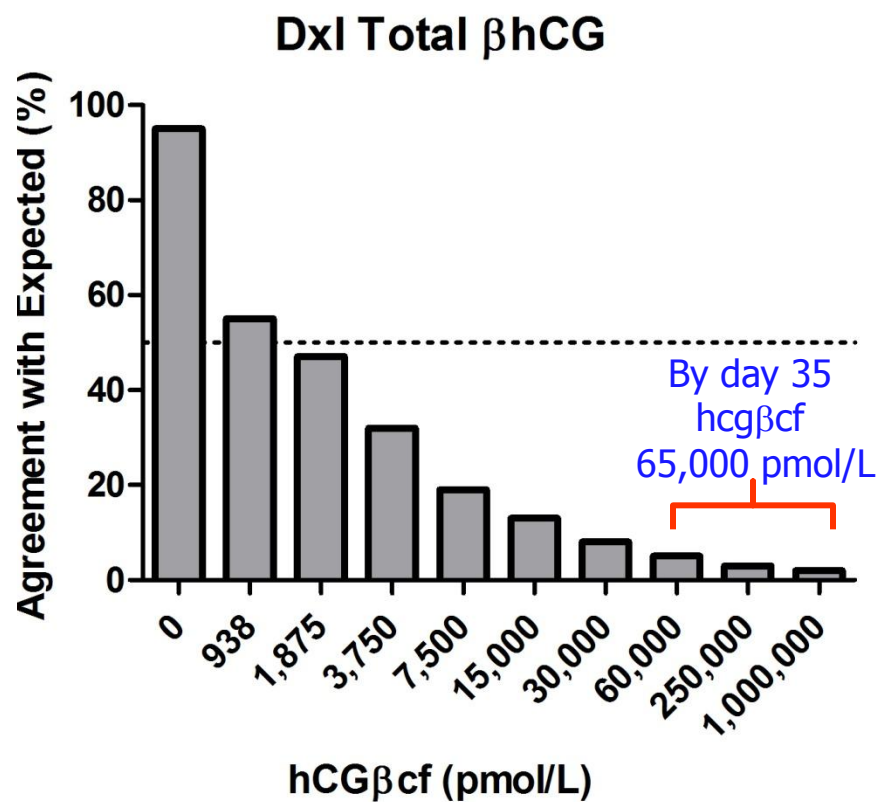
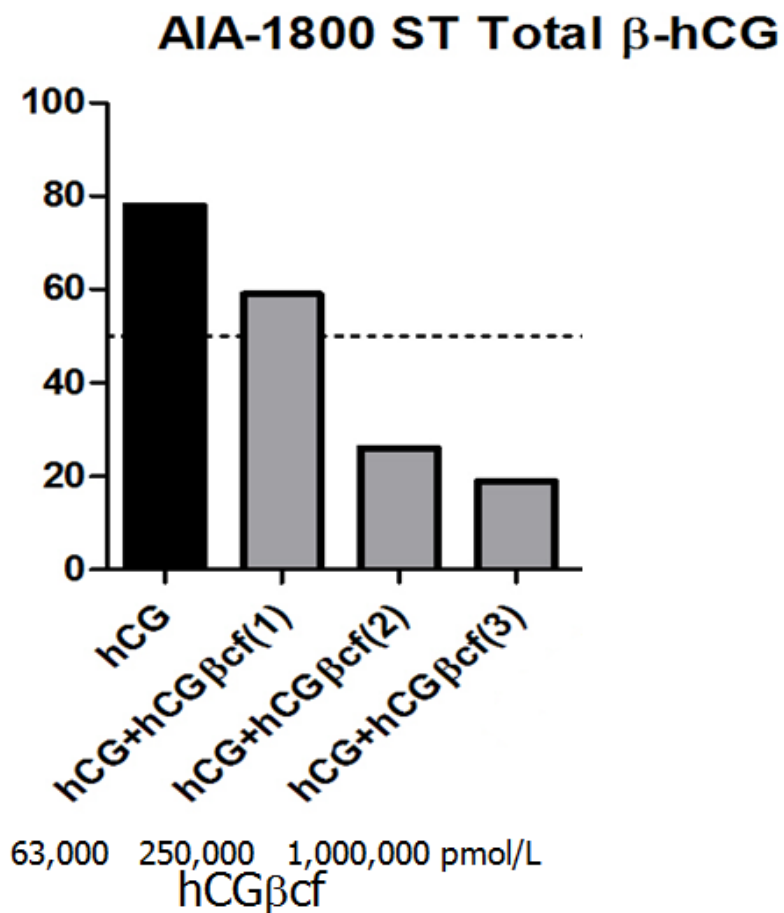


# What about Quantitative Assays?

# hCG Variant Effect on Quantitative Tests

- Advia Centaur<sup>®</sup> Total hCG (Siemens)
- AIA-1800 ST Total  $\beta$ -hCG (Tosoh Bioscience)
- Architect<sup>®</sup> Total  $\beta$ -hCG (Abbott Laboratories)
- Cobas<sup>®</sup> e411 hCG Stat (Roche Diagnostics)
- Dimension<sup>®</sup> RxL<sup>®</sup> hCG (Siemens)
- DxI<sup>®</sup> Total  $\beta$ hCG (Beckman Coulter)
- ✓ ■ Modular Analytics e170 hCG+ $\beta$  (Roche Diagnostics)
- ✓ ■ Immulite 2000 hCG
- Vitros<sup>®</sup> ECi Total  $\beta$ -hCG II (Ortho Clinical Diagnostics)

# hCG Variant Effect on Quantitative Tests







# Analytical Sensitivity

# Analytical Sensitivity Serum Quantitative

<b>Instrument</b>	<b>IU/L</b>
Roche emodule	0.1
Immulite 2000	0.4
Beckman Dxl	0.5
Abbott Architect	1.2
Siemens Centaur	2.0
Vitros ECI	2.39

## **Interpretation:**

<5 Non-pregnant  
≥5 Pregnant

OR

<5 Non-pregnant  
5-25 Indeterminate  
>25 Pregnant

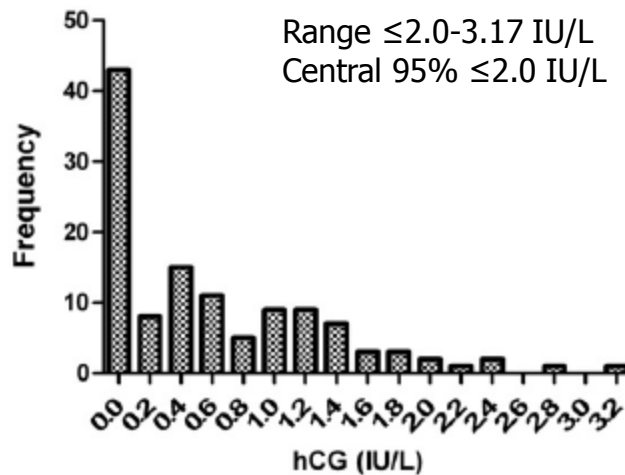
# Analytical Sensitivity Urine Quantitative

**Immulite 1000:**

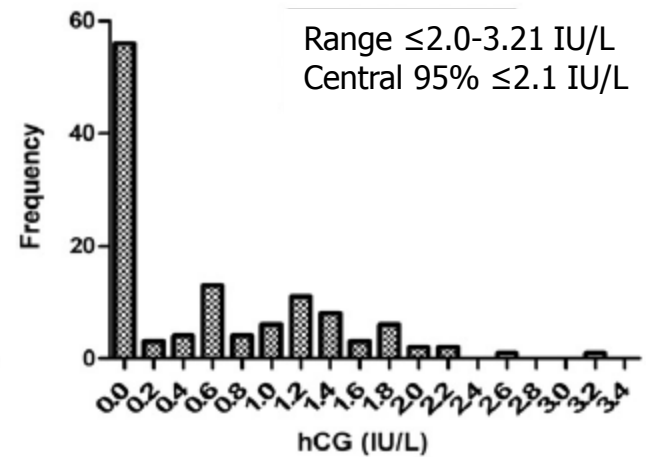
LOQ: 2 IU/L

CV: <11%

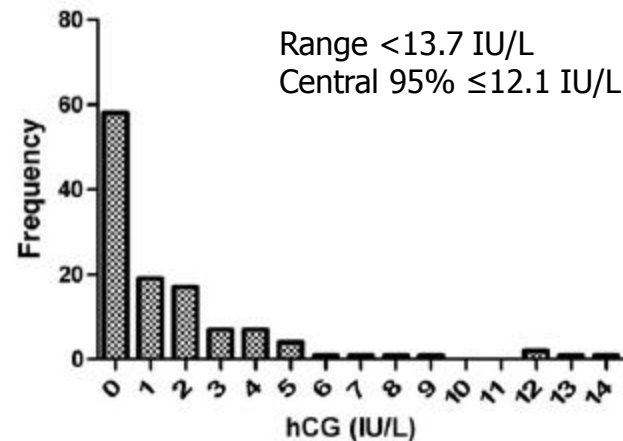
**A** Reference Interval Males 20 - 70 years



**B** Reference Interval Women < 55

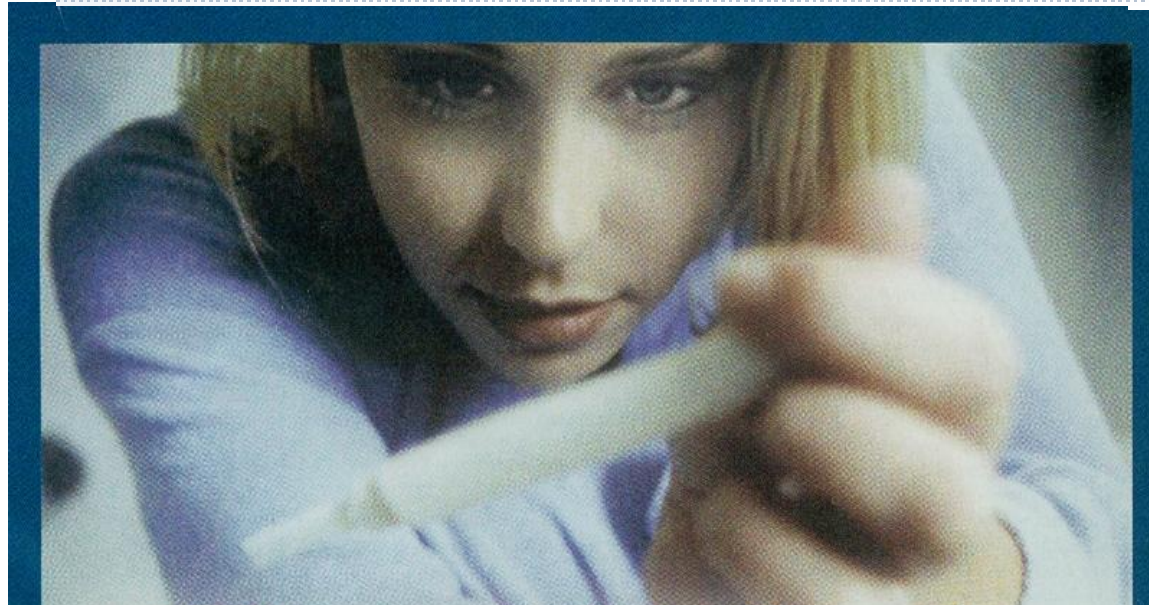


**C** Reference Interval Women > 55



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The take home message:

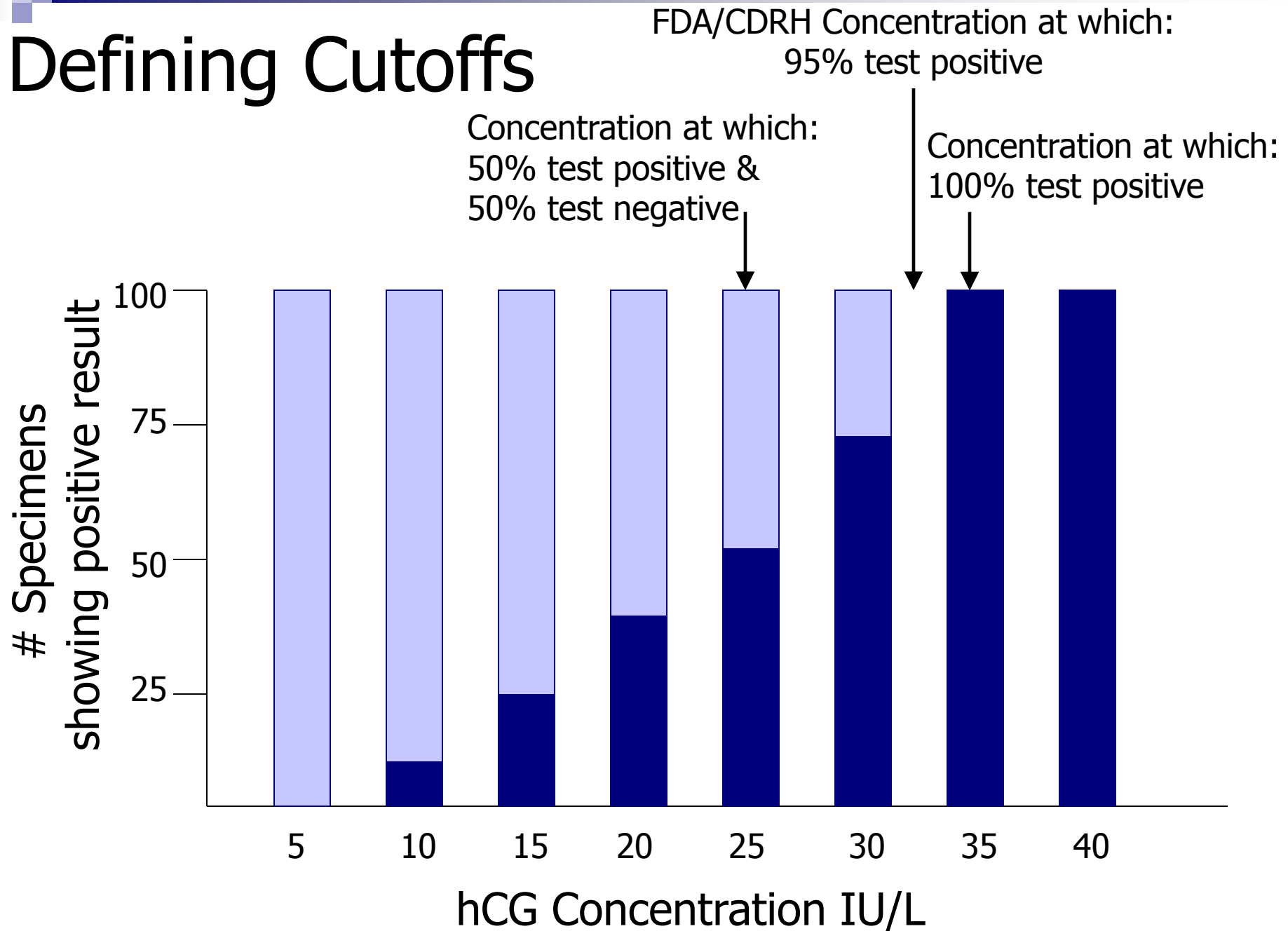
- 1) Analytical sensitivity varies with brand
- 2) Cutoffs often not in agreement with manufacturer's claimed cutoffs.

# Analytical Sensitivity

## POC Devices

Device	Read at, min	Regular hCG, IU/L				Manufacturer's hCG limit, IU/L
		6.3	13	25	50	
Clear Blue Easy	1	+	+	++	++	50
Target Early Pregnancy Test	5	±	+	+	++	100
American Fare Easy to Read	3	-	+	++	++	~50
First Response Early Result E.P.T.	3	- <sup>b</sup>	+	+	++	40
Answer	2	-	-	+	++	100
Fact Plus Pro	3	-	- <sup>b</sup>	± <sup>b</sup>	±	100
Fact Plus Select	3	-	-	+	+	25
Equate <sup>o</sup>	1	-	-	+	+	100
Walgreens E.P.T.	5	-	-	±	++	50
Walgreens One Step	3	-	-	+	++	100
Inverness Medical E.P.T.	3	-	-	+	++	100
Longs Pregnancy Test	3	-	-	+	+	50
Rite Aid One Step	3	-	-	+	++	25
Confirm	2	-	-	-	+	

# Defining Cutoffs



# Analytical Sensitivity

## POC & OTC Devices

Median Concentration  
(IU/L)

3/3 devices test positive  
(n=11 patients)

### POC Device

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

### OTC Device

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

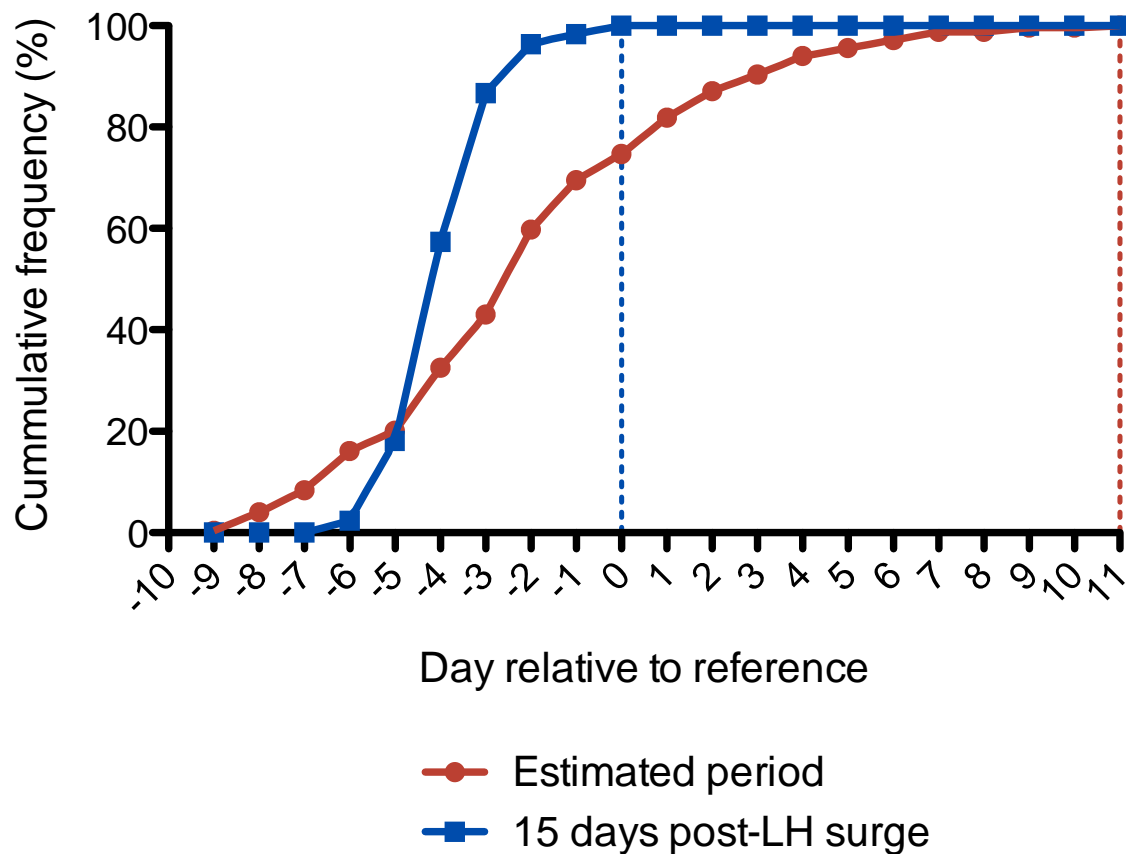
Debate-how low should we go?



# 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?

Day relative to missed period	Achieved pregnancy, %	hCG median, mIU/mL	First Response manual, %	First Response Gold digital, %	EPT manual, %	EPT Certainty digital, %	ClearBlue Easy manual, %	ClearBlue Easy digital, %
Study 5. Pregnant woman, 40 total								
0	100	41	100	98	55	65	58	60
+3	100	154	100	95	80	80	75	75

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

# How early can urine hCG tests detect pregnancy?

Day relative to missed period	Achieved pregnancy, %	hCG median, mIU/mL	First Response manual, %	First Response Gold digital, %	EPT manual, %	EPT Certainty digital, %	ClearBlue Easy manual, %	ClearBlue Easy digital, %
Study 5. Pregnant woman, 40 total								
0	100	41	100	98	55	65	58	60
+3	100	154	100	95	80	80	75	75
Study 6. Pregnant woman, 80 total								
-6	29	2.1	25	25	0	0	0	0
-5	40	2.9	33	25	5.0	5.0	5.0	5.0
-4	76	5.2	58	42	6.3	6.3	8.8	3.8
-3	88	12	74	68	14	18	27	12
-2	92	21	76	81	29	31	29	28
-1	99	40	93	91	42	55	57	51
0	99	70	96	96	53	68	67	51
+1	100	143	100	96	64	71	74	69
+2	100	227	100	99	77	79	81	77
+3	100	302	100	100	80	86	87	84
+4	100	534	100	100	100	100	100	100

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  $\beta$ -hCG concentrations on day 11.

EMP-3

Pregnancy outcome	Miscarriage	Term delivery
Number of pregnancies	33	186
Minimum/maximum $\beta$ -hCG (mIU/mL)	2-42	<u>4-235</u>
Mean $\beta$ -hCG (mIU/mL)	16.5	27.7
Median (mIU/mL)	16.9	25.1
95% confidence interval (mIU/mL)	12.9-20.2	<u>24.7-30.6</u>

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

EMP

Pregnancy outcome	Miscarriage	Term delivery
Number of pregnancies	33	173
Minimum/maximum $\beta$ -hCG (mIU/mL)	11-184	<u>32-365</u>
Mean $\beta$ -hCG (mIU/mL)	73.1	141.7
Median (mIU/mL)	61.2	129.0
95% confidence interval (mIU/mL)	58.0-88.2	<u>131.3-152.1</u>



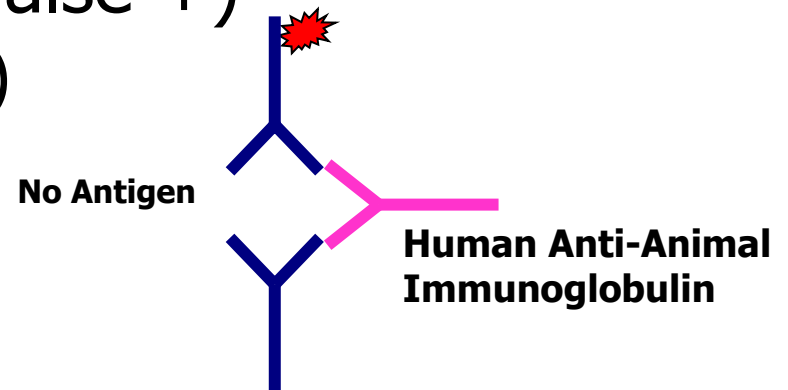
# Clinical Specificity/Limitations

# 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-idiotypic (false + or -)



# The Abbott HCG Story

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

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**

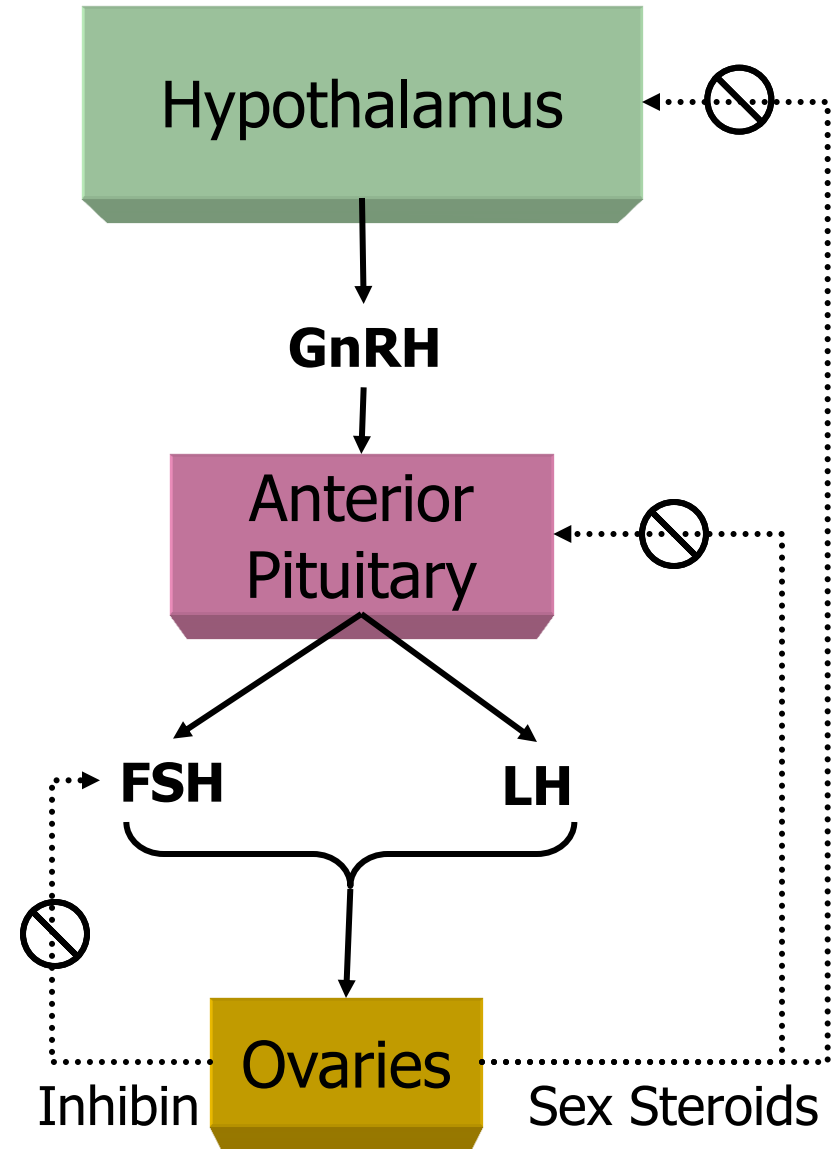
Purified non-specific animal immunoglobulins

- **Perform using different assay**

- **Measure urine hCG**

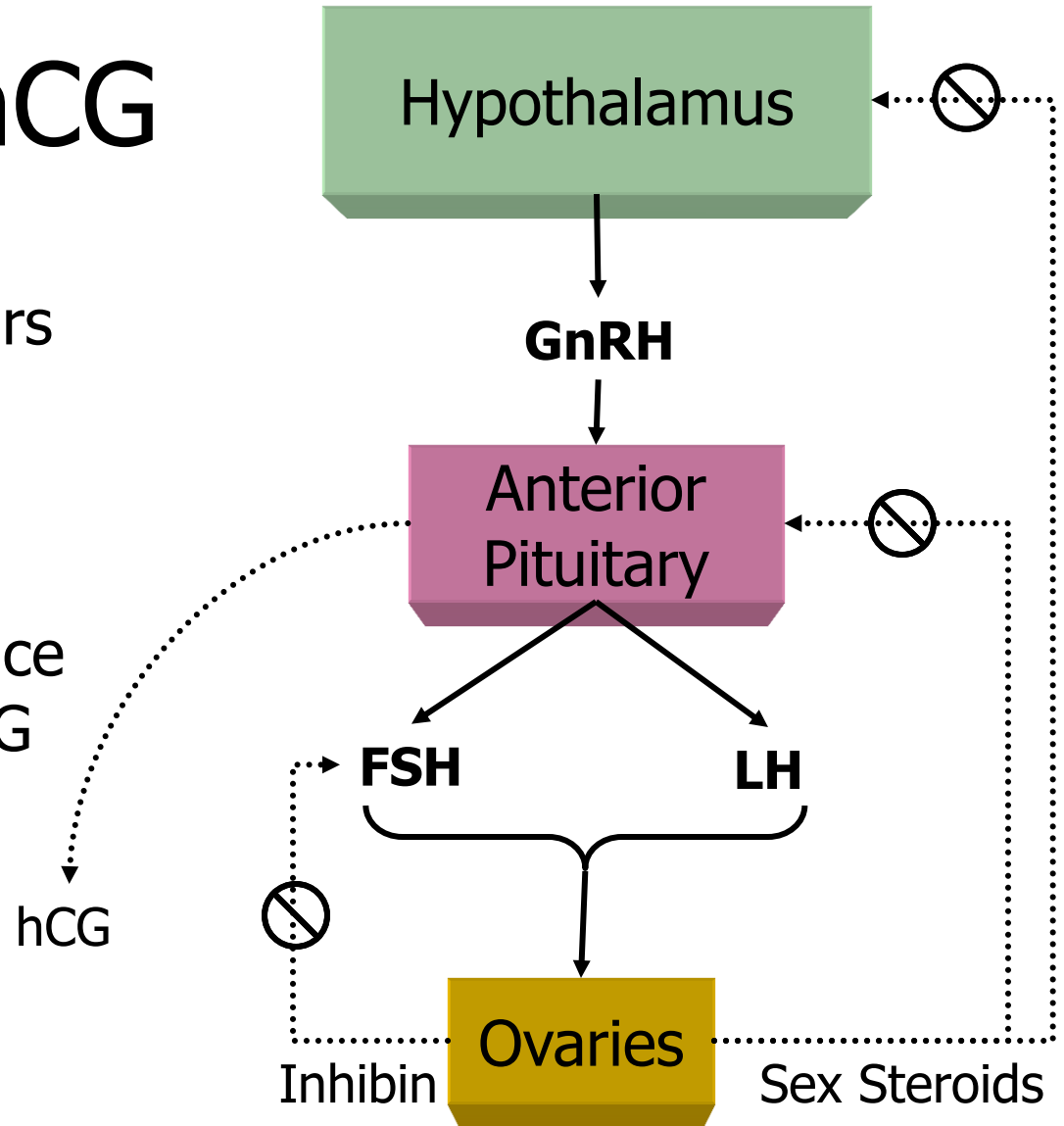
## 2) Pituitary hCG

- First reported 30 years ago
- Gonadotrope cells of pituitary gland produce small amounts of hCG

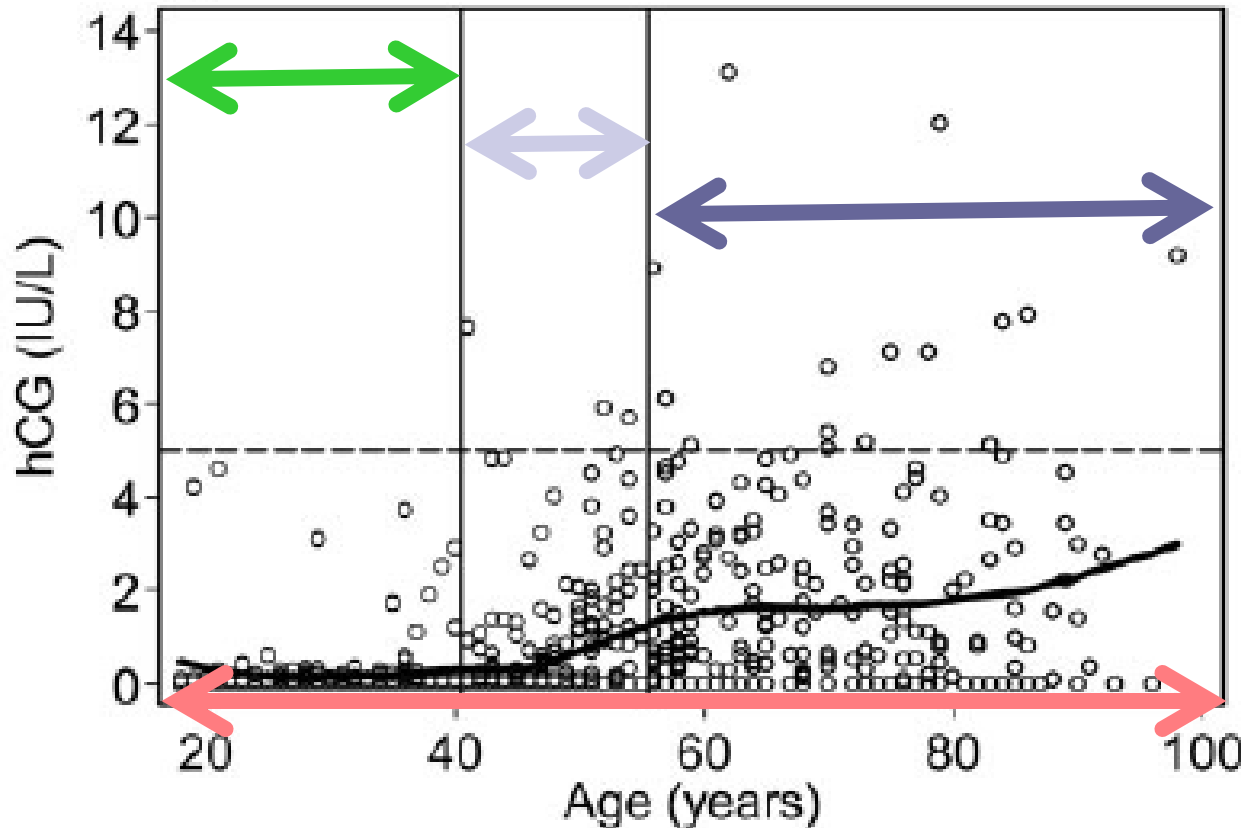


## 2) Pituitary hCG

- First reported 30 years ago
- Gonadotrope cells of pituitary gland produce small amounts of hCG



# hCG is Correlated with Age



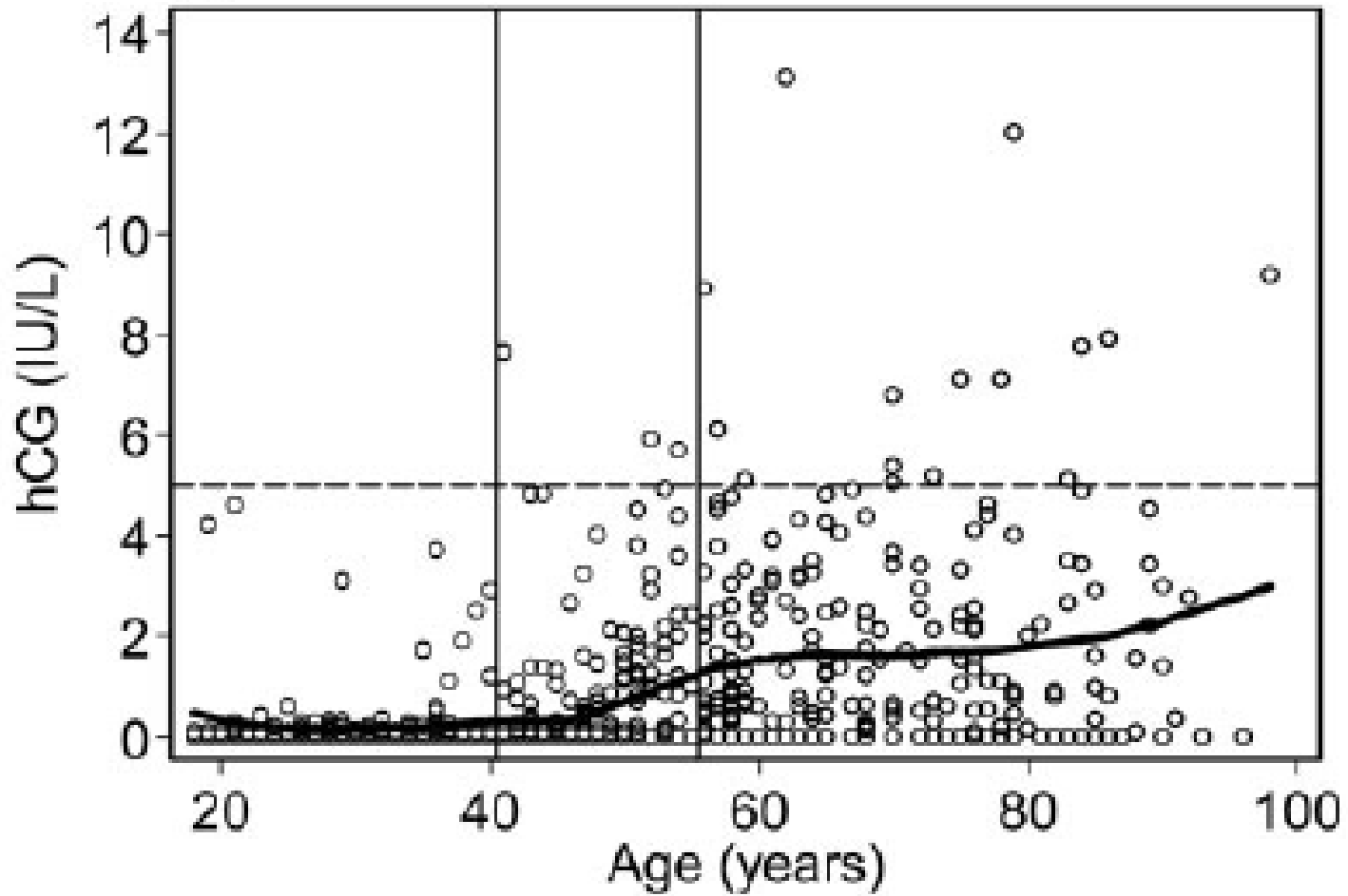
$r = 0.034, p = 0.60$

$r = 0.156, p = 0.02$

$r = 0.038, p = 0.55$

$r = 0.333, p < 0.0001$

# hCG is Correlated with Age

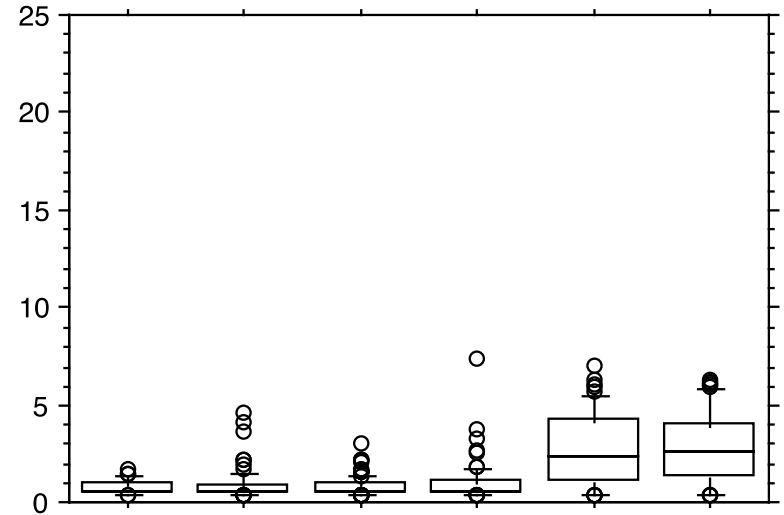
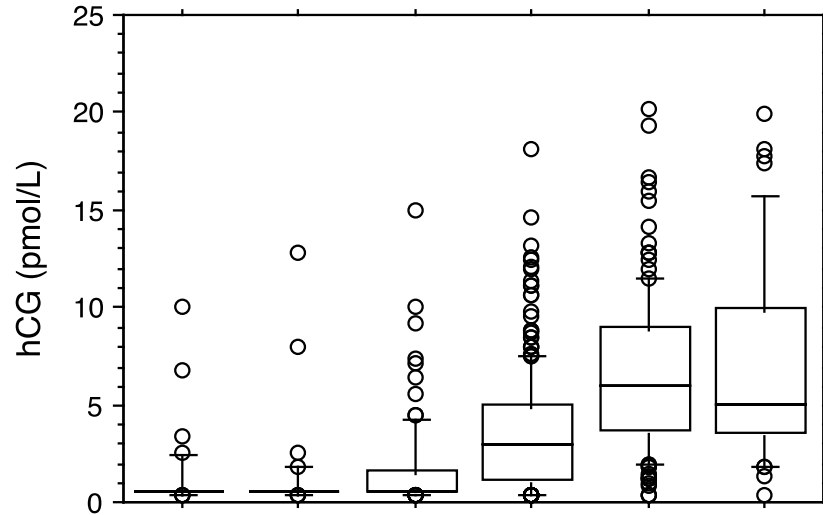


# Serum in Nonpregnant Women and Men

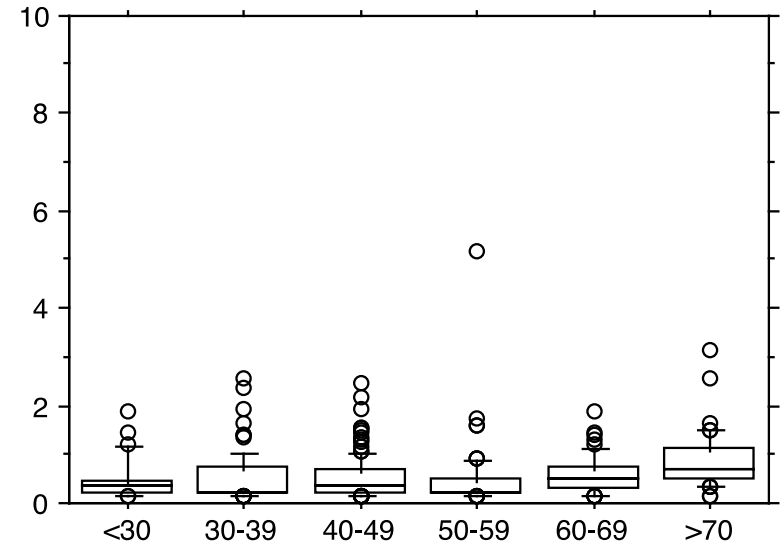
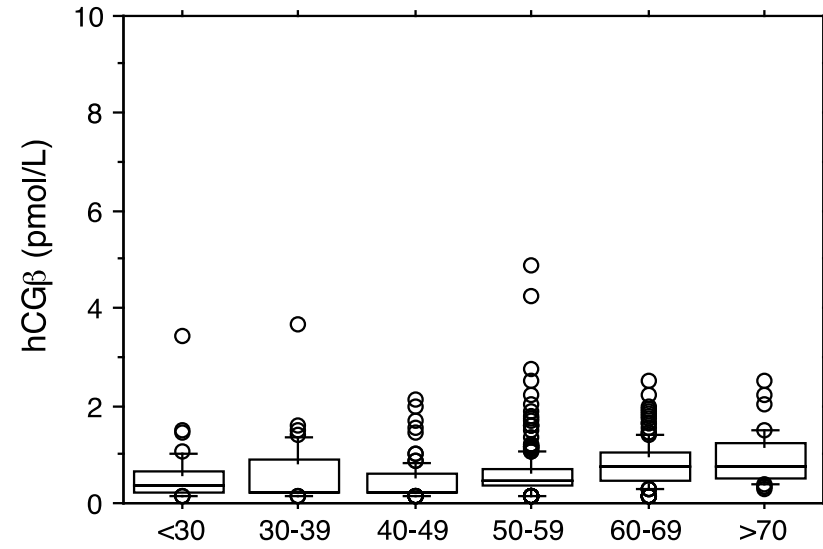
FEMALES

MALES

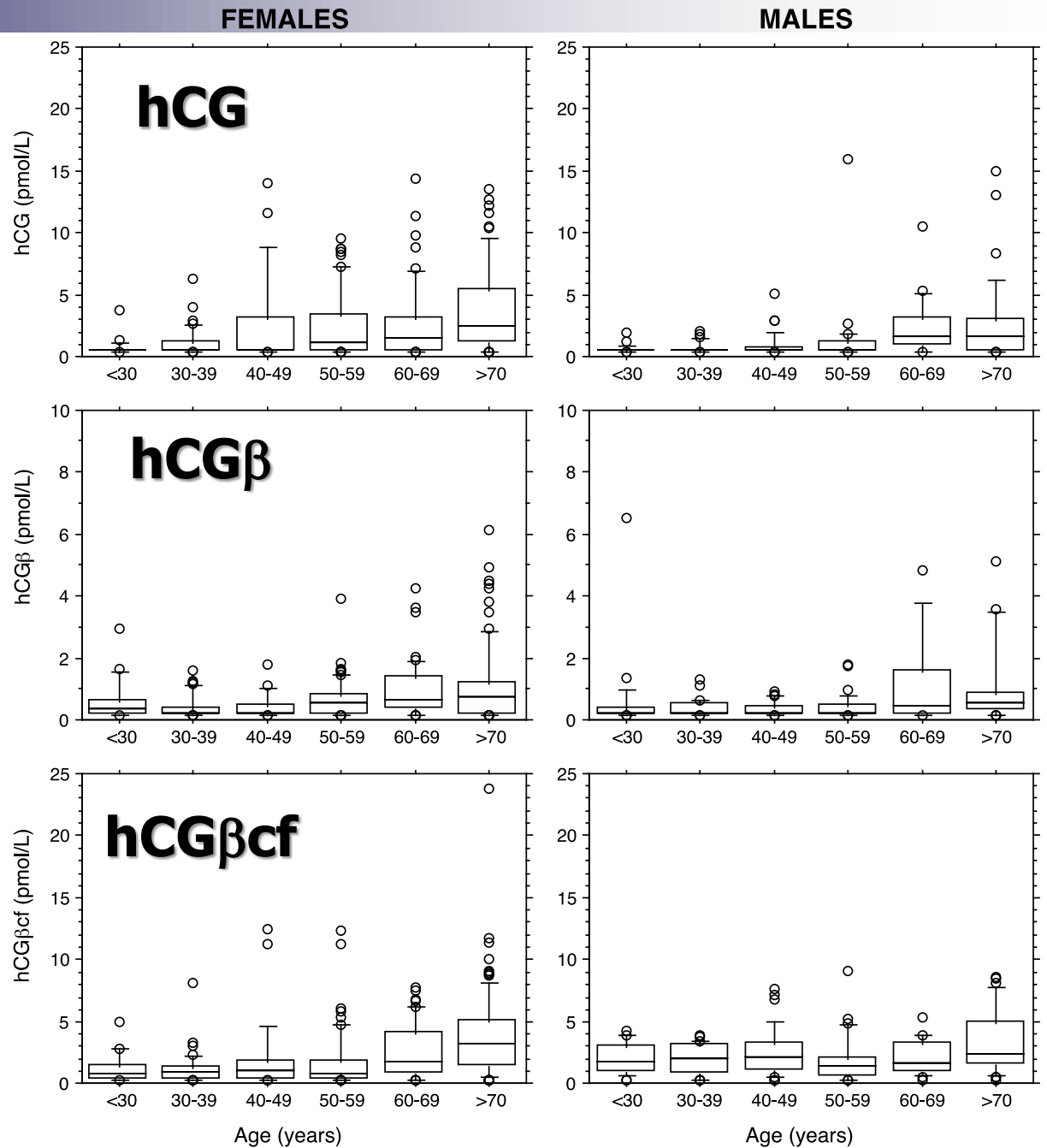
**hCG**



**hCG $\beta$**



# Urine in Nonpregnant Women and Men







The NEW ENGLAND  
JOURNAL of MEDICINE

**Normal Production of Human Chorionic Gonadotropin  
in Menopause**

Cole, L. A., Sasaki, Y., Muller, C. Y.  
*NEJM*. 2007; 356:1184-1186.

Hormone Replacement Therapy for 2 weeks

# hCG Reference Intervals by Age

hCG >5.0 IU/L  
Peri-menopausal, N=3  
Post-menopausal, N=16

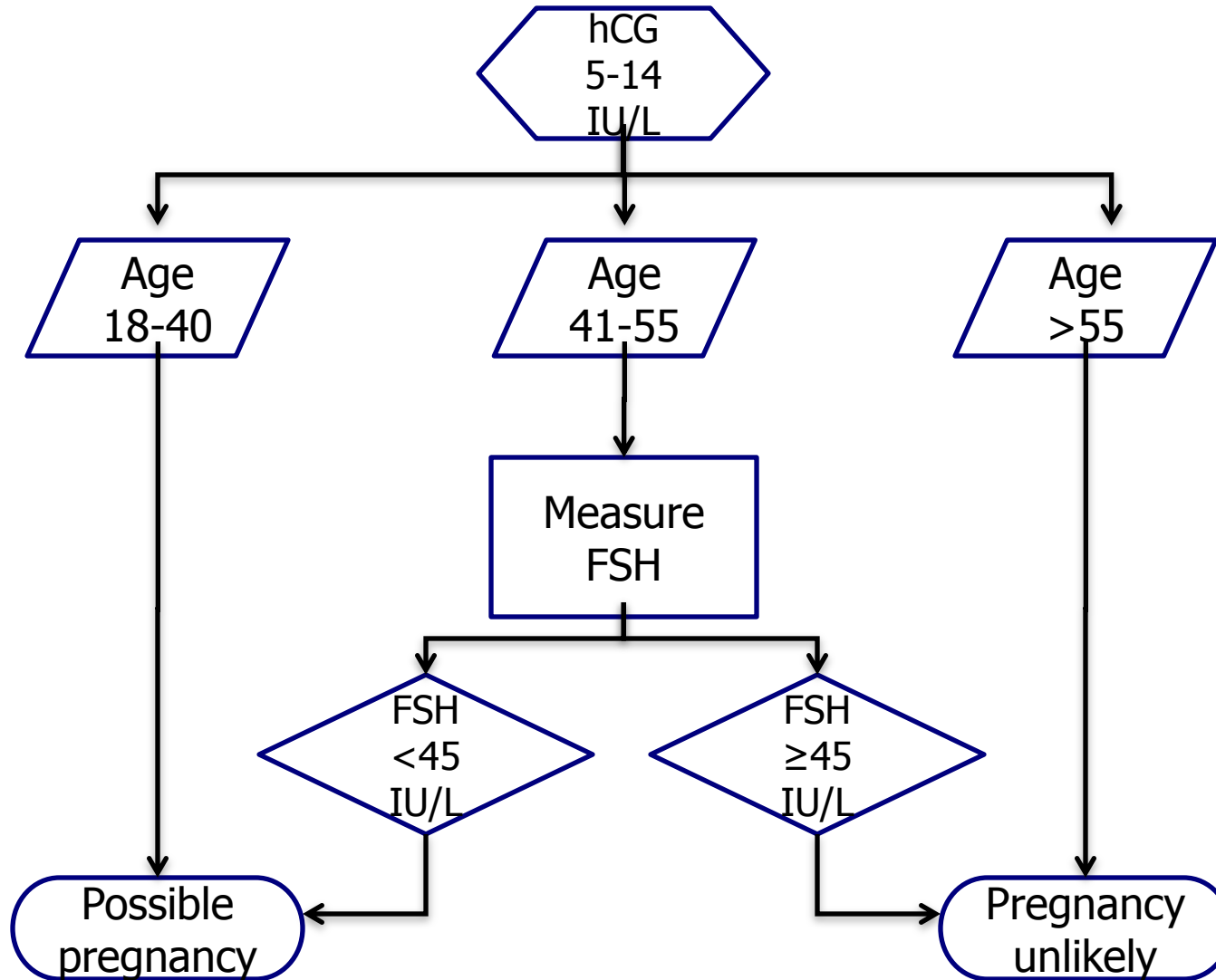
**Table 1. hCG concentration ranges and the 97.5 percentile values for the nonpregnant cohorts in the study.**

Nonpregnant cohort	n	hCG range, IU/L	97.5 percentile, IU/L	P
Premenopausal, 18–40 years	240	<2.0 to 4.6 <b>5</b>	2.5	
Perimenopausal, 41–55 years	240	<2.0 to 7.7 <b>8</b>	4.8	0.07 <sup>a</sup>
Postmenopausal, >55 years	240	<2.0 to 13.1 <b>14</b>	7.7	<0.0001 <sup>b</sup>

<sup>a</sup> Compared with the nonpregnant premenopausal cohort.

<sup>b</sup> Compared with the nonpregnant premenopausal and nonpregnant perimenopausal cohorts.

# Pituitary hCG vs pregnancy



# 3) Exogenous hCG

May 8, 2009 02:00 PM in [Basic Science](#) | [8 comments](#)

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

By [John Matson](#)



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*.

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## 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

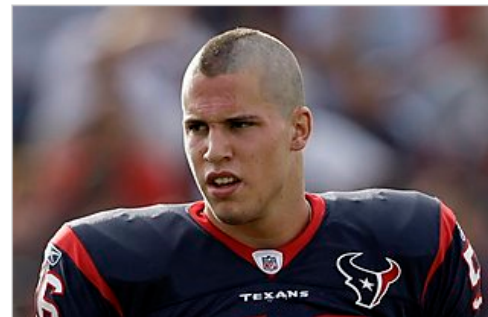
NEW YORK May 11, 2010 (AP)

The Associated Press

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



FILE - In this Aug. 3, 2009, file photo Houston Texans linebacker Brian Cushing look son during an... (AP)

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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# HCG weight-loss products are fraudulent, FDA says

Updated 1/23/2011 6:58 PM | Comments  92 | Recommend  31 | E-mail | Save | Print | Reprints & Permissions |  RSS



 Enlarge [www.simplyhcg.com](http://www.simplyhcg.com)

Big sales online: Simplyhcg.com is one of many Web-based companies selling HCG diet products.






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.

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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](http://quackwatch.org), says, "The bottom line is there is no reason to think the product works."   
 ta from l.collective-media.net...

# 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 hCG $\beta$
- 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



# Acknowledgements

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