

The Analytical & Clinical Complexities of Human Chorionic Gonadotropin Tests



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Disclosures

David G. Grenache has no relevant financial interests to disclose

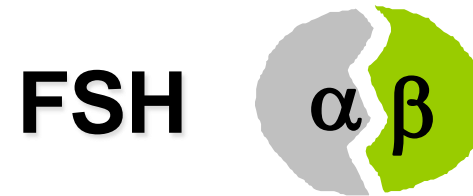
hCG tests are FDA cleared only for the detection of pregnancy

Objectives

- *Describe* the synthesis, function, and variants of hCG
- *Discuss* the clinical utility of hCG testing in the diagnosis and management of uterine and ectopic pregnancy and malignancy
- *Describe* the causes of persistently low hCG results and *explain* investigations that can be used to identify them
- *Describe* analytical issues associated with hCG assays and *identify* troubleshooting techniques

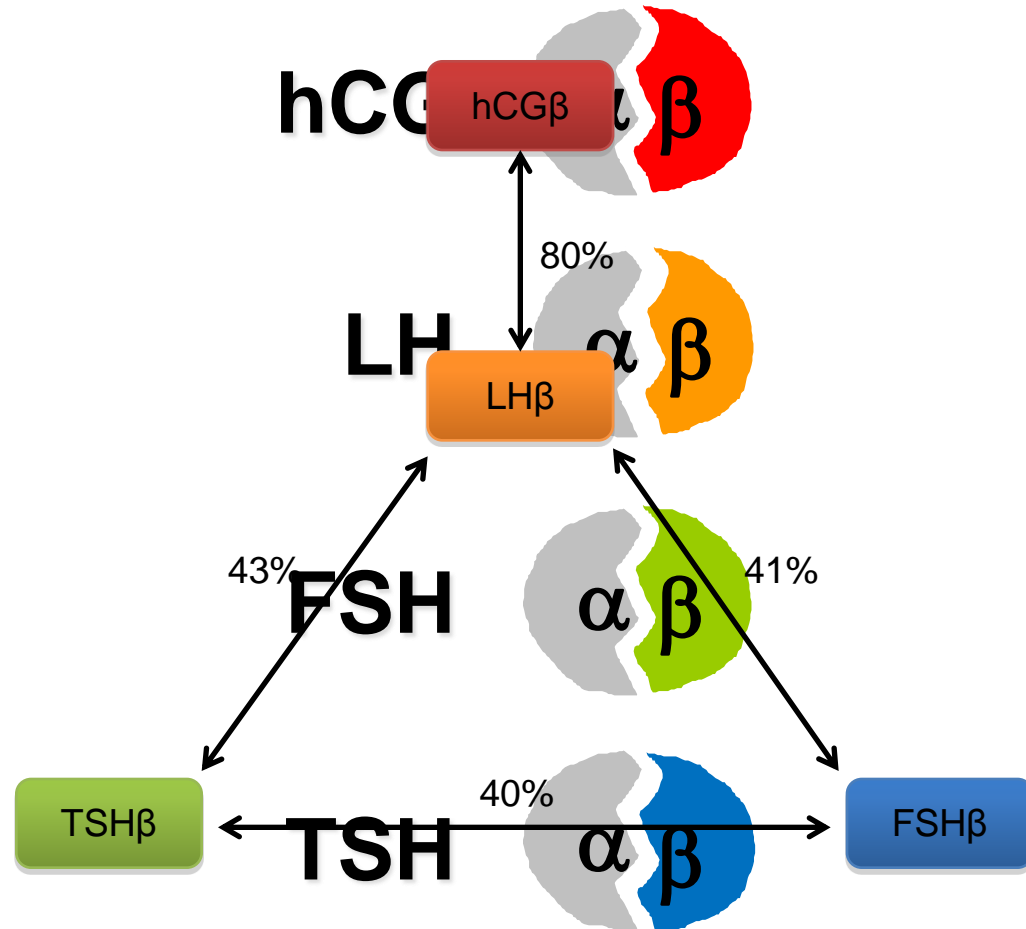
Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family



Human Chorionic Gonadotropin (hCG)

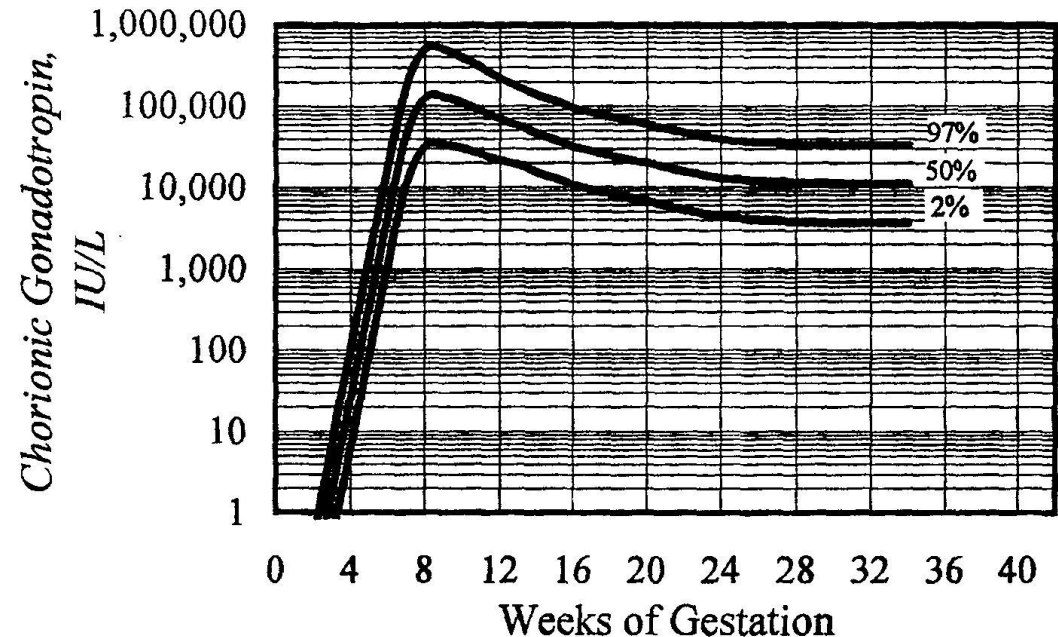
- Glycoprotein hormone family



Adapted from Vassart G, et al. *Trends Biochem Sci* 2004; 29:119-126

hCG Synthesis

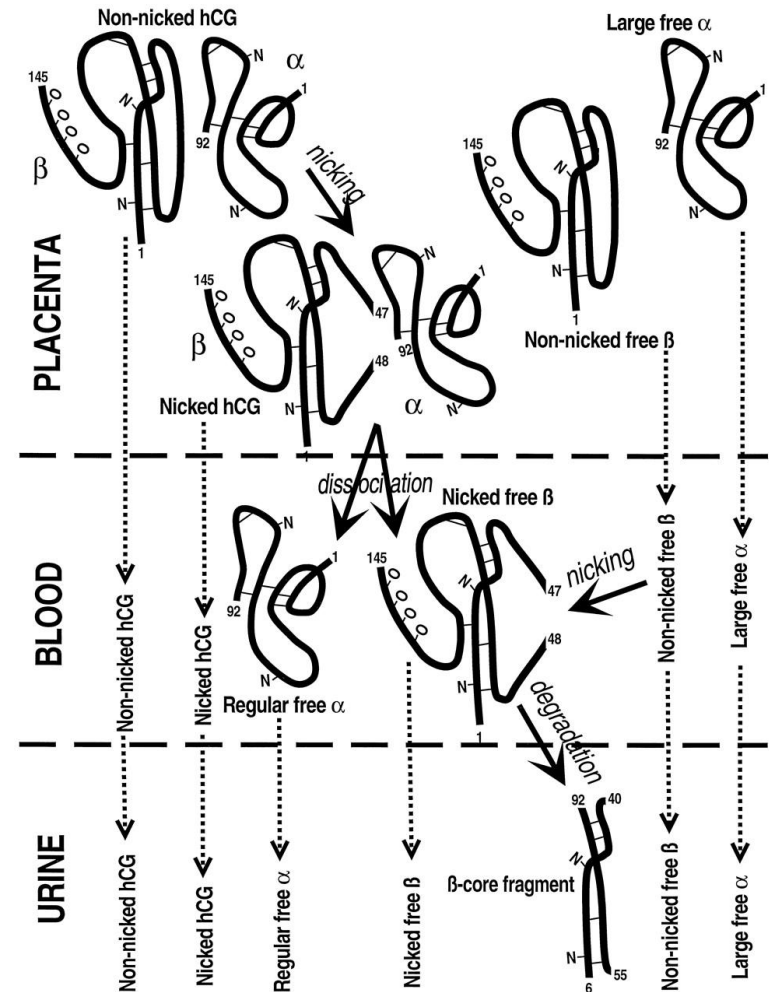
- Synthesized by syncytiotrophoblasts
- Serum concentrations increase progressively in early pregnancy
 - Peak at 7 – 9 weeks of gestation
- Decrease until ~24 weeks then plateau



Tietz Textbook of Clinical Chemistry, 4th, 2006

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)



Cole L. *Clin Chem* 1997;43:2233-2243

Intact hCG

- Non-covalently associated α and β subunits
- Biologically active
 - Extends functional life of corpus luteum
 - Maintains high progesterone concentrations in early pregnancy
 - Thyrotropic at very high concentrations

Nicked hCG (hCGn)

- Single cleavage in β subunit by leukocyte elastase
- Leads to disassociation of dimer and loss of biological activity
- Peak concentrations occur in conjunction with peak intact hCG
 - ~9% of total hCG in pregnancy

Free β Subunit

- Present in non-nicked (hCG β) and nicked forms (hCG β n)
- Peak concentration at ~8 weeks (1% of total)
- High concentrations in trophoblastic disease, germ cell tumors, and other malignancies
 - Intact hCG may not be detectable

Beta Core Fragment (hCG β cf)

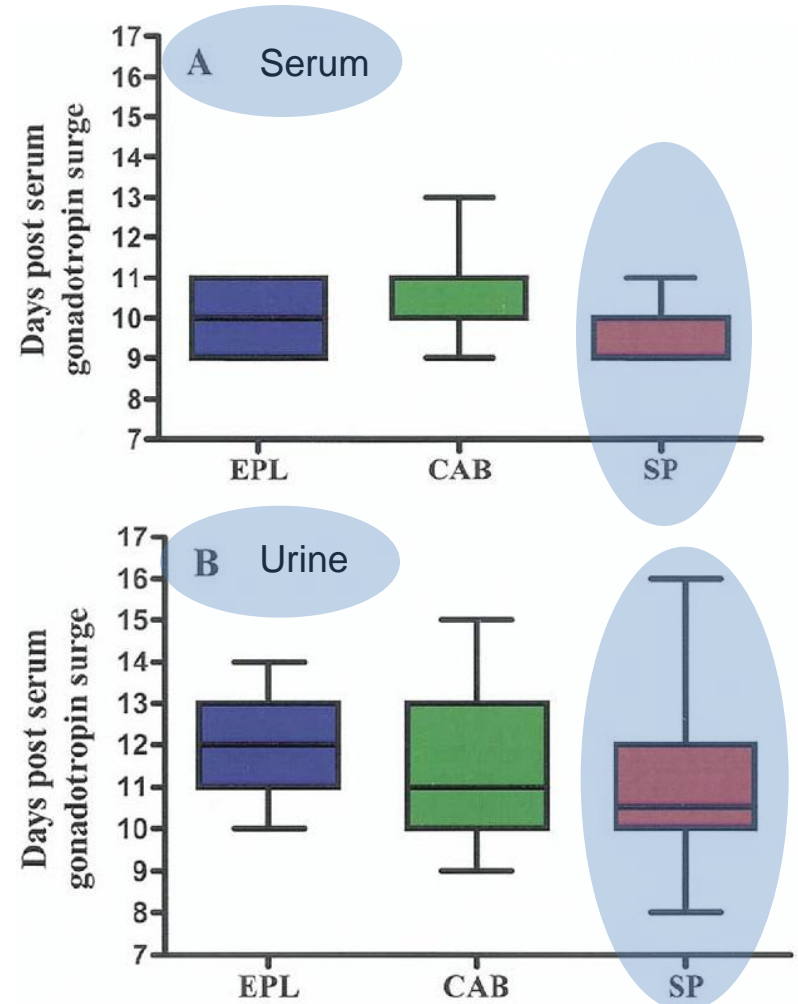
- Terminal degradation product of beta subunit
- Predominant hCG molecule in pregnancy urine
- Not detectable in serum

Clinical Uses of hCG Assays

- Diagnose pregnancy
 - Only use for which hCG tests are FDA cleared
- Diagnose ectopic pregnancy
- Down syndrome screening
- Tumor marker
 - hCG β may be most abundant or the only variant produced

Diagnose Pregnancy

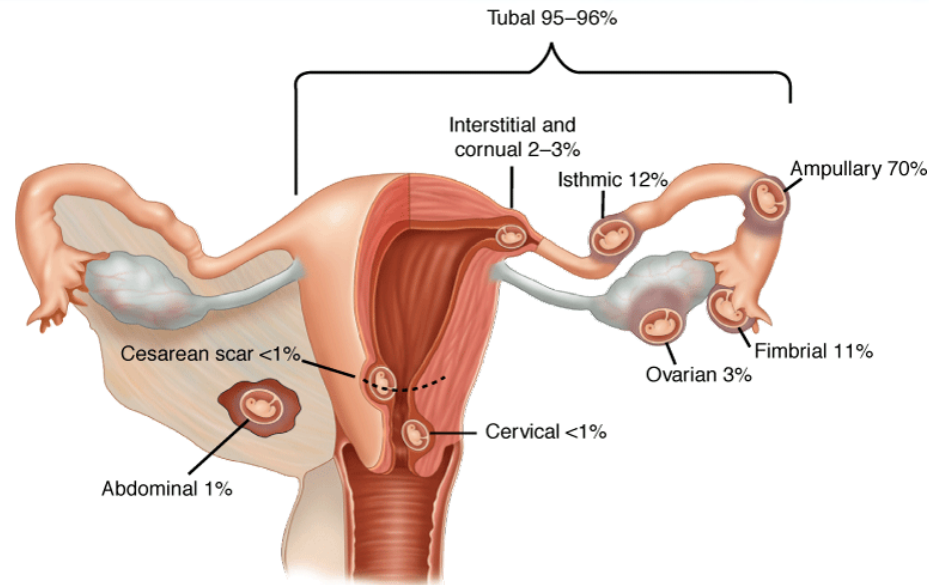
- Pregnancy diagnosis involves history & physical exam in conjunction with hCG testing
- Serum hCG detectable 9-11 days after conception (1-3 days before expected menses)
- Urine hCG usually detectable 1-2 days later (highly variable)



Lohstroh P, et al. *Fertil Steril* 2005;83:1000-1111

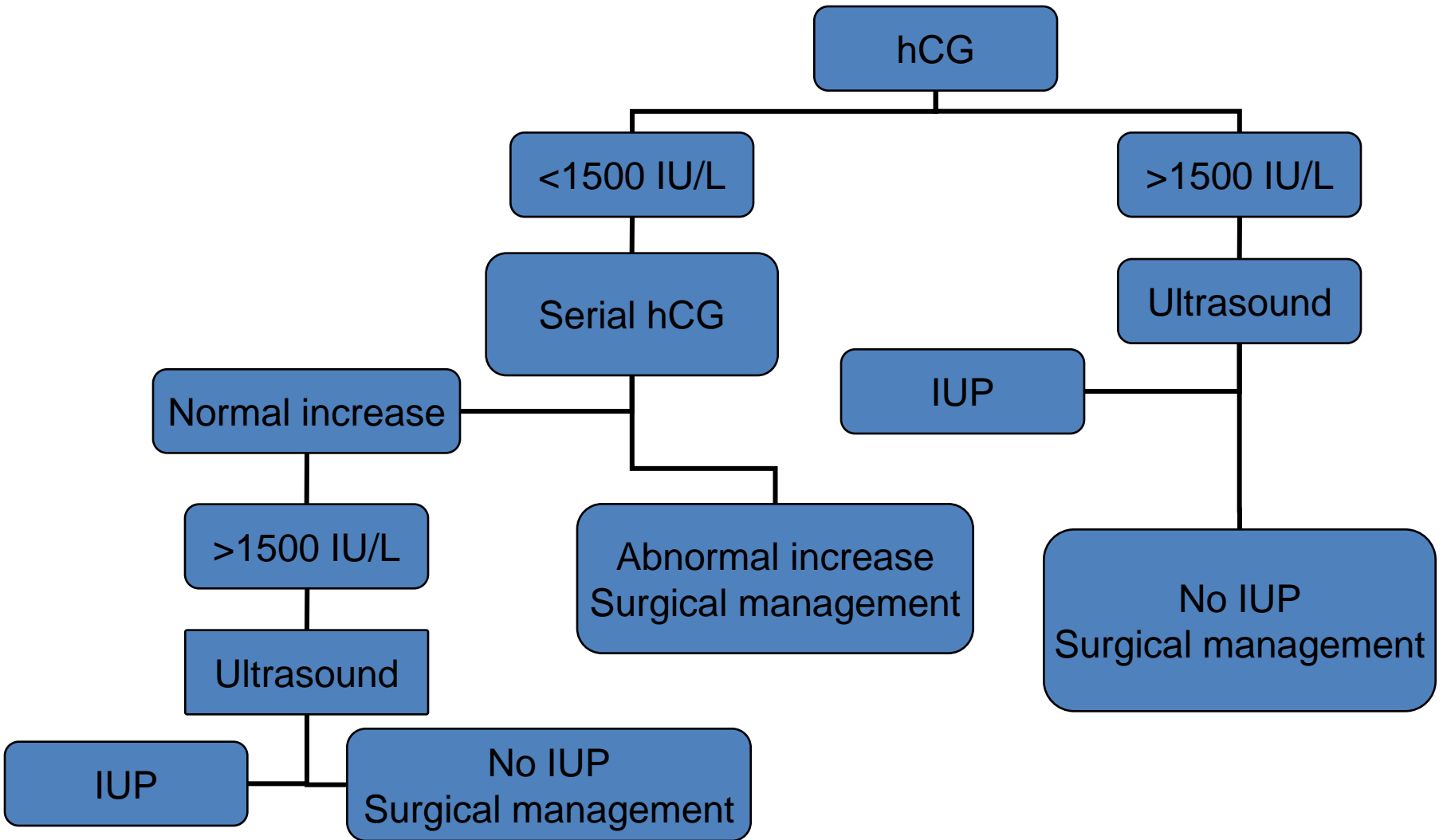
Ectopic Pregnancy

- Extrauterine implantation of blastocyst
 - 98% occur in fallopian tube
- Incidence is estimated at 2% of all pregnancies and is responsible for 5% of maternal deaths
- Classic symptoms include abdominal pain (95%) and vaginal bleeding (70%) but some have no symptoms until rupture
- Diagnostic tools
 - Serial hCG (prolonged doubling time, 87% sensitive)
 - Transvaginal ultrasound (90% sensitive)



Source: Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY: *Williams Obstetrics, 23rd Edition*: <http://www.accessmedicine.com>
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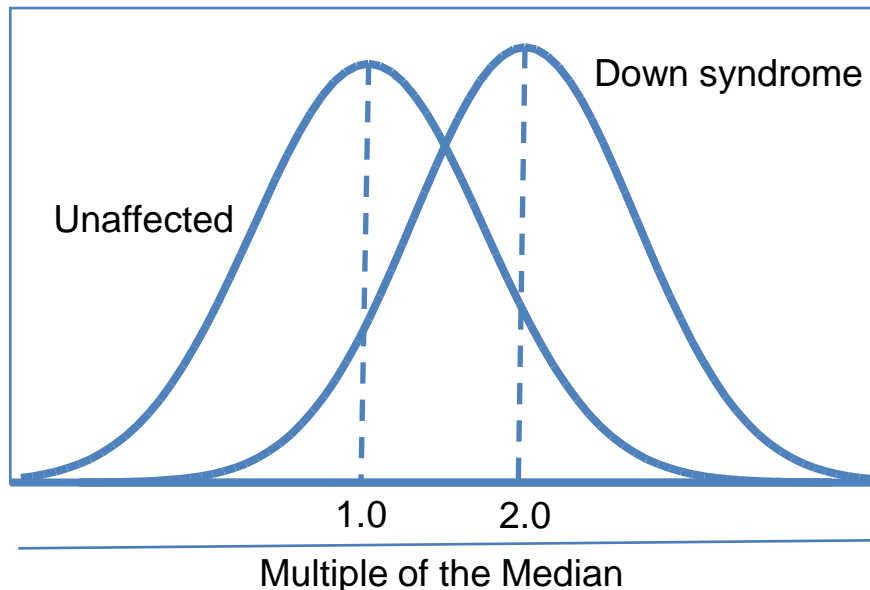
Diagnose Ectopic Pregnancy



Adapted from Barnhart K, et al. *Obstet Gynecol* 1994;84:1010-1015

Down Syndrome Screening

- 2nd trimester screening (16-18 weeks)
 - Triple screen: hCG, AFP, uE3
 - Quadruple screen: hCG, AFP, uE3, Inhibin A
- 1st trimester screening (11-13 weeks)
 - hCG or hCG β , PAPP-A, nuchal translucency (ultrasound)



hCG as Tumor Marker

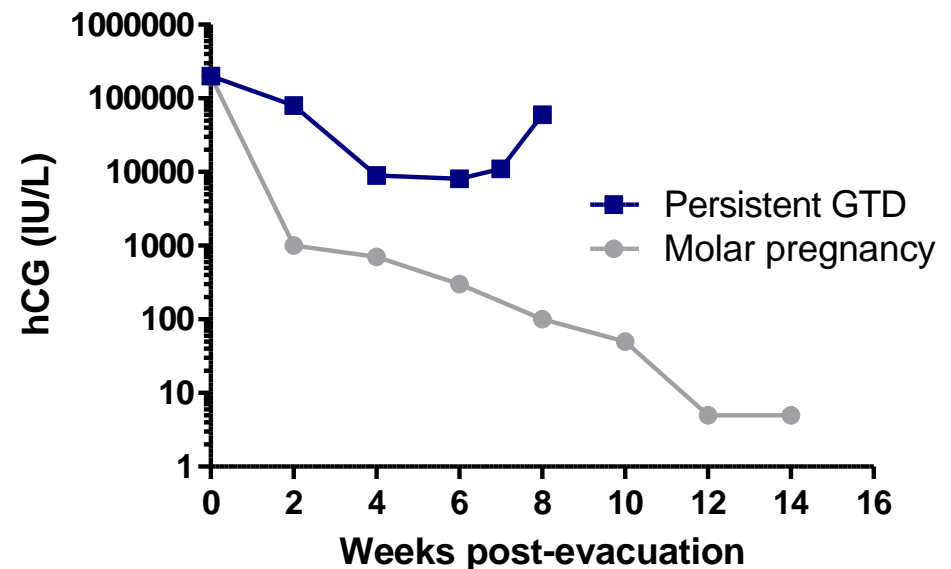
- hCG produced by all gestational trophoblastic diseases and often by germ cell tumors
- Case reports of hCG produced by
 - Melanomas
 - Breast cancers
 - GI carcinomas
 - Lung cancers
 - Ovarian cancers
 - Others

Gestational Trophoblastic Disease (GTD)

- Heterogeneous group of interrelated lesions derived from an aberrant fertilization event
 - Hydatidiform mole (partial and complete)
 - Choriocarcinoma
 - Placental site trophoblastic tumor
- All produce hCG and hormone is used in diagnosis and to monitor response to therapy
- Very responsive to chemotherapy and treatment is most often single-agent therapy with methotrexate

hCG in GTD

- Concentrations may exceed 100,000 IU/L
- Assess tumor mass
 - [hCG] correlates with the number of viable tumor cells
- Monitor therapy
 - Successful treatment leads to progressive decline of hCG
 - A rise or plateau suggests recurrence or persistence of disease
- Detect recurrence
 - ACOG recommends f/u hCG for 6 months

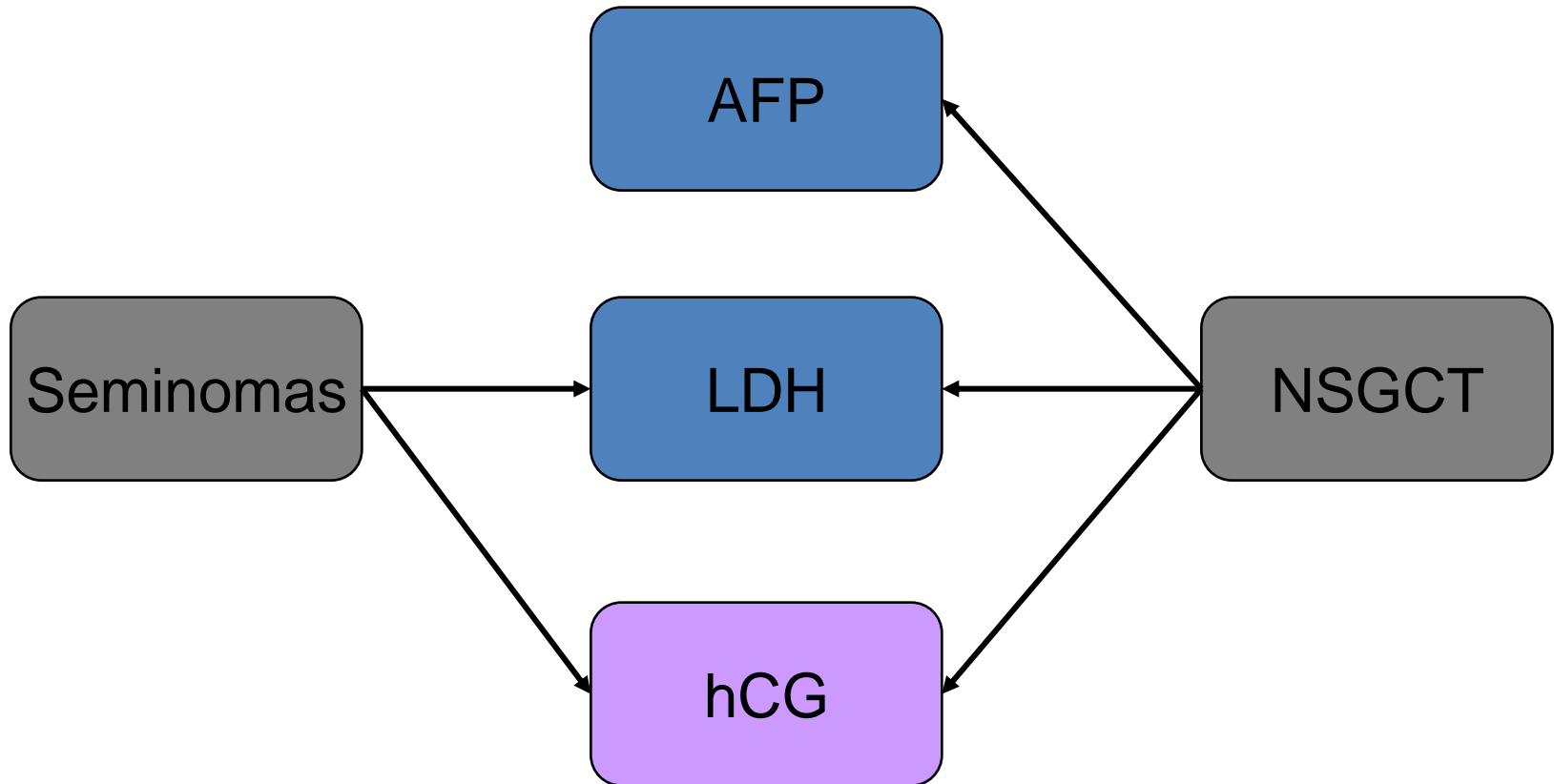


Adapted from Pastorfide, et al. *Am J Obstet Gynecol* 1974;118:293 and 1974;120:1025

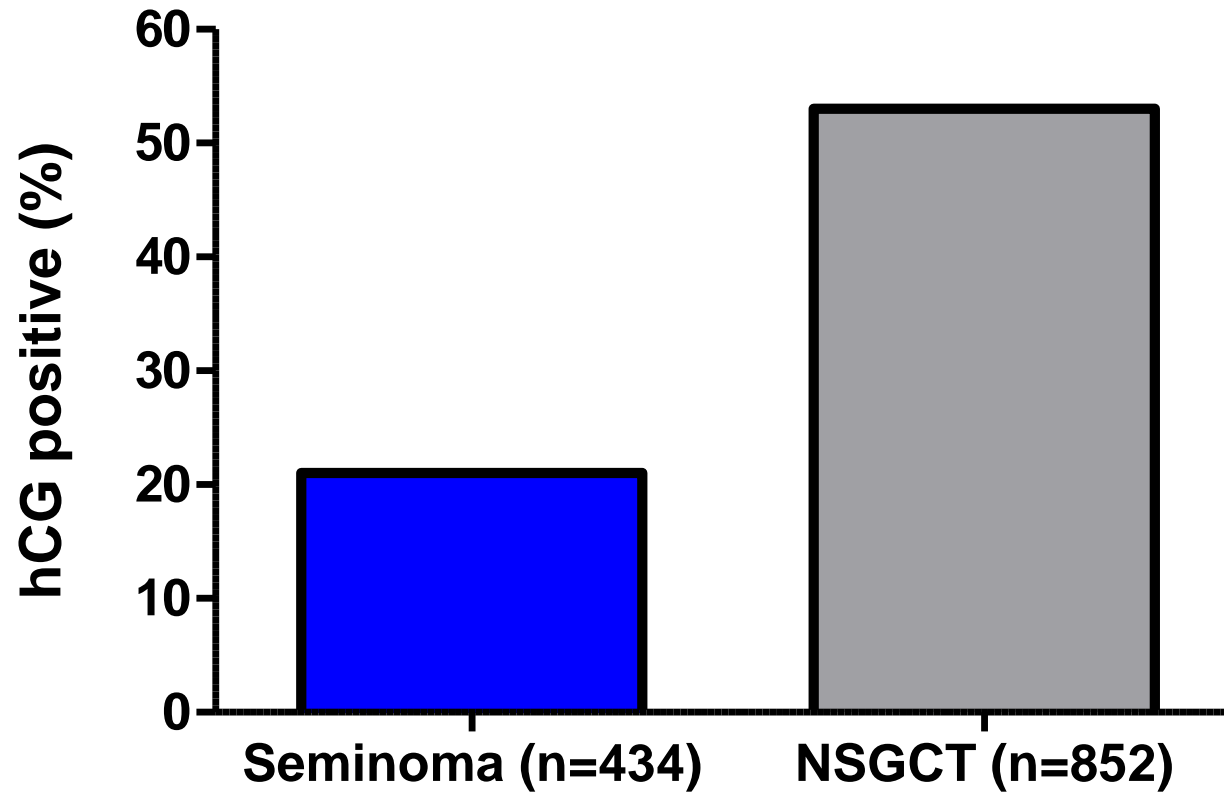
Germ Cell Tumors

- Neoplasms derived from germ cells
 - 90% of testicular tumors (99% are malignant)
 - 20% of ovarian tumors (>90% are benign)
 - Extragonadal if no evidence of primary tumor in gonads
- Testicular cancer
 - Seminomas
 - Nonseminomatous germ cell tumors

Testicular Cancer Tumor Markers

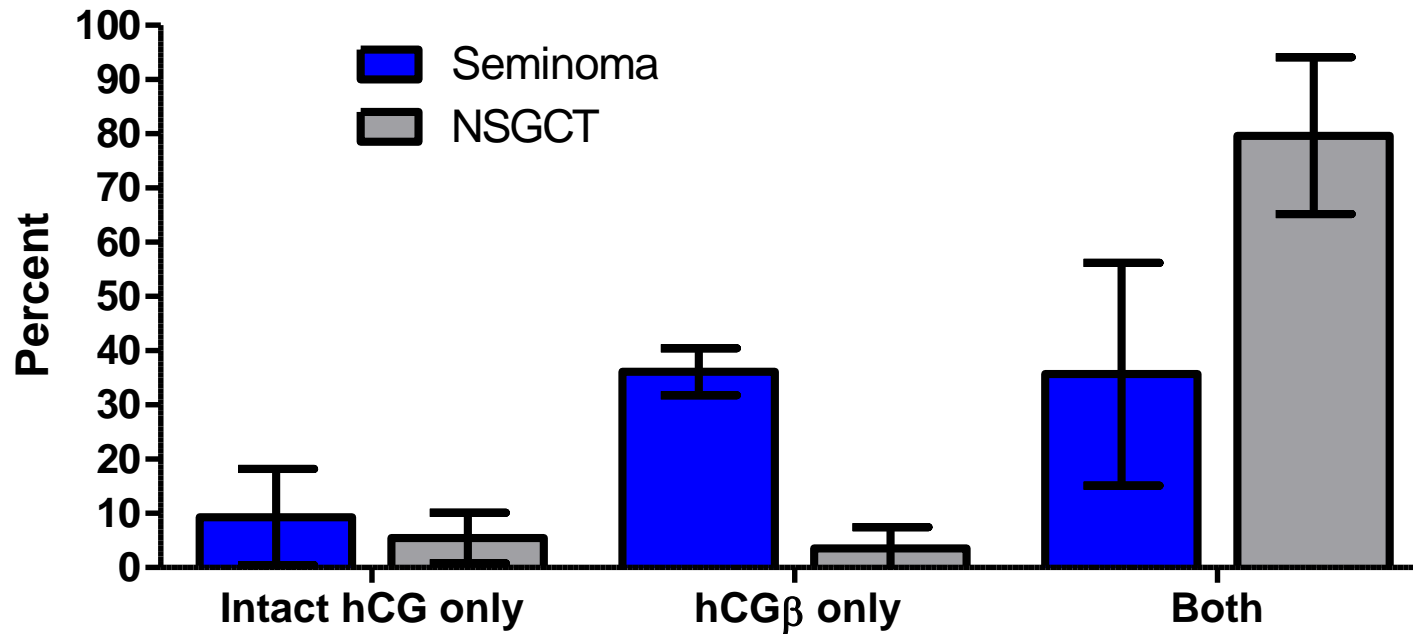


hCG in Testicular Cancer



Adapted from Germa-Lluch JR, et al. *Eur Urol* 2002;42:553-563

hCG β in Testicular Cancer



Adapted from:

Saller B, et al. *Clin Chem* 1990;36:234-239

Hoshi S, et al. *Int J Urol* 2000;7:218-223

Lempiainen A, et al. *Clin Chem* 2008;54:1840-1843

Persistently Low hCG

- Low concentrations of hCG that persist for months to years
 - hCG often <50 IU/L
- Uncommon event attributed to
 - Interfering antibodies
 - Pituitary hCG

Persistent Low hCG – The Clinical Problem

- hCG tests performed on women prior to interventions that could harm fetus
 - hCG cutoff of <5.0 IU/L used for pregnancy diagnosis
- Standardized protocols result in the use of hCG testing even in women who are unlikely to be pregnant (e.g. menopausal, hysterectomy)
- Positive results create clinical confusion, may delay needed therapies, or result in unnecessary therapy

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

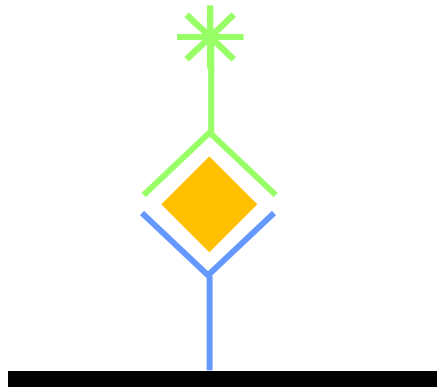
Seattle Post-Intelligencer

Saturday, June 30, 2001

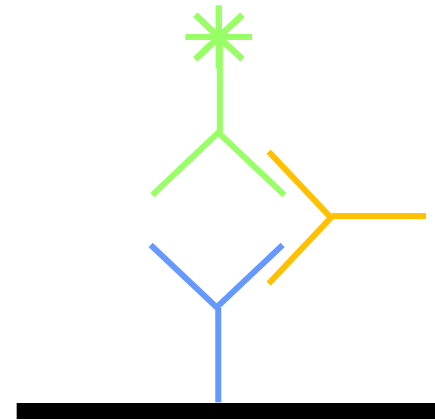
Case Report

- Patient consulted doctor about irregular bleeding between menstrual periods
 - Positive serum hCG but no apparent intra-uterine pregnancy
- 11 month ordeal to identify cause
- Treatments
 - Laparoscopy for presumed ectopic pregnancy (none)
 - D&C (normal)
 - Chemotherapy, single and multi-agent (no hCG change)
 - Hysterectomy (no hCG change)
 - Surgery for removal of lung nodules found by CT (no disease)
- hCG eventually found to be false-positive due to interfering antibody in patient's serum
- <http://www.legalwa.org/> (docket number 99-2-27090-8)

Mechanism of Interference - Positive



Real hCG present



Interfering antibody cross-links reagent antibodies

Falsely increased result

Detection

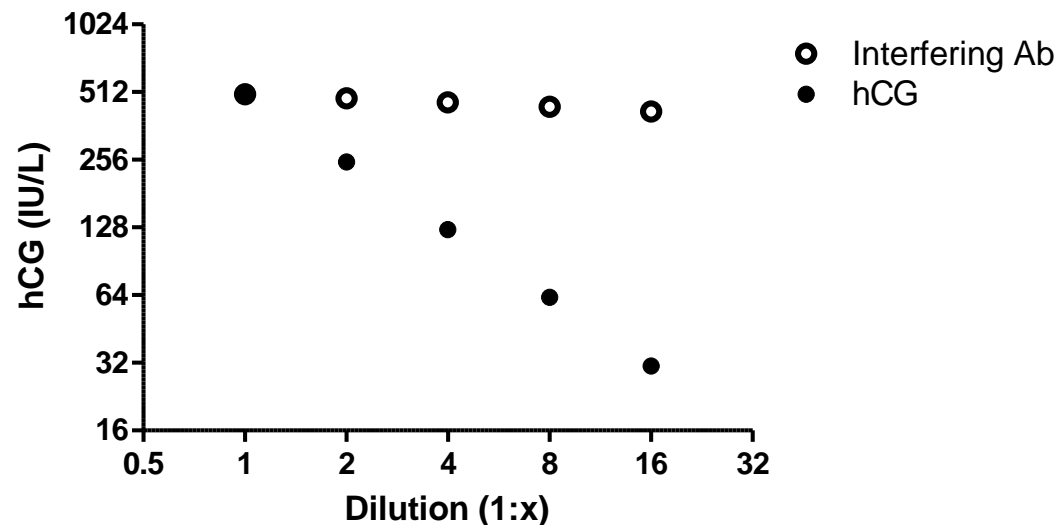
- Suspicion of interfering antibodies should be high when immunoassay results are inconsistent with the clinical scenario
- Lab personnel are frequently unaware of clinical condition of patient
 - Difficult for lab to identify independently
- What to do when asked “could this result be falsely increased?”

Urine hCG

- Real hCG should be detectable in the urine
- Antibodies too large to be filtered by kidney
- Negative urine hCG suggests interfering antibody present

Serial Dilution

- Interfering antibodies are reactive against the assay reagents and not the measured analyte
- Serial dilution of specimen may not produce the expected linear response



Different Assay

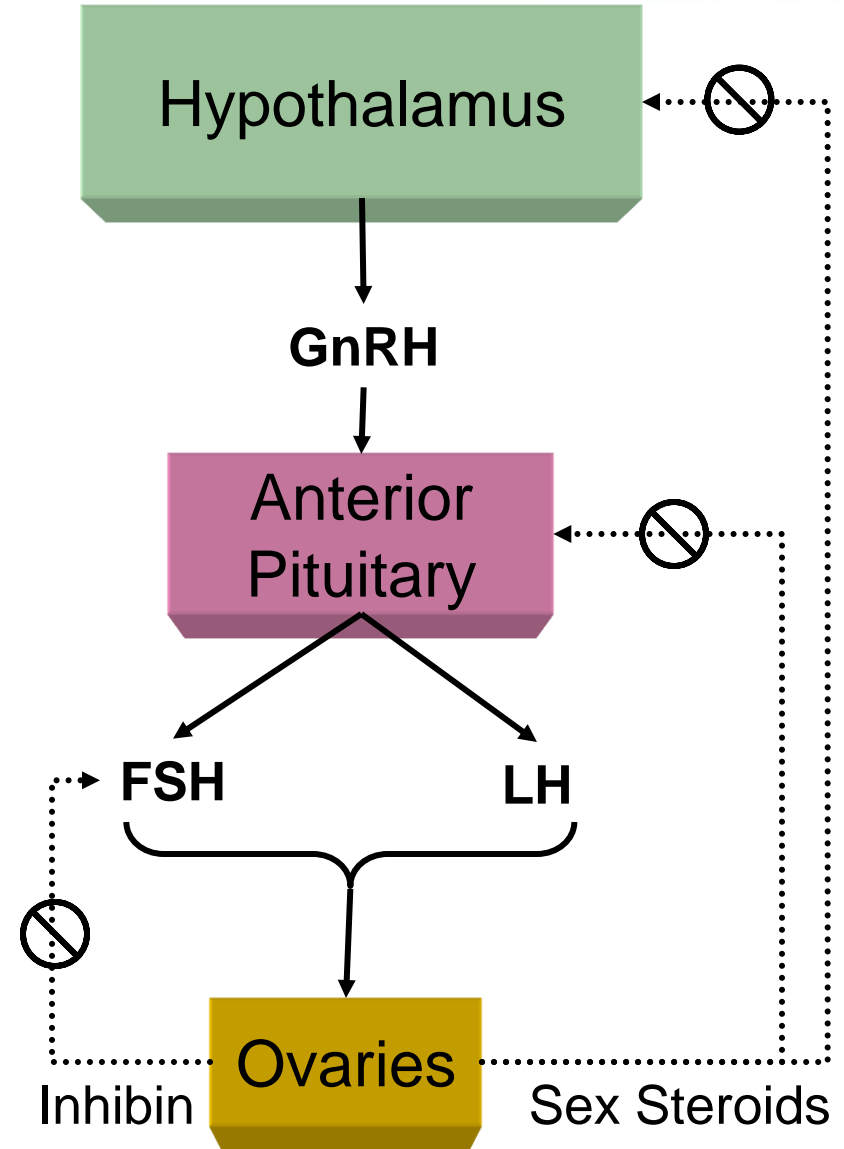
- Repeat test using an immunoassay that utilizes antibodies produced from a different animal species
- Not fool-proof as some interfering antibodies are not species-specific
 - May cross react with multiple animal species and interfere with multiple assays

Blocking Agents

- Remove interfering antibody through use of material that binds to it
 - Substantial change in concentration after use suggests interfering antibody present
- Non-immune globulin from the same species of animal used to produce the assay antibodies
- Effectiveness depends on interfering antibody class, specificity, and concentration
- Immunoassay reagents often contain blocking agents

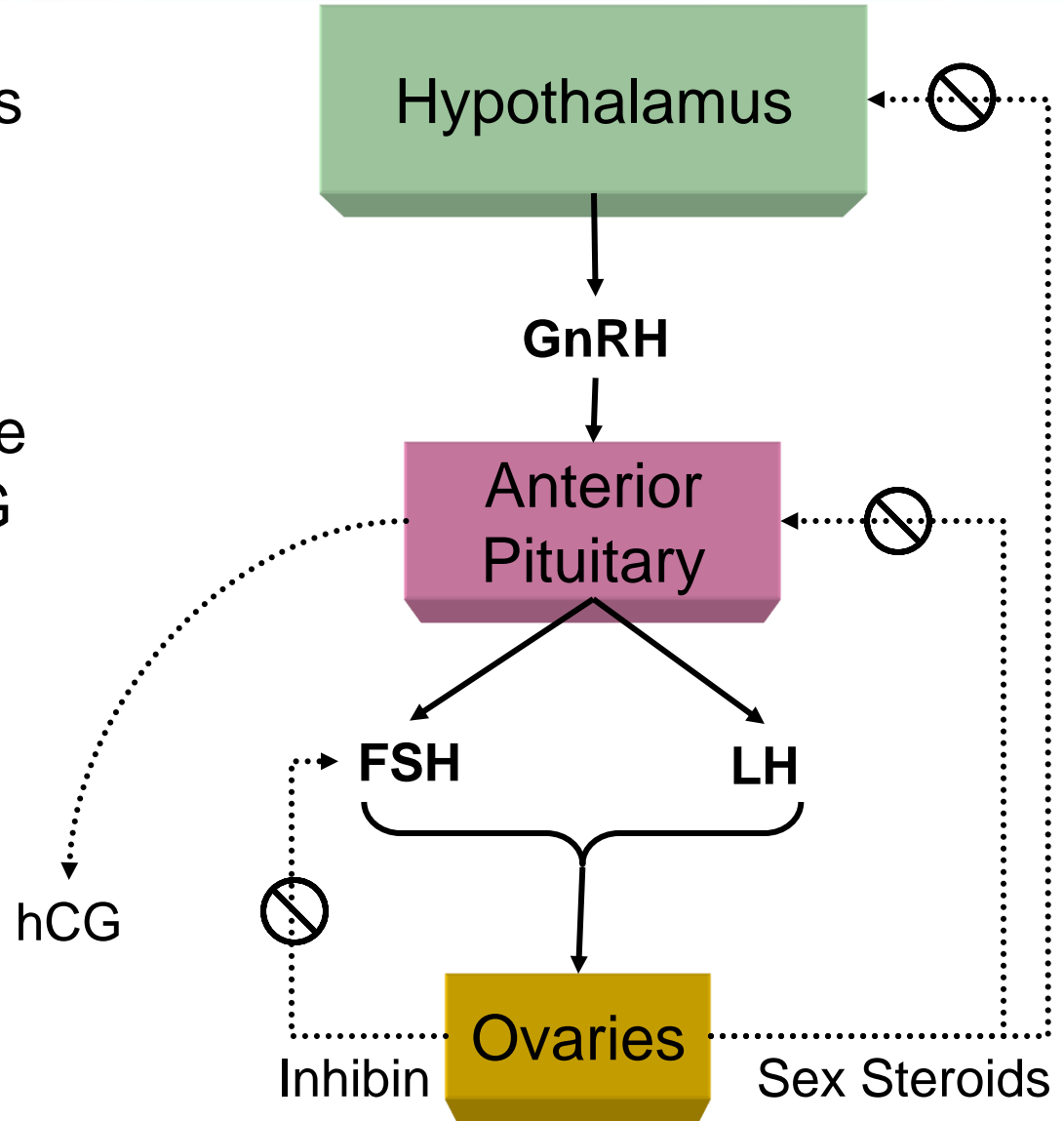
Pituitary hCG

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



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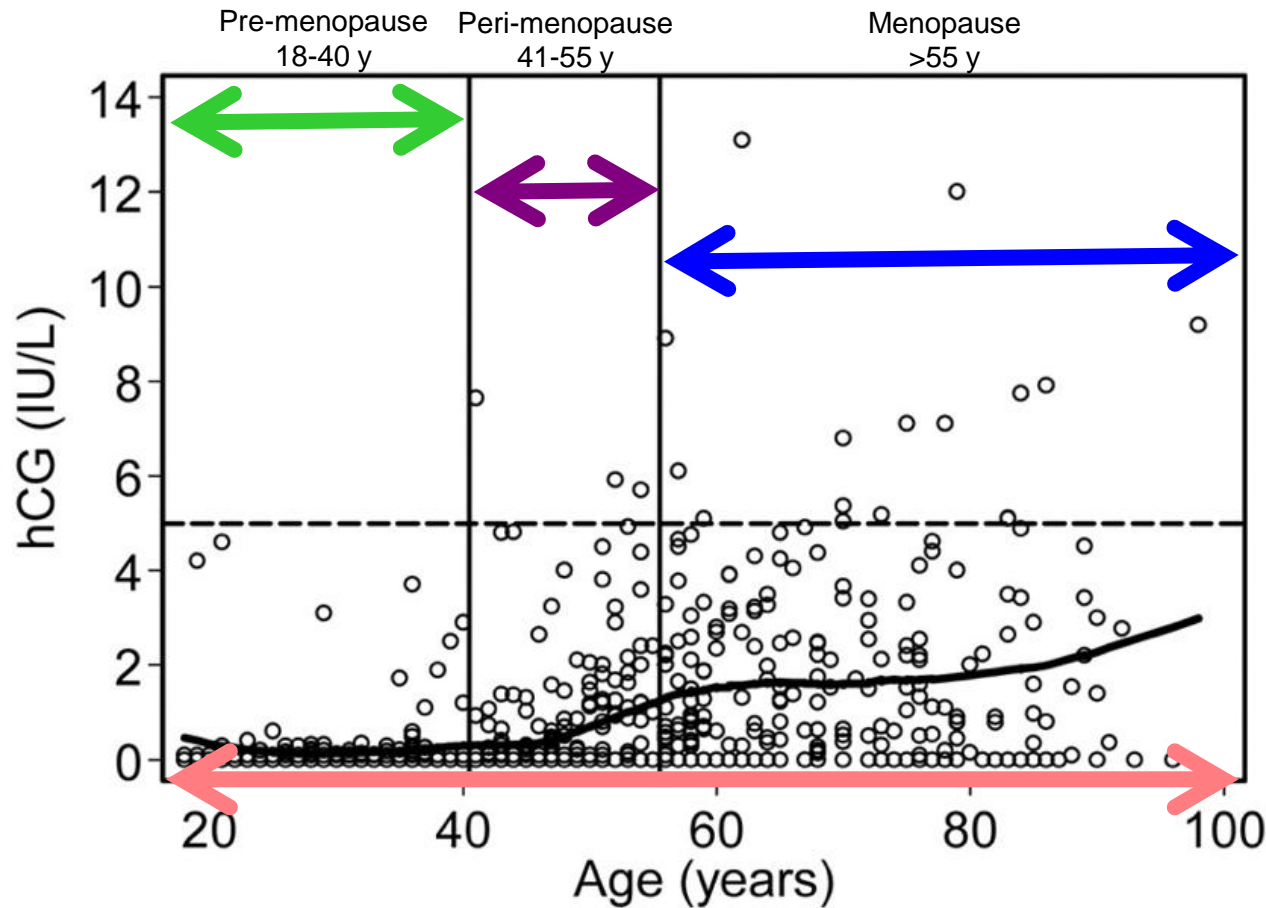


Case Report

- JP is a 49-year-old female with ESRD secondary to polycystic kidney disease. She has been on peritoneal dialysis for 2 years and is admitted for a deceased-donor renal transplant
- Pre-transplant screening reveals an hCG of 12 IU/L
- The renal team calls to ask what this means

hCG increases with age in non-pregnant women

Snyder J, et al. *Clin Chem* 2005;51:1830-1835



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 5 | 2.5 | |
| Perimenopausal, 41–55 years | 240 | <2.0 to 7.7 8 | 4.8 | 0.07 ^a |
| Postmenopausal, >55 years | 240 | <2.0 to 13.1 14 | 7.7 | <0.0001 ^b |

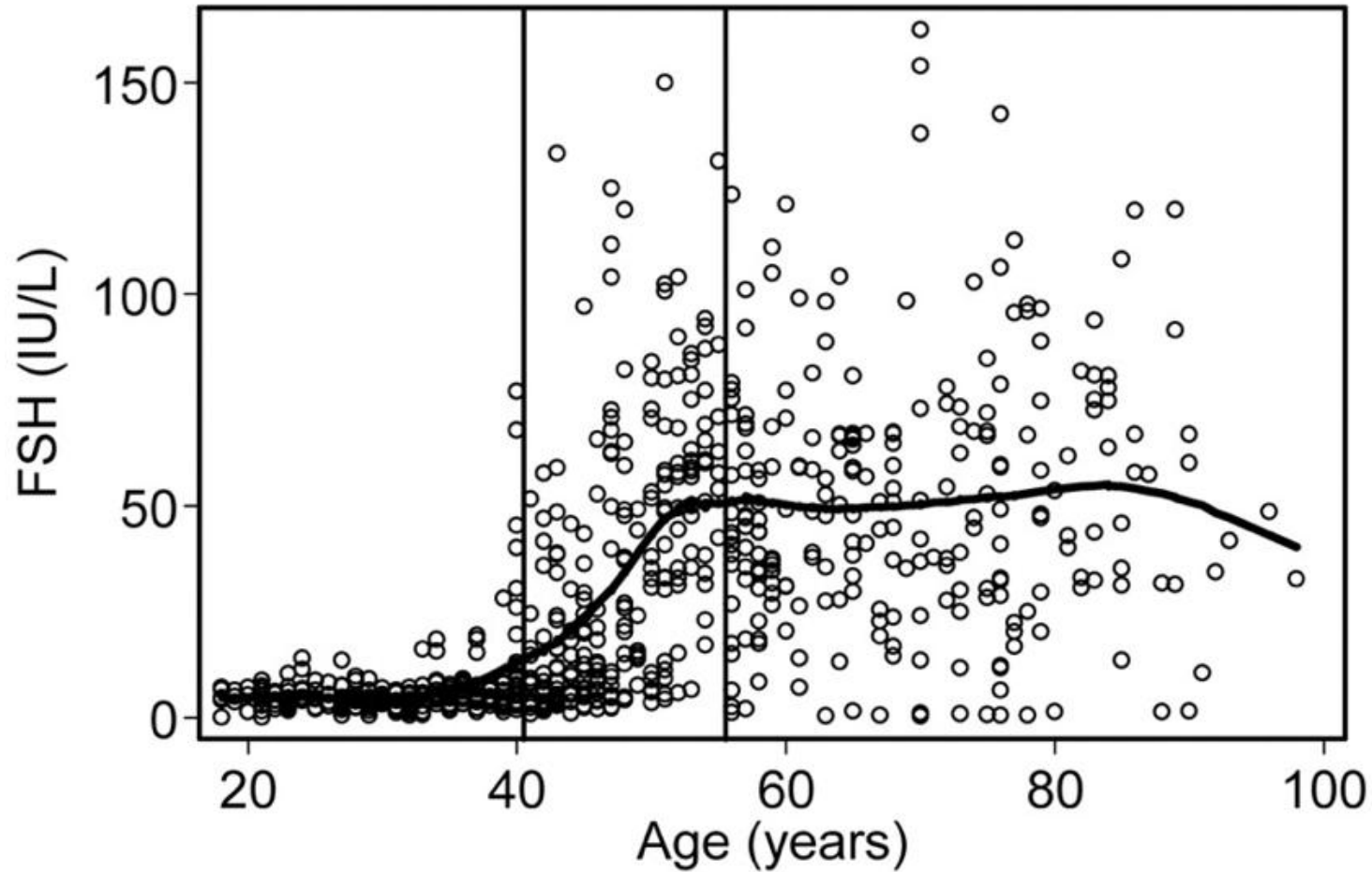
^a Compared with the nonpregnant premenopausal cohort.

^b Compared with the nonpregnant premenopausal and nonpregnant perimenopausal cohorts.

Snyder J, et al. *Clin Chem* 2005;51:1830-1835

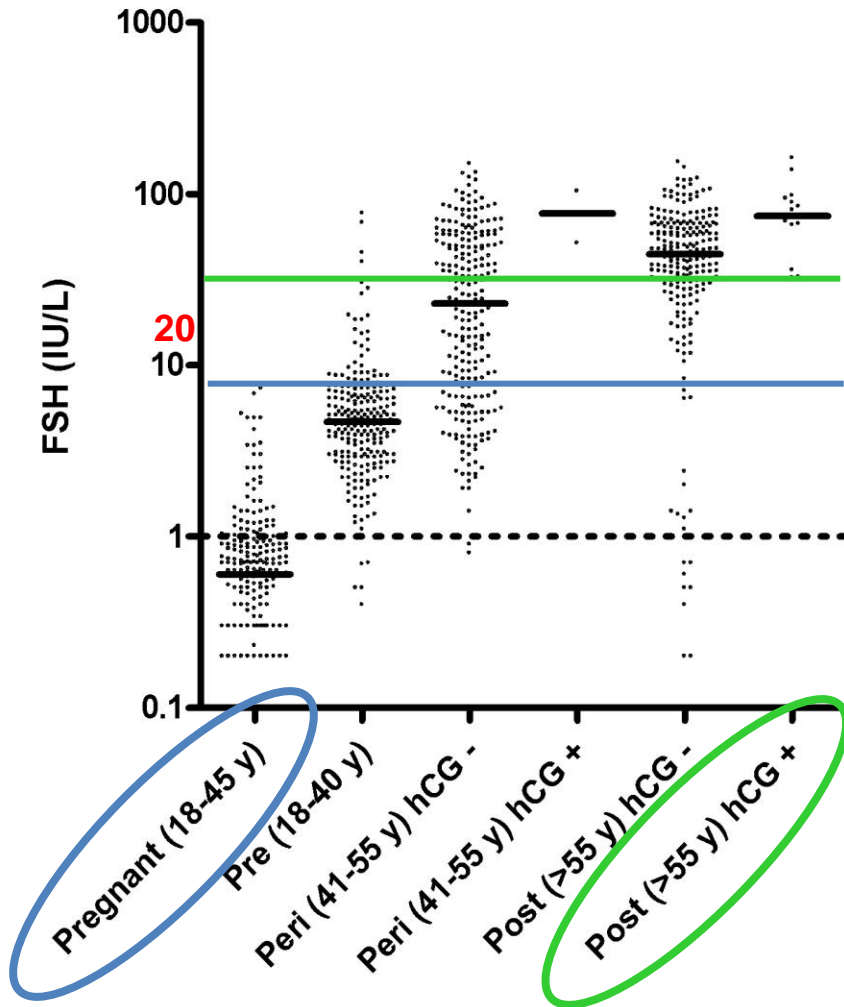
FSH vs. Age

Snyder J, et al. *Clin Chem* 2005;51:1830-1835



FSH by Cohort & hCG Status

Snyder J, et al. *Clin Chem* 2005;51:1830-1835



- FSH cutoff of 20 IU/L differentiated hCG of pregnancy from pituitary hCG
- Only 3 peri-menopausal patients with low concentration of hCG

Validation of FSH Cutoff

Gronowski A, et al. *Clin Chem* 2008;54:652-656

- 100 peri-menopausal women with hCG 5-14 IU/L
 - 77 hCG not of placental origin
 - 23 hCG of placental origin
 - 17 resolving abortion/miscarriage
 - 4 GTD
 - 2 early pregnancies

| Cutoff | Sensitivity | Specificity |
|---------|-------------|-------------|
| 20 IU/L | 83% | 84% |
| 45 IU/L | 100% | 76% |

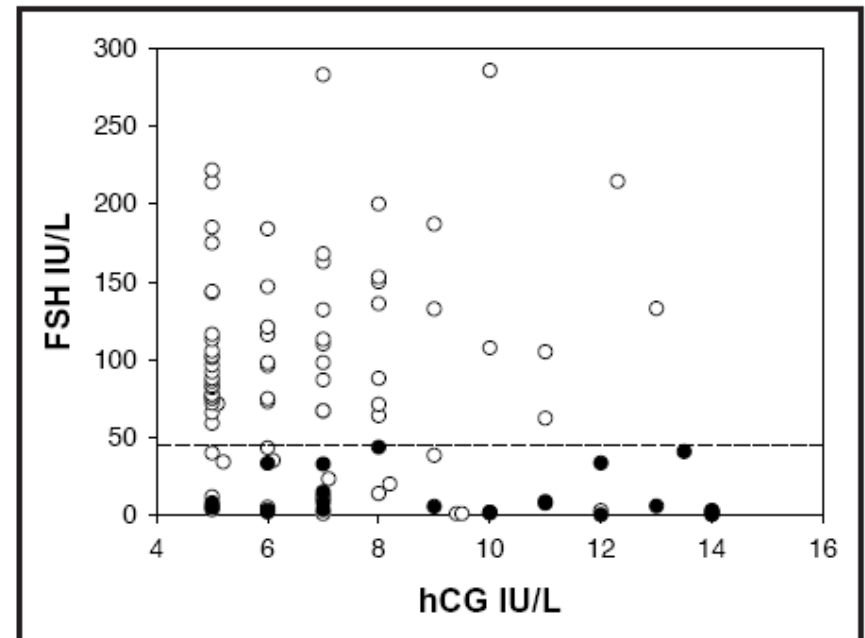
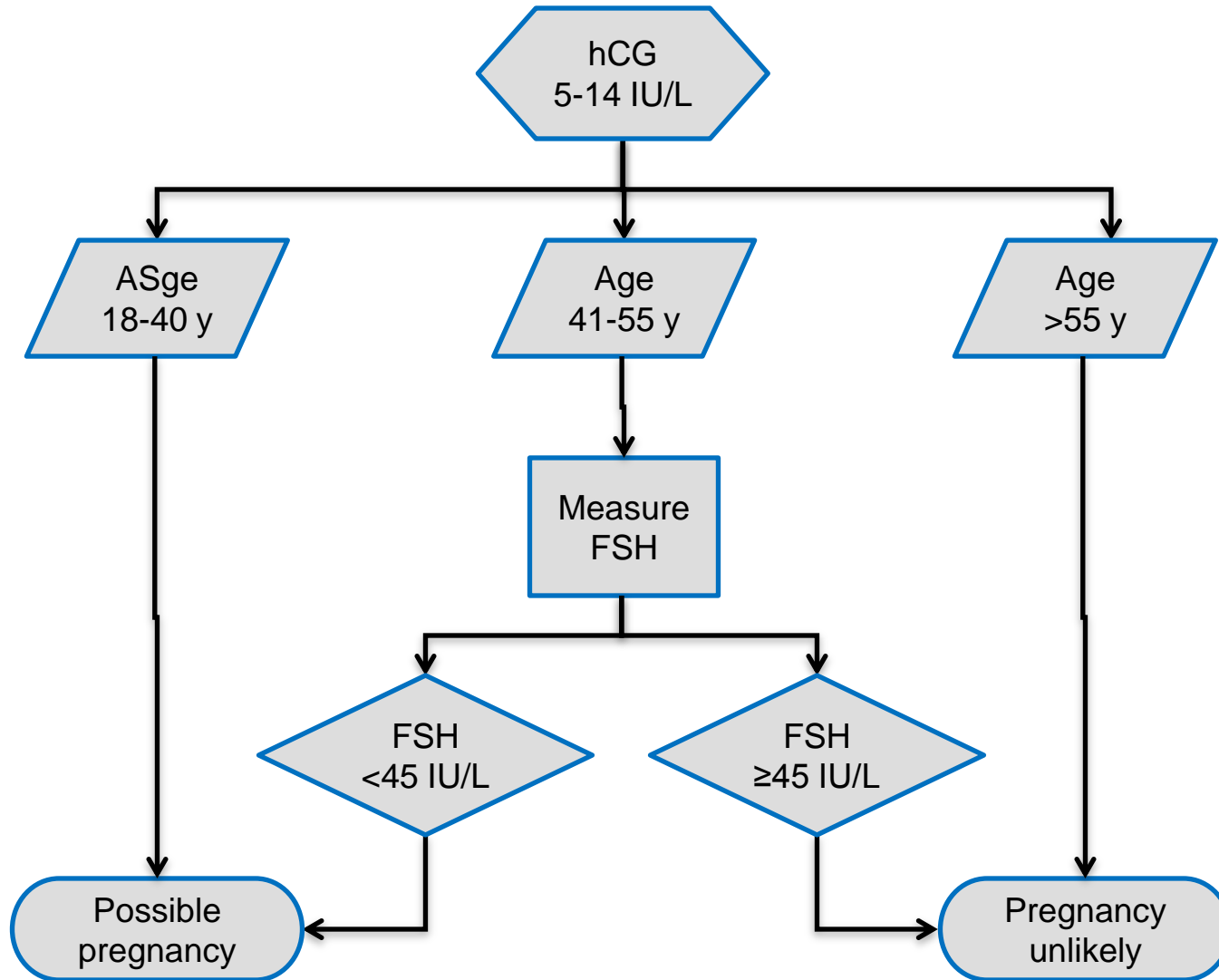


Fig. 2. Scatter plot of hCG vs FSH concentrations. Open circles represent nonplacental hCG; n = 77. Closed circles represent placental hCG; n = 23. The dashed line represents a FSH cutoff of 45 IU/L.

Interpreting Low hCG Results



Case Report Follow-up

49 yo renal transplant patient with hCG of 12 IU/L

- FSH = 215 IU/L
 - More consistent with menopausal status than pregnancy
- Renal transplant was performed

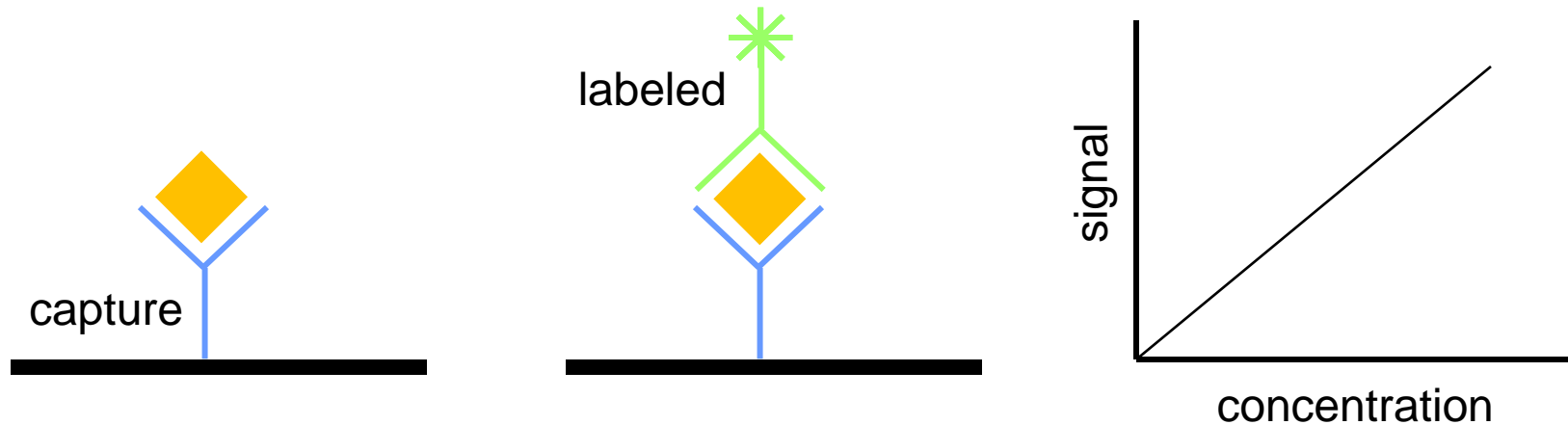
hCG Assays

| | Serum | Urine |
|---------------------------|--------------------------|--------------|
| Quantitative | Yes | No* |
| Qualitative (POCT) | Yes (but only in lab) | Yes |

*Occasionally done to investigate discrepant POCT results (results not reported).

Quantitative Assays

- Immunometric methods designed for use with serum

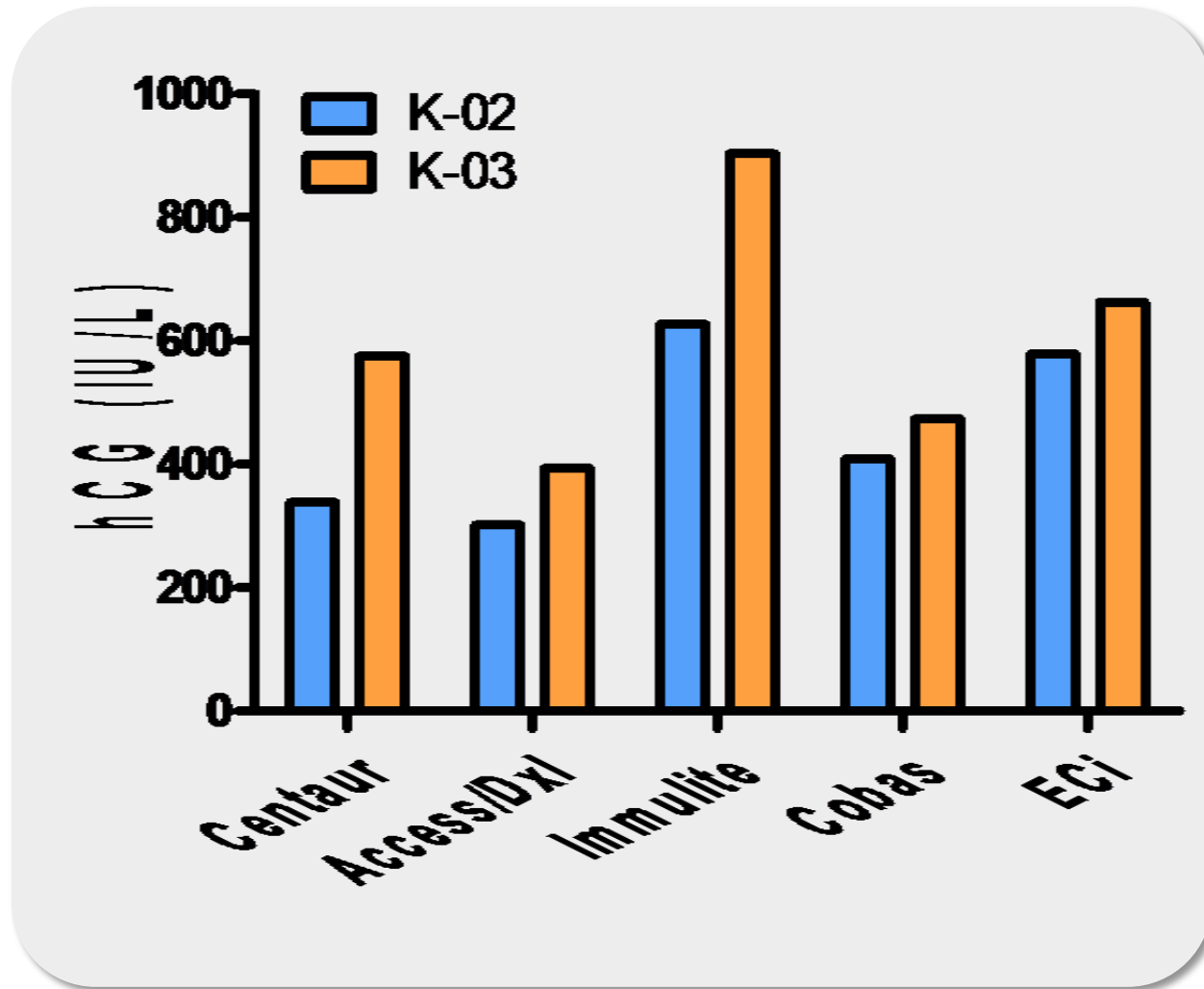


Lack of Assay Harmonization

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

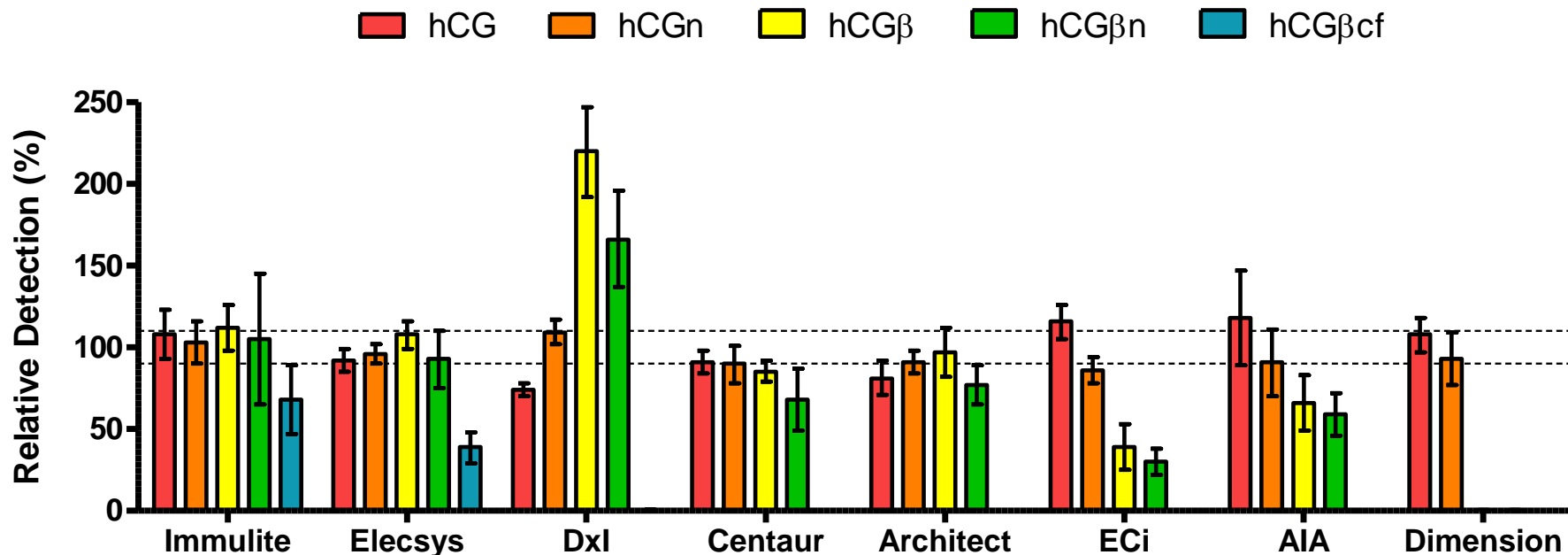
Wide variation in measured hCG concentrations and the detection of hCG variants

Variation in Measured hCG Concentrations



CAP Ligand Survey A, 2008

Variation in hCG Variant Detection



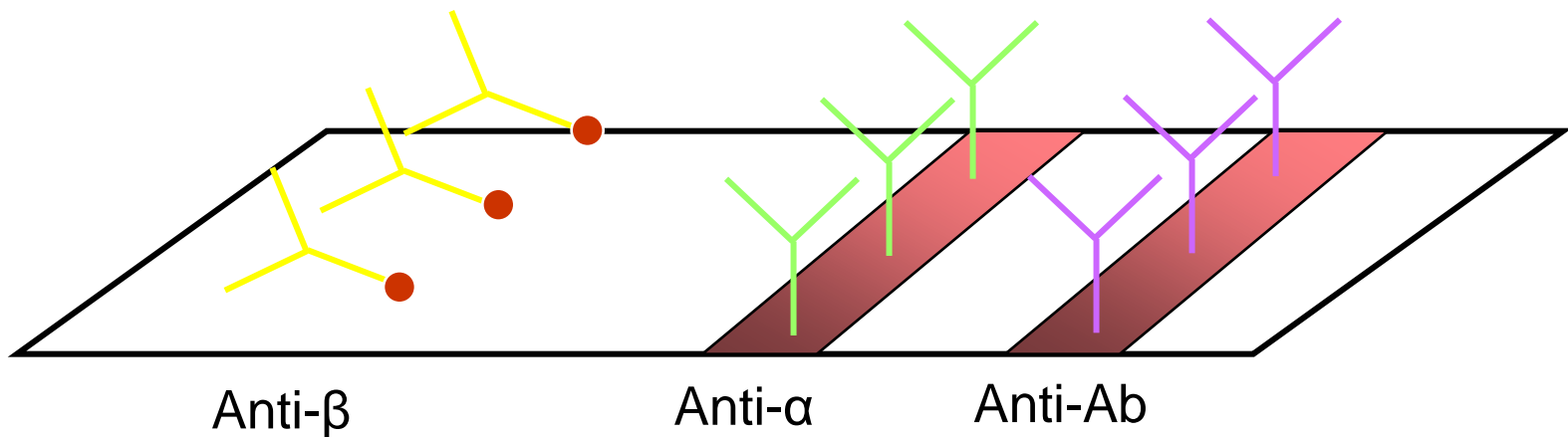
Whittington J, et al. *Clin Chim Acta* 2010;411:81-85

Clinical Impact of Variation

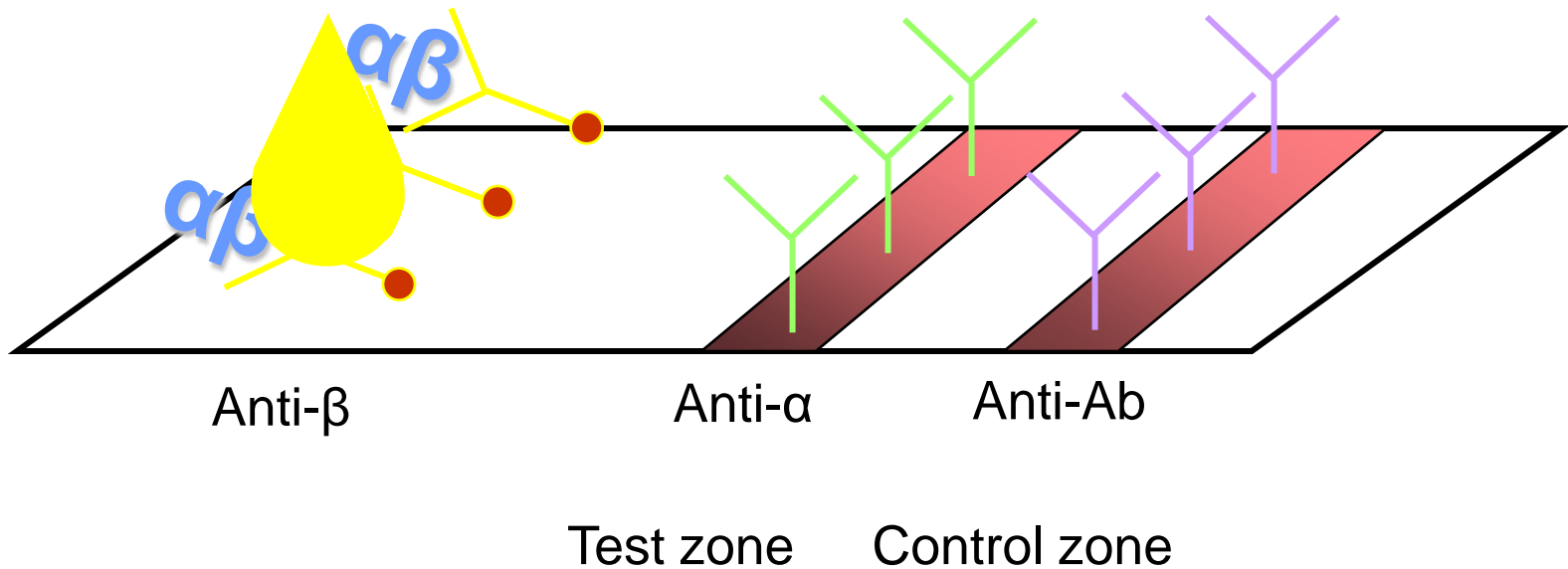
- Minimal when only a single measurement is required
 - Pregnancy diagnosis
 - Down syndrome screening
- Significant when serial measurements are required
 - Ectopic pregnancy
 - Oncology applications (tumor marker)

Qualitative Assays

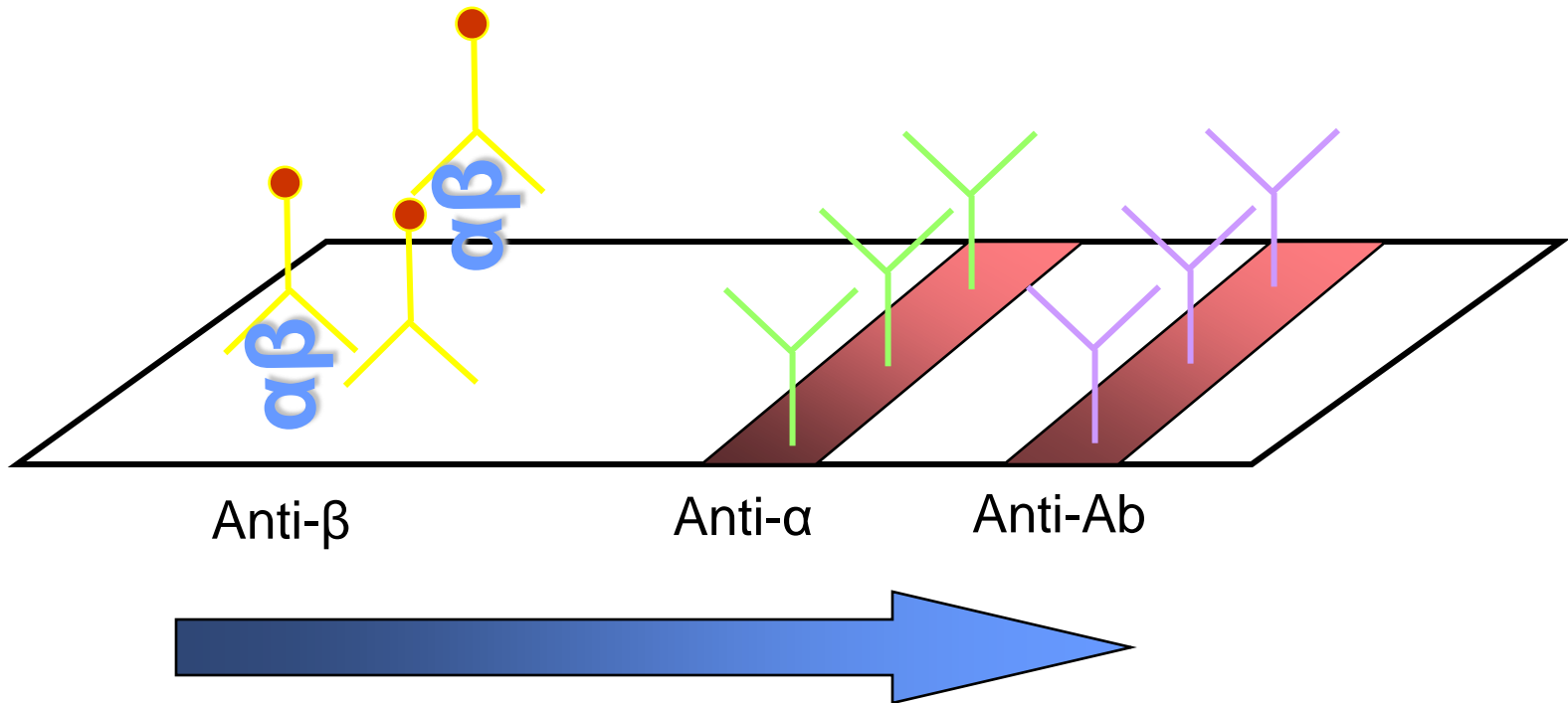
- All can be performed with urine (waived) and some with serum (moderately complex)
- Majority of current tests are immunochromatographic



Qualitative Assays



Qualitative Assays



Variation Among Qualitative Assays

- Used for pregnancy detection so are designed to detect dimeric hCG variants (hCG and hCGn)
- Some detect additional, unexpected variants

Analytical Specificity (POC)

Table 1. Characteristics of 6 qualitative CG devices and results of qualitative and quantitative urine tests using various CG isoforms.

| | Qualitative CG device | | | | | | Elecsys ^a , IU/L, pmol/L ^b |
|---|-----------------------|--------------------|-------------------|--------------------|--------------------|--------------------|---|
| | Sure-Vue | CIIntest | QuickVue+ | Osom | hCG Combo | ICON II | |
| Capture antibody specificity, type ^c | Anti- α (u) | Anti -CG dimer (m) | Proprietary (p) | Anti- α (m) | Anti- α (m) | Anti- α (m) | Anti- β (m) |
| Label antibody specificity, type ^c | Anti-CG dimer (u) | Anti- β (m) | Anti- β (m) | Anti- β (m) | Anti- β (m) | Anti- β (m) | Anti- β (m) |
| 4th IS-CG | 10/10 | 10/10 | 10/10 | 5/5 | 10/10 | 10/10 | 1220 |
| CGn | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | NA ^d |
| CG β | 10/10 | 10/10 | 10/10 | 0/10 | 10/10 | 10/10 | 2263 |
| CG β n | 10/10 | 10/10 | 10/10 | 0/10 | 10/10 | 10/10 | 7800 |
| CG β cf | 0/10 | 10/10 | 6/10 | 0/10 | 10/10 | 0/10 | 2336 |
| CG α | 0/10 | 0/10 | 0/10 | 0/5 | 0/10 | 0/10 | 8800 |
| | | | | | | | 630 |
| | | | | | | | 3300 |
| | | | | | | | 815 |
| | | | | | | | 10 200 |
| | | | | | | | <2.0 |
| | | | | | | | 8400 |

Sigel C and Grenache DG. *Clin Chem* 2007;53:989-990

False-negative Urine hCG Test Result

Gronowski A, et al. *Clin Chem* 2009;55:1389-1394

- 18 yo female with vaginal spotting and cramping and 3 months pregnant
- Negative urine hCG POC in ED
- Serum hCG: 419,680 IU/L
- Ultrasound shows live intrauterine pregnancy
- Negative urine hCG POC in lab
 - Positive when diluted 1:5
 - Urine hCG: 176,498 IU/L



Hypothesis

Non-dimeric hCG variant binding to only one of the assay antibodies and preventing “sandwich” formation

hCG Variant Effect

Gronowski A, et al. *Clin Chem* 2009;55:1389-1394

- hCG variants in patient's urine
 - hCG 0.7 $\mu\text{mol/L}$ (21%)
 - hCG β 0.05 $\mu\text{mol/L}$ (1%)
 - hCG βcf 2.6 $\mu\text{mol/L}$ (78%)

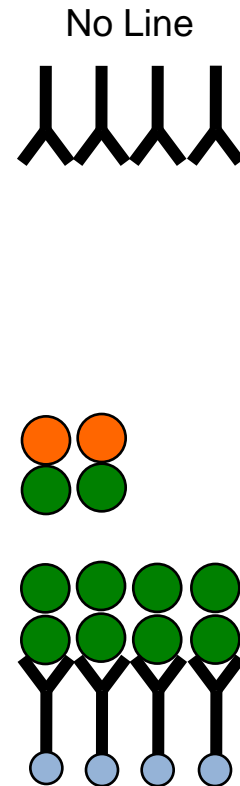
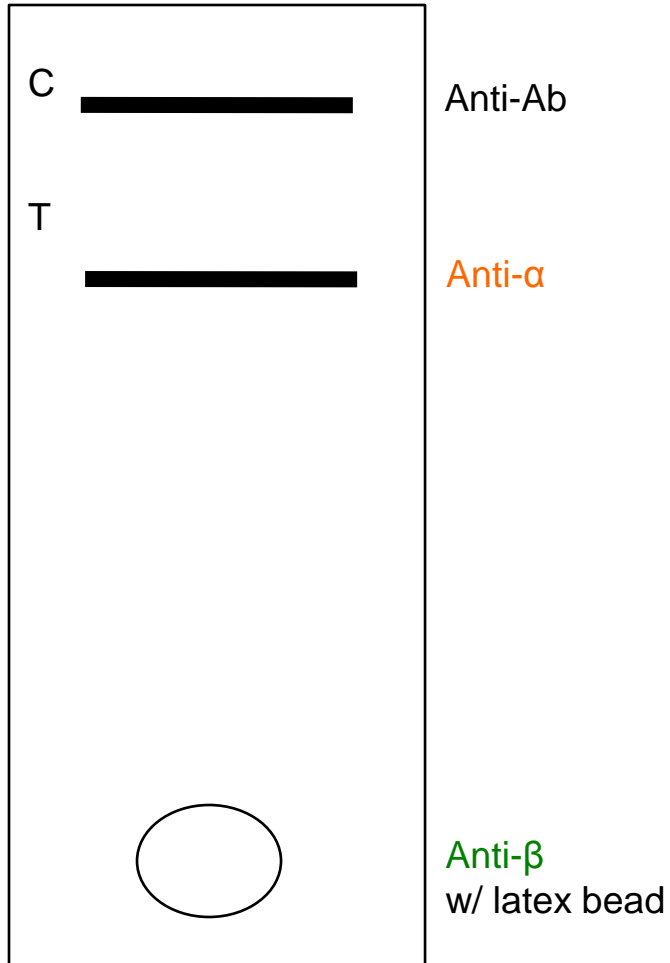


hCG: 17,800 IU/L
hCG βcf : 0.04 $\mu\text{mol/L}$



hCG: 17,800 IU/L
hCG βcf : 1.0 $\mu\text{mol/L}$

hCG Variant Effect



Clinical Implications

- hCG β cf is major hCG variant in urine after ~35 days of pregnancy
 - Accounts for up to 90% of hCG immunoreactivity in urine
- Caution when testing women at >35 days of gestation
 - False negative results can occur
- Bottom line
 - Positive result: good evidence patient is pregnant
 - Negative result: does not mean patient is not pregnant

Summary

- hCG is a heterogeneous molecule that exists as numerous variants
- hCG is used clinically in the diagnosis of pregnancy (uterine & ectopic), in Down syndrome screening, and as a tumor marker
- Persistently low hCG results may be due to interfering antibodies or pituitary hCG and could have significant consequences if interpreted incorrectly
- Both quantitative and qualitative hCG assays show considerable variability in the detection of hCG variants
- Qualitative urine hCG test results can be falsely negative due to elevated concentrations of non-dimeric hCG variants
- Laboratorians must know the analytical specificity of the hCG assays used in their laboratory



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