Identifying the Pregnant Patient

There's More to Know than "Yes" or "No"

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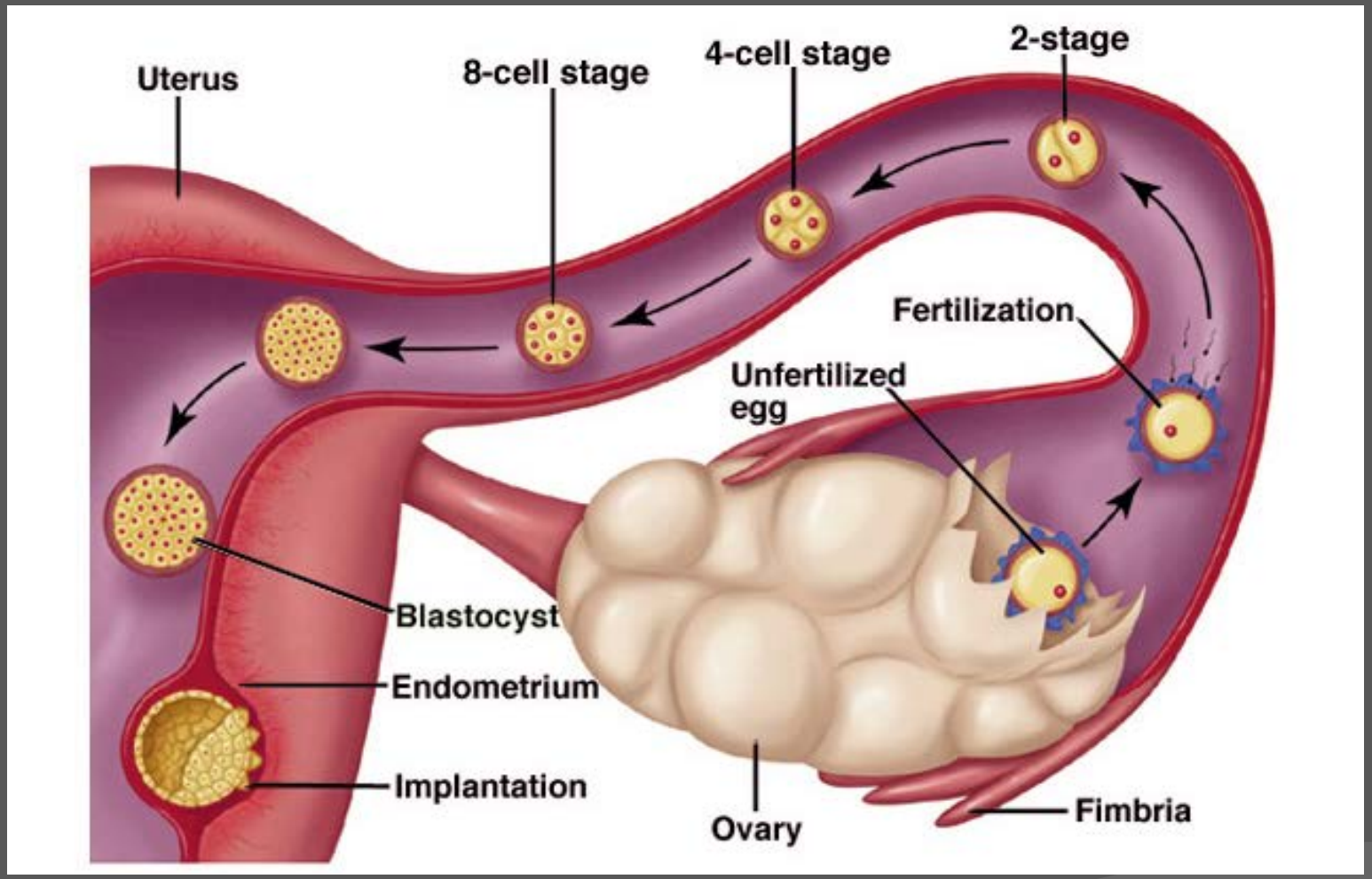
- Intellectual property/Royalty Income: None

Learning Objectives

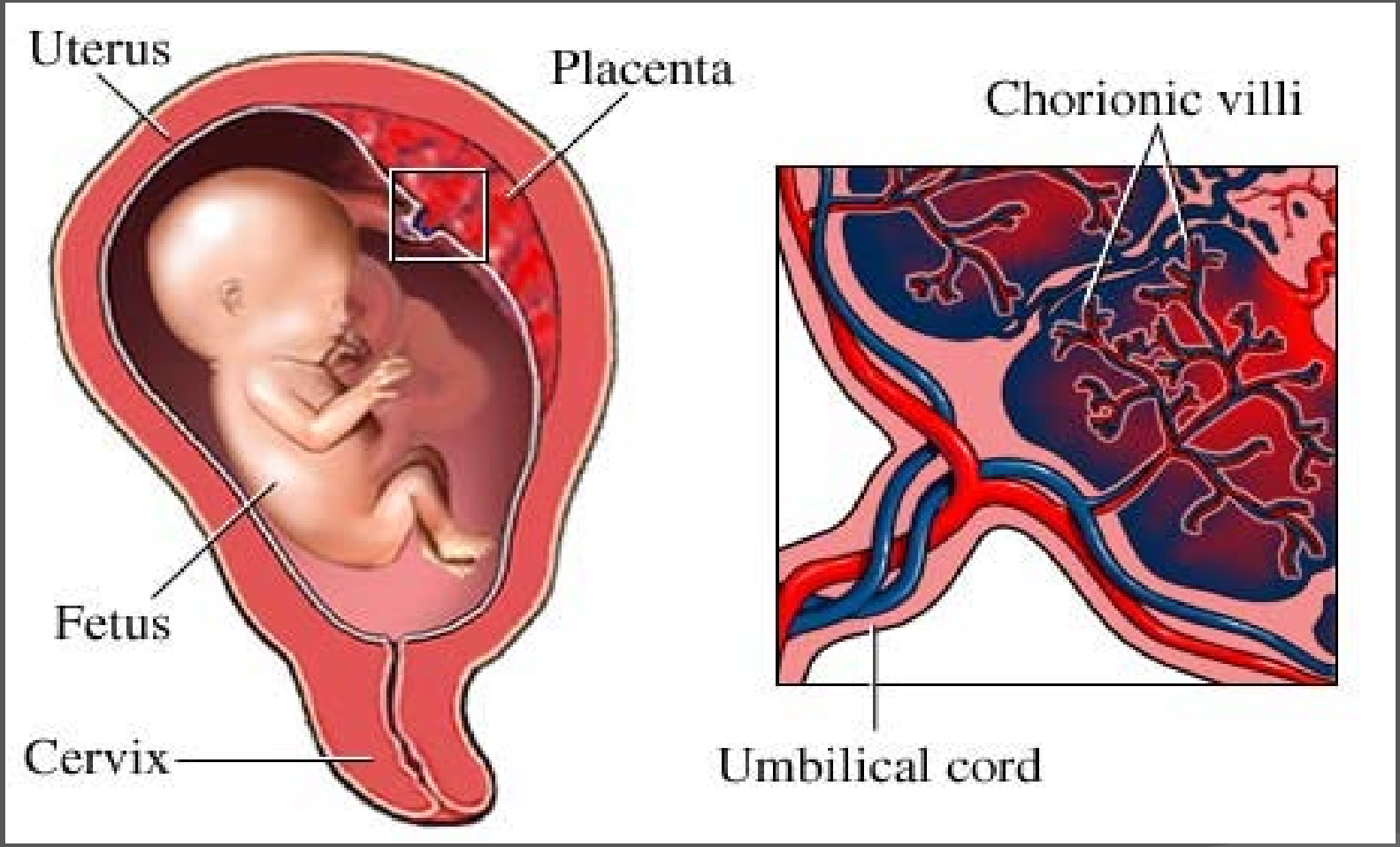
- Describe the biochemistry and function of hCG
- Explain the limitations of qualitative point-of-care hCG tests and causes of erroneous results
- Evaluate the effect that qualitative point-of-care hCG tests have on turnaround time and patient length of stay

Fertilization & Implantation



http://www.mhhe.com/socscience/sex/common/ibank/ibank/0112.jpg

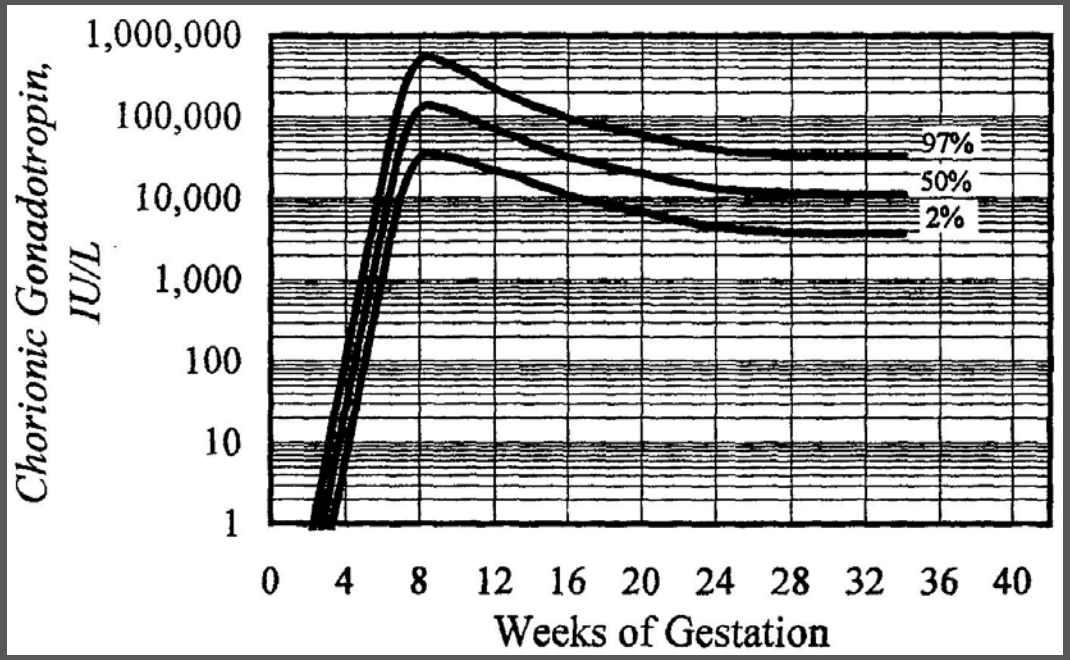
Chorionic Villus



http://www.nucleusinc.com

hCG Concentrations During Pregnancy

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



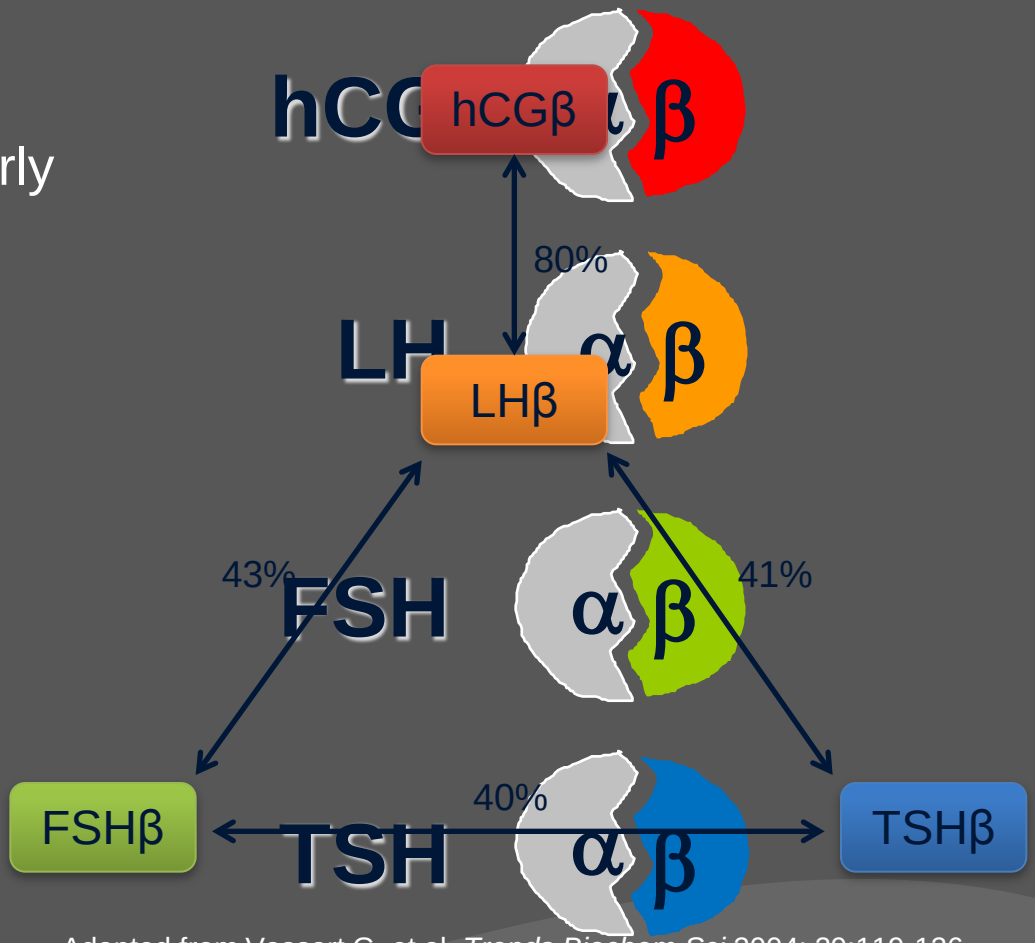
Tietz Textbook of Clinical Chemistry, 5thed, 2012

hCG

- Glycoprotein hormone family

hCG

- Glycoprotein hormone family
- Maintains progesterone in early pregnancy



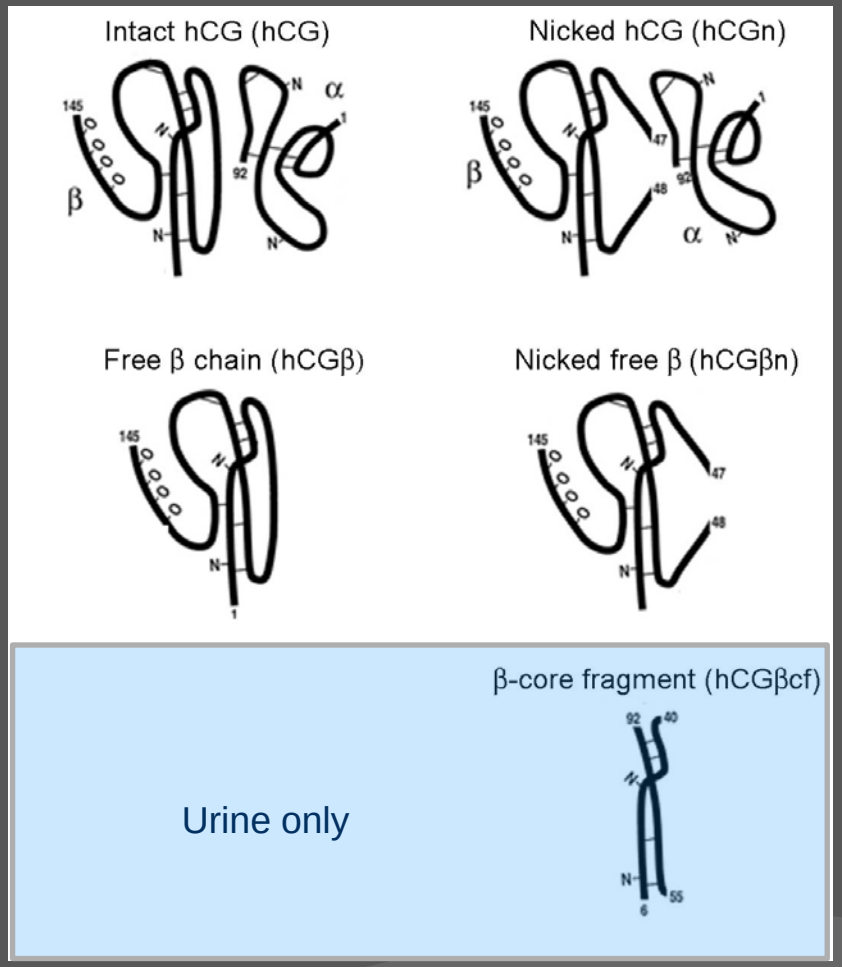
Adapted from Vassart G, et al. *Trends Biochem Sci* 2004; 29:119-126

hCG Variants

- Numerous molecular forms of hCG present in pregnancy serum
  - Dissociated or degraded molecules
  - No biologic activity

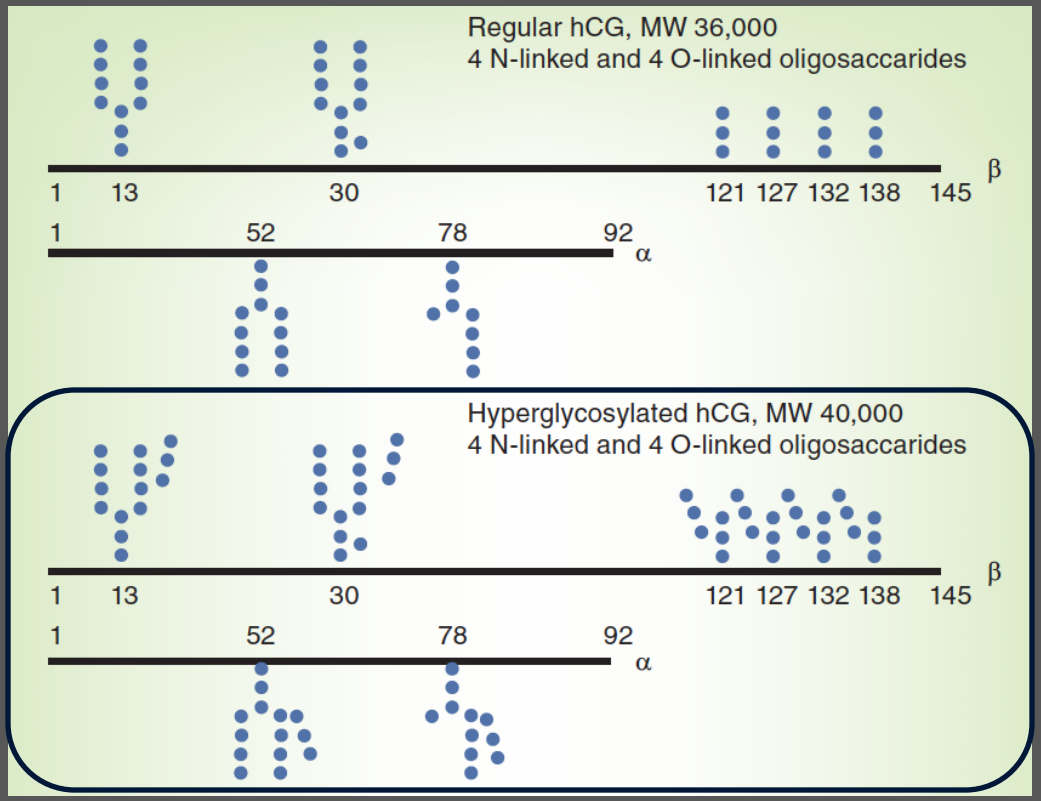
- Key β-containing variants
  - Intact hCG
  - Nicked hCG
  - Free β subunit
  - Nicked free β subunit
  - β-core fragment (urine)

Serum & Urine



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

Hyperglycosylated hCG (hCG-H)

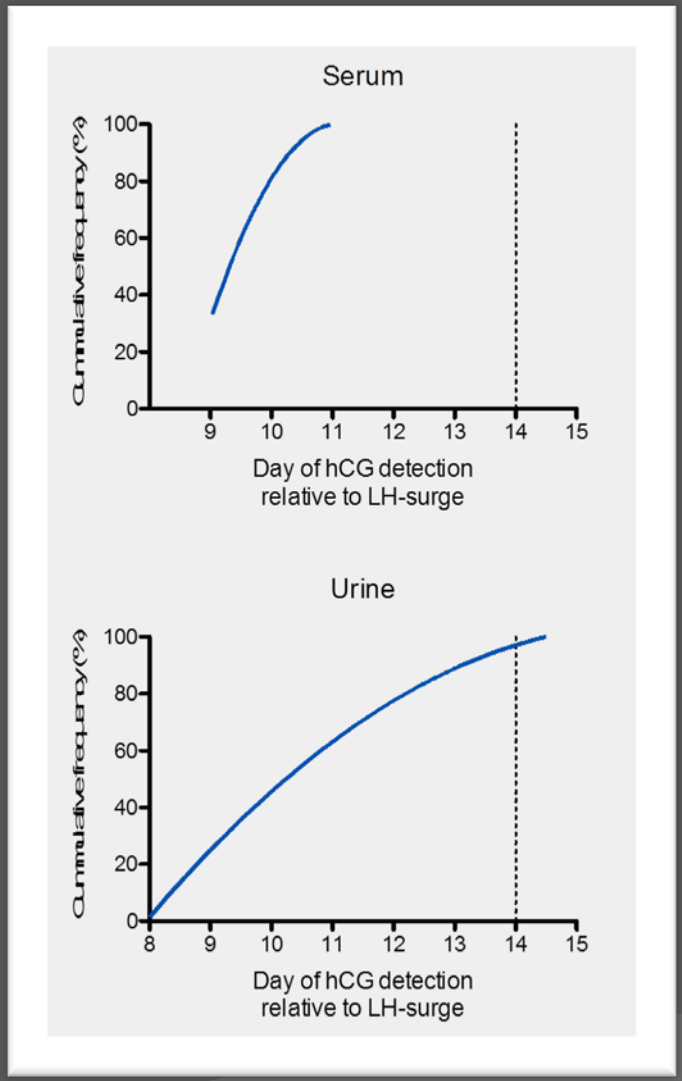


Cole, L. *Expert Rev Mol Diag* 2009;9:721-747

- Principal hCG variant produced in early gestation
- Larger oligosaccharide side chains compared to regular hCG
  - Molecular mass increased by 11%
- Synthesized by invasive cytotrophoblasts
  - Implantation blastocysts

hCG in Normal Pregnancy

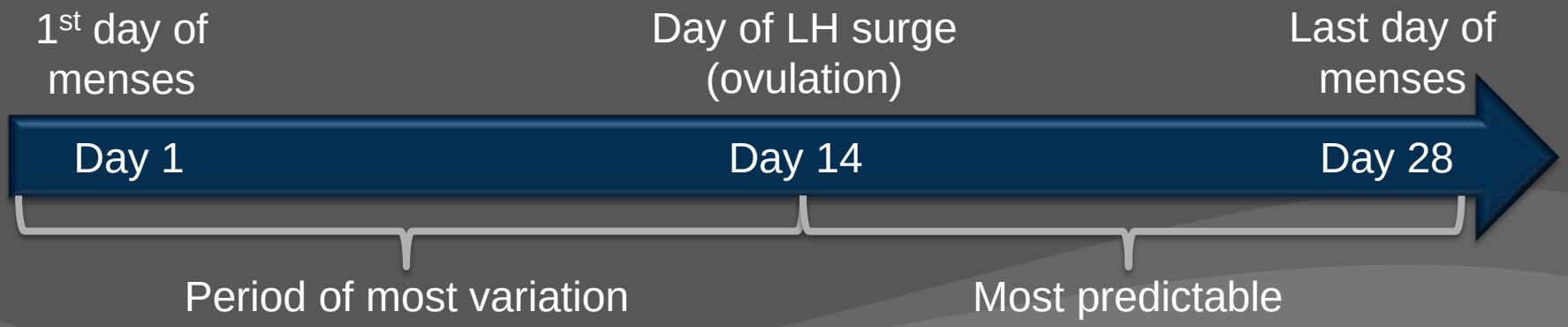
- Pregnancy diagnosis involves history & physical exam in conjunction with hCG testing
- Serum hCG detectable 9-11 days after LH surge
  - ~3-5 days before expected menses
- Urine hCG detectable around same time or soon after
  - More variable than serum



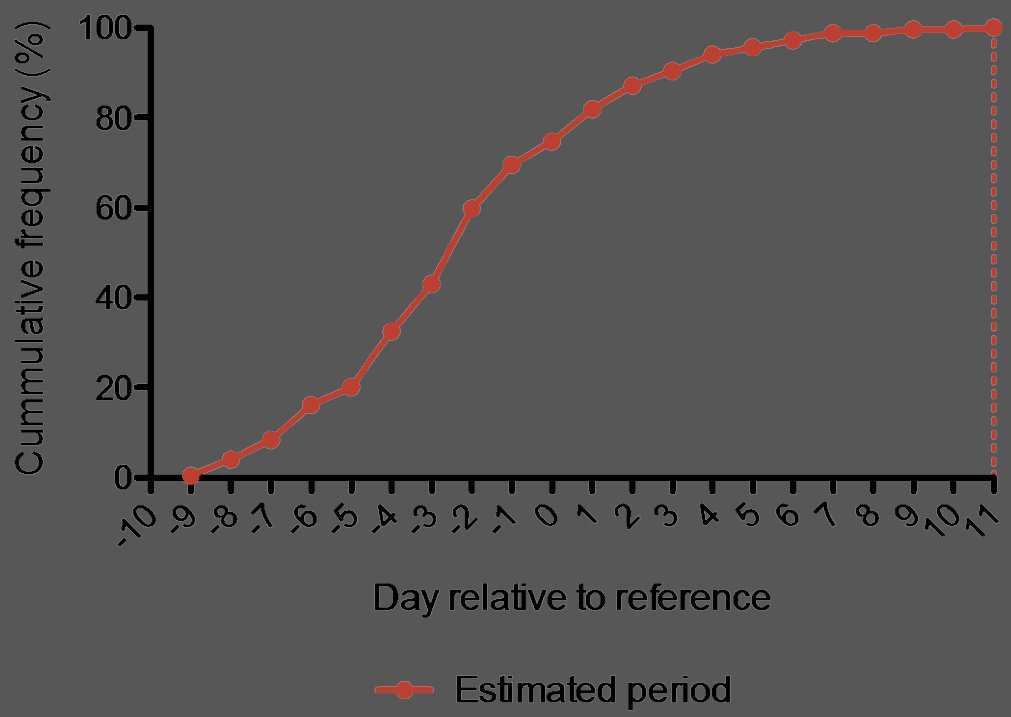
Adapted from Lohstroh P, et al. *Fertil Steril* 2005;83:1000-1111

How early can urine hCG detect pregnancy?

- Depends on several variables
  - Length of menstrual cycle
  - Time from ovulation to fertilization
  - Time from fertilization to implantation
  - How expected day of menses is determined
    - Average cycle length
    - Days relative to LH surge or LH peak



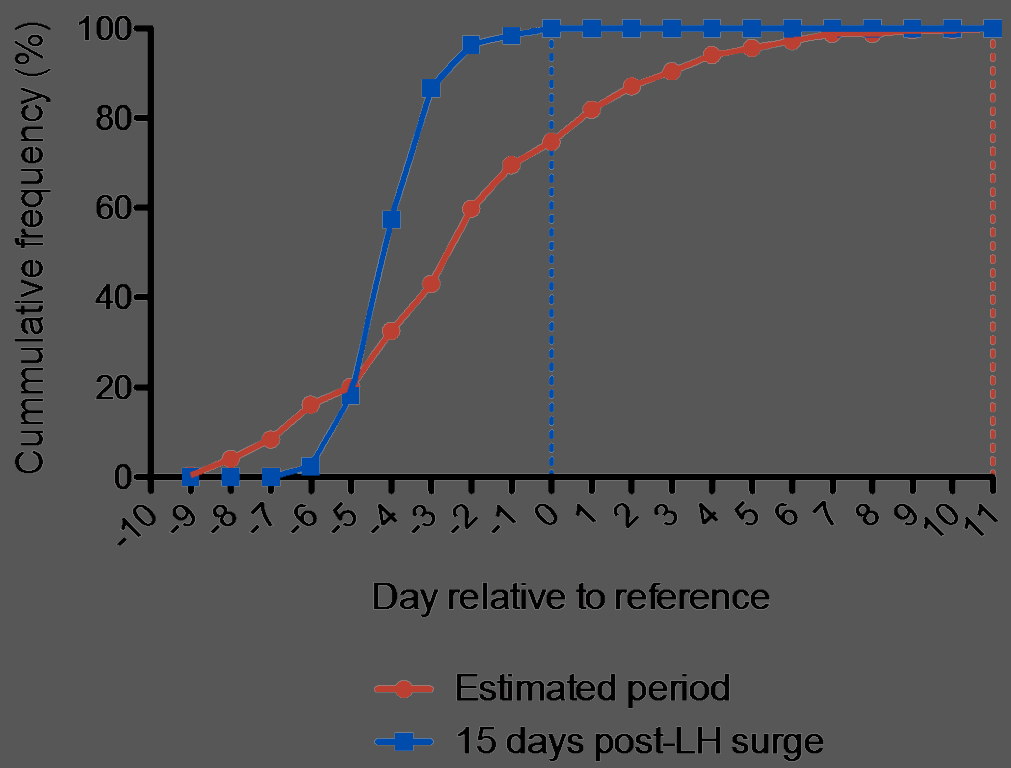
How early can urine hCG detect pregnancy?



- 2,715 samples from 86 women achieving pregnancy
- OTC hCG test
- Day 0 is day of expected menses

Adapted from Johnson SR, et al. *Curr Med Res Opin* 2009;25:741-748

How early can urine hCG detect pregnancy?



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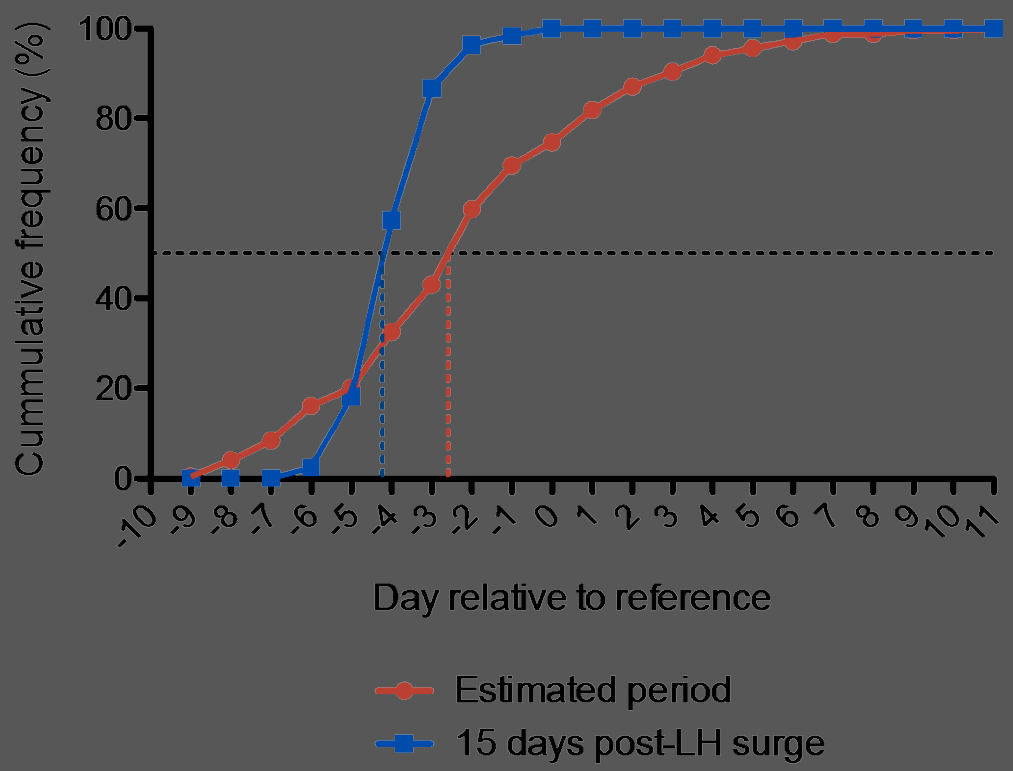


- 2,715 samples from 86 women achieving pregnancy
- OTC hCG test
- Day 0 is day of expected menses

Adapted from Johnson SR, et al. *Curr Med Res Opin* 2009;25:741-748

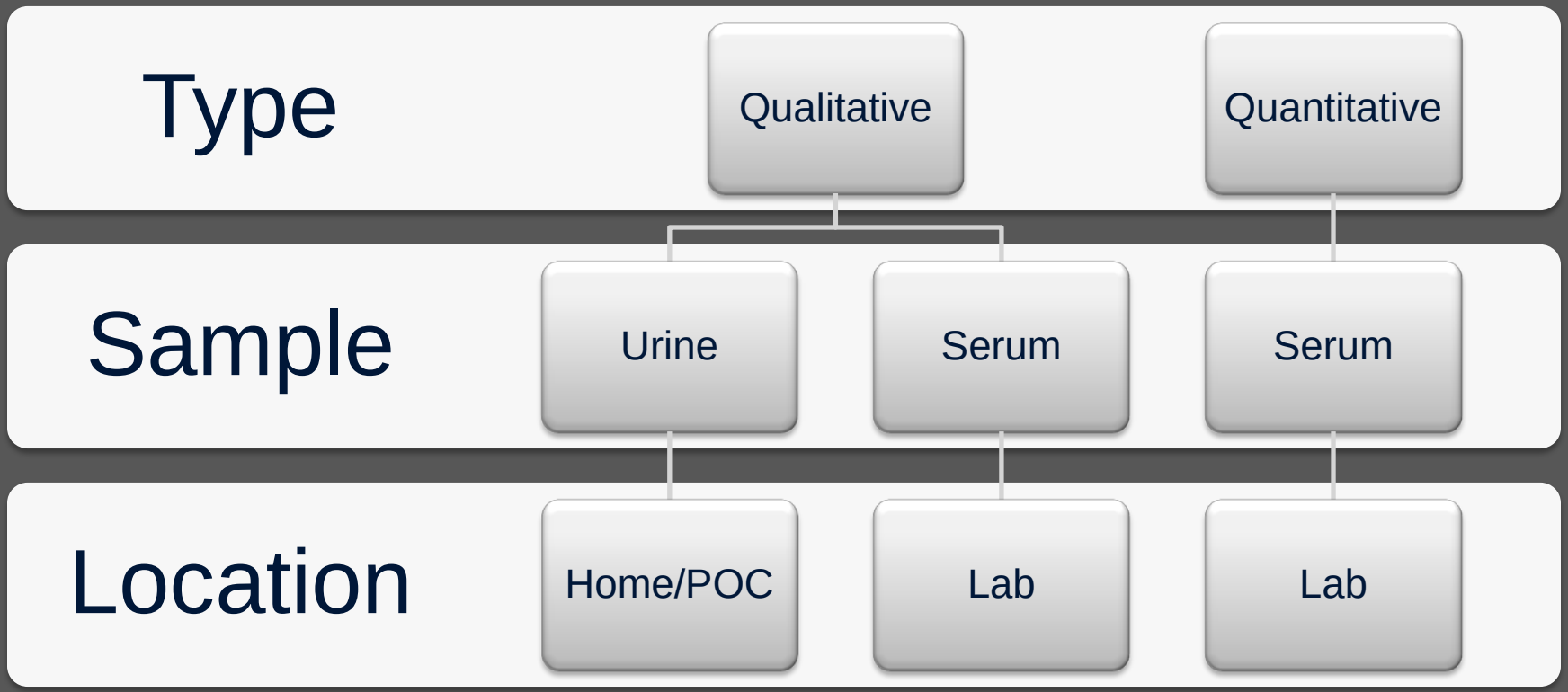
Clinical Utility of hCG as a Test for Pregnancy

Identify if symptoms (abdominal pain, vaginal bleeding, vomiting, etc.) are due to pregnancy

Prevent radiation exposure

Prevent administration of teratogenic medications

Types of hCG Tests

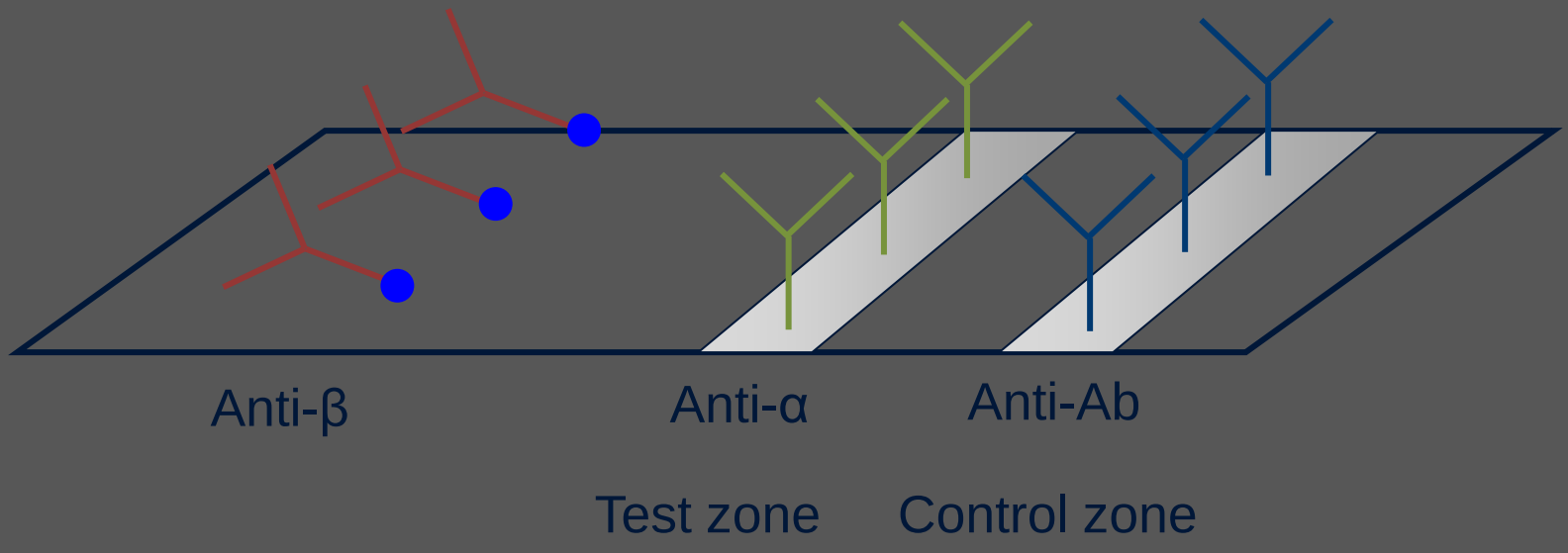


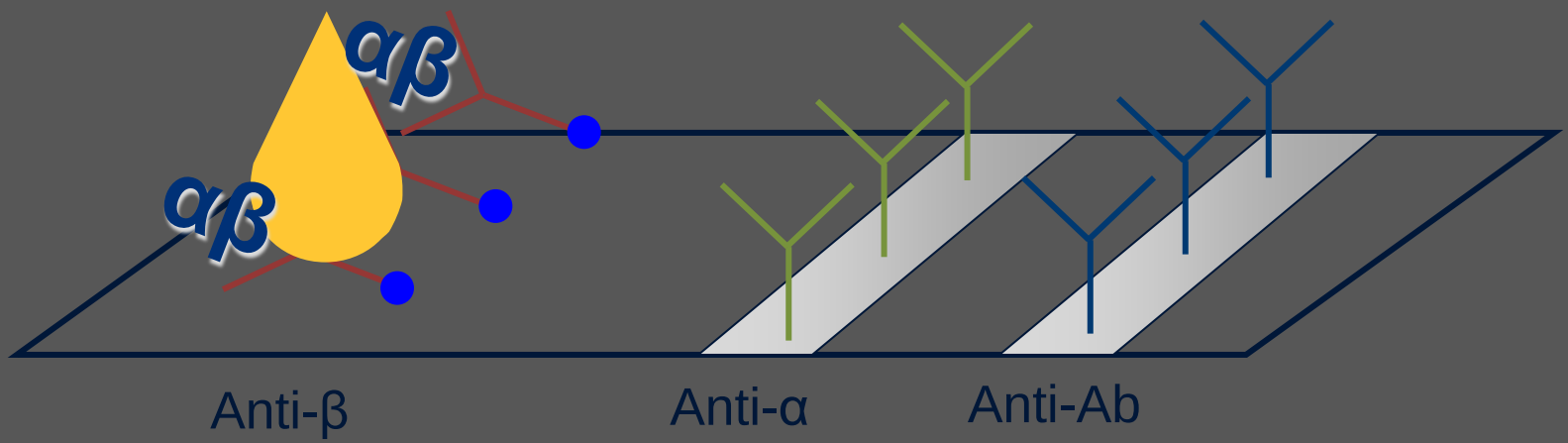
Qualitative hCG Tests

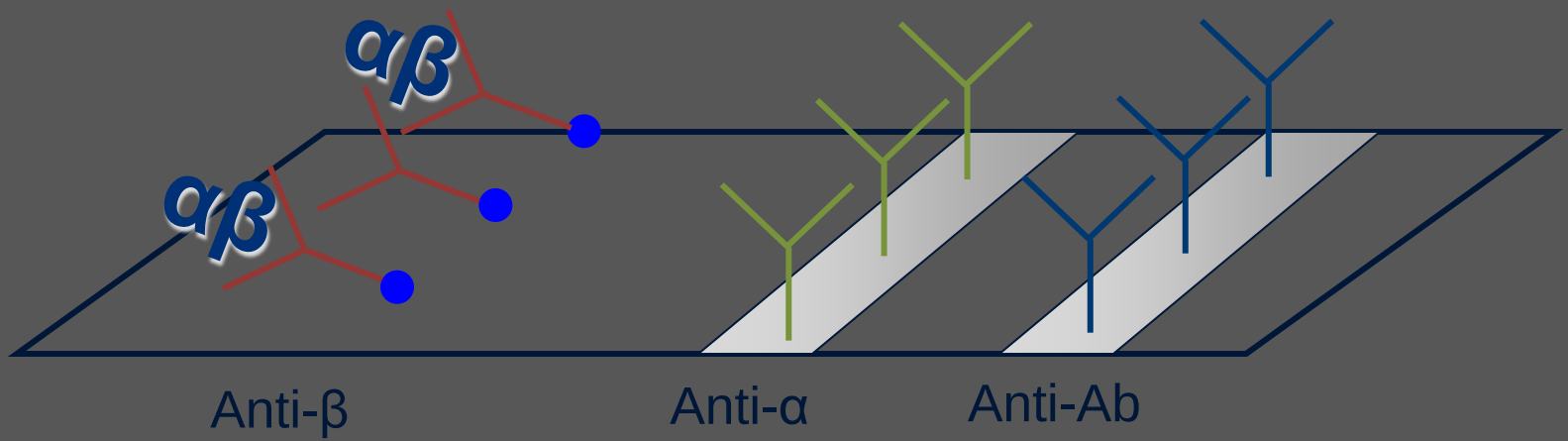
- How do they work?
- What hCG variants can they detect?
- What is the lowest hCG concentration they detect?
- What are the analytical sources of error?
- Does their use affect outcomes?

How do they work?

- All can be performed with urine (waived) and some with serum (moderately complex)




αβ
αβ
Anti-β
Anti-α
Anti-Ab
Test zone
Control zone


αβ
αβ
Anti-β
Anti-α
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Test zone
Control zone

What hCG variants can they detect?

Analytical Specificity: POC Devices

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

	Qualitative CG device						
	Sure-Vue	Clinitest	QuickVue+	Osom	hCG Combo	ICON II	Elecsys[a], IU/L, pmol/L[b]
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 NA[d]
CGn	10/10	10/10	10/10	10/10	10/10	10/10	2263 7800
CGβ	10/10	10/10	10/10	0/10	10/10	10/10	2336 8800
CGβn	10/10	10/10	10/10	0/10	10/10	10/10	630 3300
CGβcf	0/10	10/10	6/10	0/10	10/10	0/10	815 10 200
CGα	0/10	0/10	0/10	0/5	0/10	0/10	<2.0 8400

Sigel C, et al. *Clin Chem* 2007;53:989-990

Analytical Specificity: OTC Devices

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

Cervinski M, et al. *Clin Chim Acta* 2009;406:81-85

What is the lowest hCG concentration they detect?

Analytical Sensitivity: Lack of Consensus

- Disagreement regarding required analytical sensitivity of qualitative hCG tests
  - "Tests with a detection limit no lower than 25 mIU/mL are preferred." (Stenman U, et al. *IVD Technology*, 2003)
  - "…a cut-off value of 5 IU/L instead of 20 IU/L or higher (is optimal)." (Terwijn M, et al. *Clin Chim Acta* 2013)

- Does setting make a difference?
  - Results of OTC and POC hCG tests are used differently

- Claimed detection limits are 20 – 25 IU/L (urine) and 10 – 25 IU/L (serum)

Qualitative point-of-care and over-the-counter urine hCG devices differentially detect the hCG variants of early pregnancy

Mark A. Cervinski [a], Christina M. Lockwood [a], Angela M. Ferguson [a], Randall R. Odem [b], Ulf H. Stenman [c], Henrik Alfthan [c], David G. Grenache [d], Ann M. Gronowski [a,*]

Clinica Chimica Acta 406 (2009) 81–85

- 10 women undergoing fertility treatment
- Single urine sample obtained from each between 1 and 10 days after day of expected menses
- Samples diluted based on intact hCG concentration with hCG-free urine
- POC and OTC devices utilized
  - Lowest dilution that gave a positive result in 3 out of 3 tests was considered the analytical sensitivity

Analytical Sensitivity of OTC & POC hCG Tests Varies Across Patients & Devices

Sample number	Lowest concentration (IU/l) at which 3/3 devices were positive									
	2	4	8	11	12	17	20	23	29	30
POC device										
Clinitest	12.5	12.5	6.3	25	6.3	12.5	12.5	12.5	12.5	12.5
Osom	25	25	6.3	25	6.3	12.5	>28 [b]	25	25	25
Quick-Vue	50	25	6.3	50	6.3	50	28	25	12.5	12.5
hCG Combo	25	25	12.5	50	6.3	50	25	50	25	25
ICON II	25	25	12.5	50	nd [c]	25	>28 [b]	50	25	25
SureVue	50	>25 [d]	12.5	100	12.5	50	>28 [b]	25	>28 [b]	>40
OTC Device										
First Response	6.3	≤1.6 [e]	0.4	≤6.3 [e]	0.4	≤1.6 [e]	3.1	≤3.1 [e]	≤1.6 [e]	6.3
Answer	≤1.6 [e]	6.3	0.8	6.3	≤1.6 [e]	≤3.1 [e]	3.1	6.3	≤1.6 [e]	6.3
Target Early Result	6.3	6.3	1.6	6.3	0.8	≤3.1 [e]	12.5	6.3	3.1	6.3
EPT Certainty	12.5	6.3	1.6	6.3	6.3	6.3	12.5	≤6.3 [e]	≤6.3 [e]	6.3
Clearblue Easy	12.5	12.5	1.6	6.3	6.3	12.5	12.5	6.3	12.5	12.5
Wal-Mart Equate	6.3	12.5	6.3	12.5	6.3	12.5	12.5	12.5	12.5	12.5

Across Patients

Across Devices

Cervinski M, et al. *Clin Chim Acta* 2009;406:81-85

Claimed detection limit: 25 IU/L for all

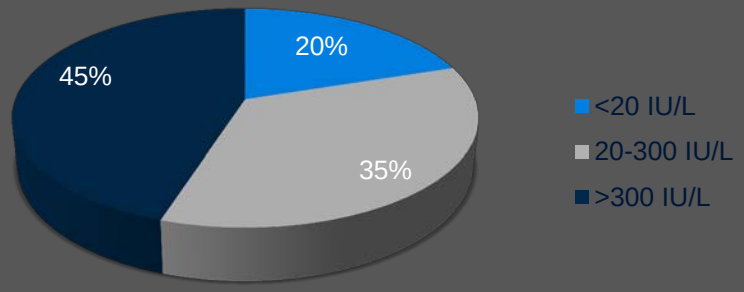
Limitations in qualitative point of care hCG tests for detecting early pregnancy

Dina N. Greene [a,*], Robert L. Schmidt [b], Sandy M. Kamer [a], David G. Grenache [b], Carolyn Hoke [a], Thomas S. Lorey [a]

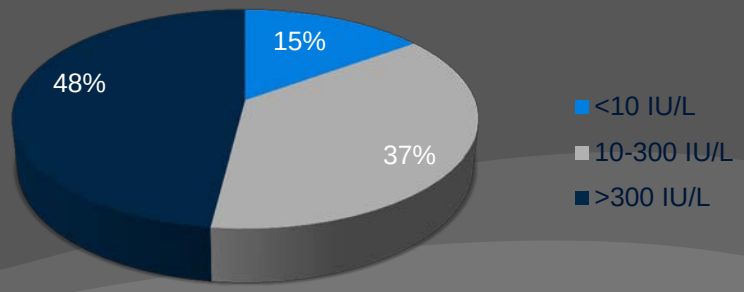
Clinica Chimica Acta 415 (2013) 317–321

- Urine and serum samples (not paired) selected based on hCG concentration determined by Siemens Immulite
- Samples tested using 2 POC hCG devices
  - OSOM hCG test (Genzyme)
  - QuickVue+ One-Step hCG Combo test (Quidel, San Diego, CA)
- Duplicate testing across 2 reagent lots by 2 individuals

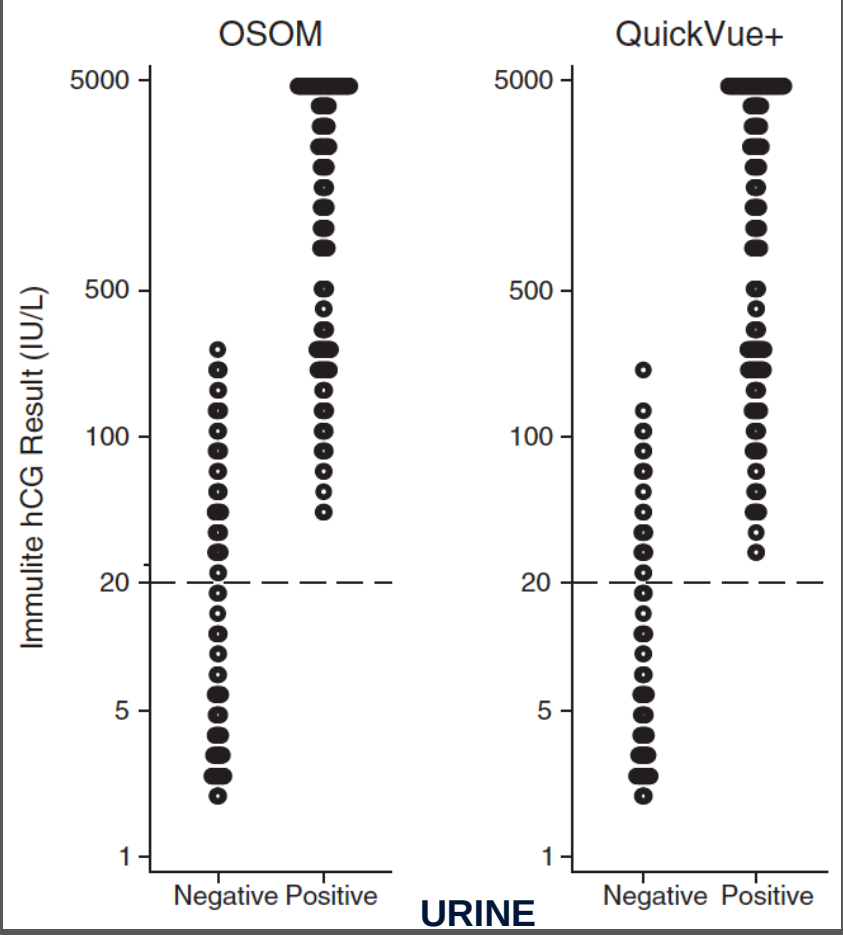
Urine (N=289)



Serum (N=269)



Analytical Sensitivity in Urine

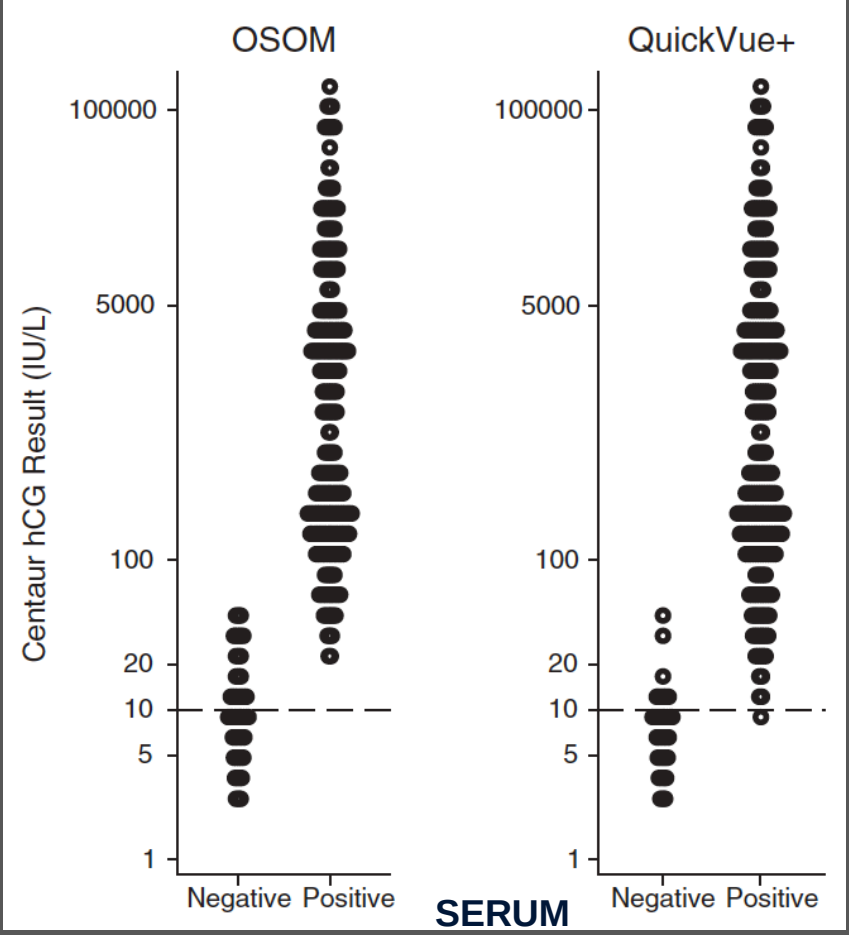


	Diagnostic Sensitivity	
	>20 IU/L	*20-300 IU/L*
OSOM	80%	53%
QuickVue+	90%	78%

	[hCG] to Achieve 99% Sensitivity	
	Empirical	*Statistical*
OSOM	225 IU/L	700 IU/L
QuickVue+	150 IU/L	290 IU/L

Greene D, et al. *Clin Chim Acta* 2012;415:317-321

Analytical Sensitivity in Serum



	Diagnostic Sensitivity	
	>10 IU/L	*10-300 IU/L*
OSOM	90%	78%
QuickVue+	96%	91%

	[hCG] to Achieve 99% Sensitivity	
	Empirical	*Statistical*
OSOM	45 IU/L	90 IU/L
QuickVue+	20 IU/L	53 IU/L

Greene D, et al. *Clin Chim Acta* 2012;415:317-321

Qualitative Detection of hCG in Early Pregnancy

- Devices perform best when hCG is >300 IU/L
- Poorer performance when hCG is 20 to 300 IU/L
  - Zone of sub-optimal performance
- Serum is a more suitable sample type for detecting pregnancy
- ~4% prevalence of samples with urine hCG 20-225 IU/L and serum hCG 10-45 IU/L in clinical settings
  - Qualitative urine hCG testing is a high volume test so even infrequent false-negative results will affect a considerable number of patients

Greene D, et al. *Clin Chim Acta* 2012;415:317-321

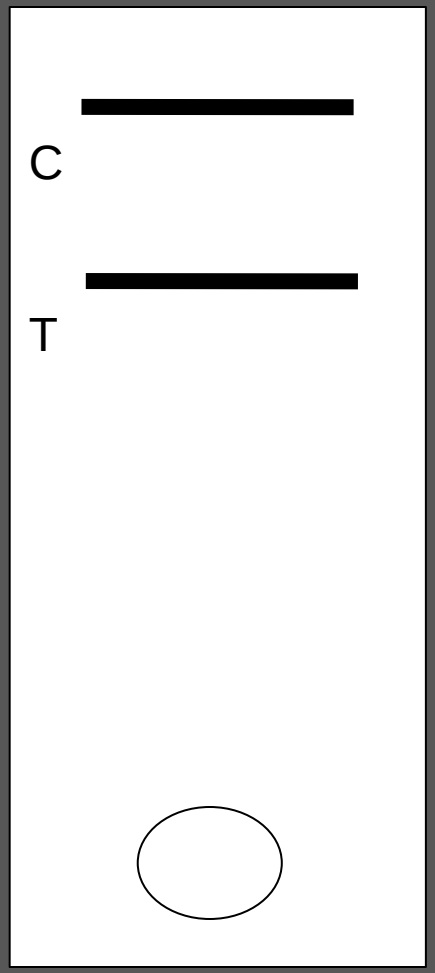
What are the analytical sources of error?

I am pregnant!

- 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

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

High-dose Hook Effect

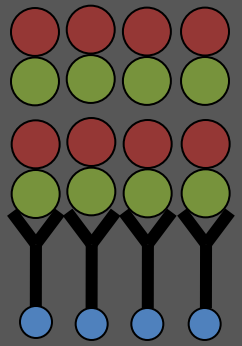


Anti-Ab

Anti-α

No Line

Anti-β
w/ latex bead



The POCT device was shown to hook at an hCG concentration of ~1,800,000 IU/L and patient's urine hCG concentration was 176,498 IU/L

Very high hCG concentration

I am pregnant!

- 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

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

hCG Variant Effect

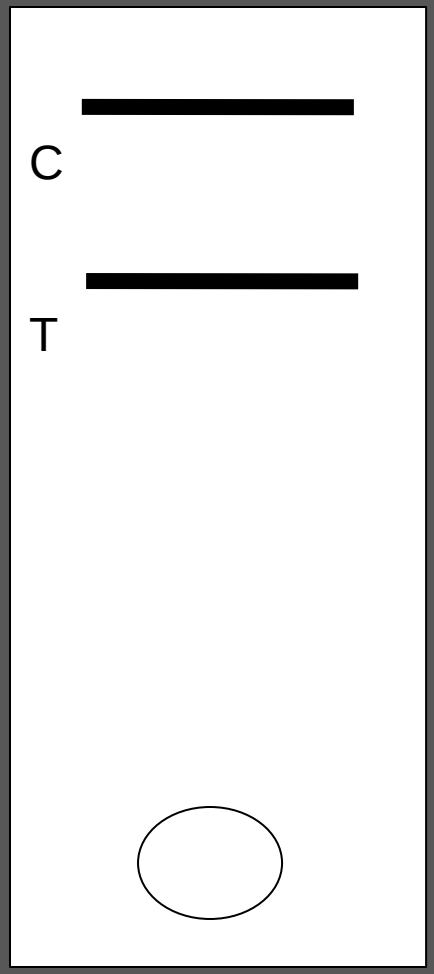
- hCG variants in patient's urine
  - hCG 0.7 µmol/L (21%)
  - hCGβ 0.05 µmol/L (1%)
  - hCGβcf 2.6 µmol/L (78%)

hCG: 17,800 IU/L
hCGβcf: 0.04 µmol/L

hCG: 17,800 IU/L
hCGβcf: 1.0 µmol/L

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

hCG Variant Effect



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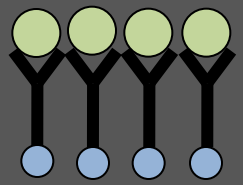


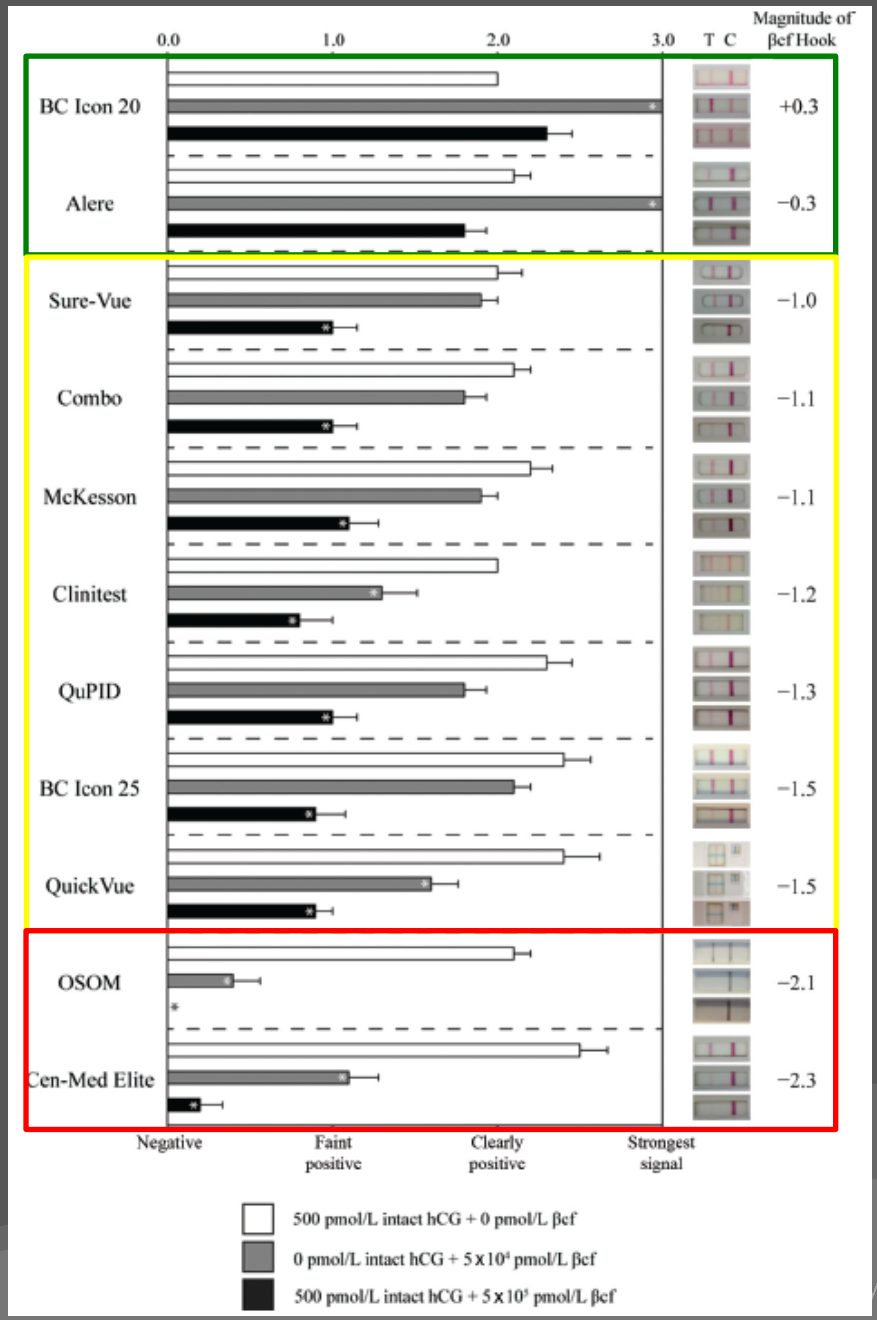
hCG

+

Very high concentration
of hCG variant

hCG Variant Effect

- Prepared urine samples
  - Intact only
  - hCGβcf only
  - Intact + hCGβcf
- Tested in duplicate with 11 POC devices
  - Each interpreted by 10 individuals (0, 1, 2, or 3)
- 9 susceptible to variant effect



Nerenz RD, et al. *Clin Chem* 2014;60:667-674

An Unlikely Pregnancy

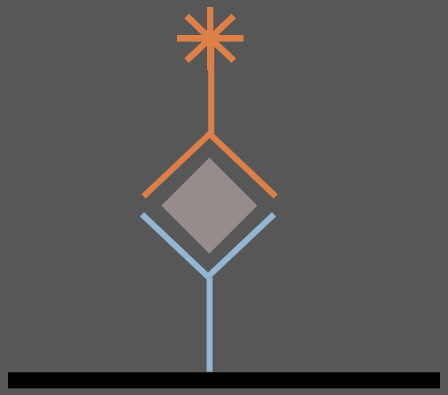
- 46-year-old woman surgical patient is 2 weeks late for her menstrual cycle but denies sexual activity

- Serum POC hCG test interpreted as positive
  - Quantitative hCG <2 IU/L

- Urine POC hCG test interpreted as negative

Greene D, et al. *Clin Chem* 2010;56:1645-1646

Interfering Antibodies



Real hCG present

Interfering antibody cross-links reagent antibodies

Falsely increased/positive result

Does their use affect outcomes?

Effect on Patient Outcome

- Urine hCG tests are used to determine pregnancy status
- Frequently assumed to improve patient care (e.g. length of stay; avoidance of contraindicated interventions)
- Remarkable lack of supportive evidence

Turnaround Time:
ED POCT vs. Lab Testing

- 476 urine samples
- Tested in ED and in the central lab

	TAT Sample collect → Charted result		TAT Sample sent → Test completed	
	ED POCT	Central Lab	ED POCT	Central Lab
Mean time (min)	7.6	67.4	7.6	32.6
p	<0.0005		<0.0005	

- Did not evaluate time required to collect urine sample

Lazarenko GC, et al. *Can J Emerg Med* 2001;3:292-295

Turnaround Time & Length of Stay: Central Lab vs. ED Lab Testing

	TAT Received → Report		ED Length of Stay	
	Central Lab	ED Lab	Central Lab	ED Lab
N	44	54	44	54
Mean time (min)	78	5	386	346
p	<0.05		0.22	
	94% reduction in time required to report a qualitative urine hCG test result		No change in LOS	

- Clinician satisfaction with TAT increased
- Did not evaluate time required to collect urine sample

Lee-Lewandroski E, et al. *Arch Pathol Lab Med* 2003;127:456-460

Length of Stay:
Central Lab vs. ED POCT

	Patients with hCG testing performed		Control group	
	Pre-POCT	Post-POCT	Pre-POCT	Post-POCT
N	991	1,103	4,133	4,568
Mean LOS (min)	364	415	286	322
Difference in mean LOS	51 (p<0.001)		36 (p<0.001)	
	p=0.33			

Plerhoples W, et al. *Am J Emerg Med* 2004;22:460-464

Length of Stay:
Central Lab vs. ED POCT

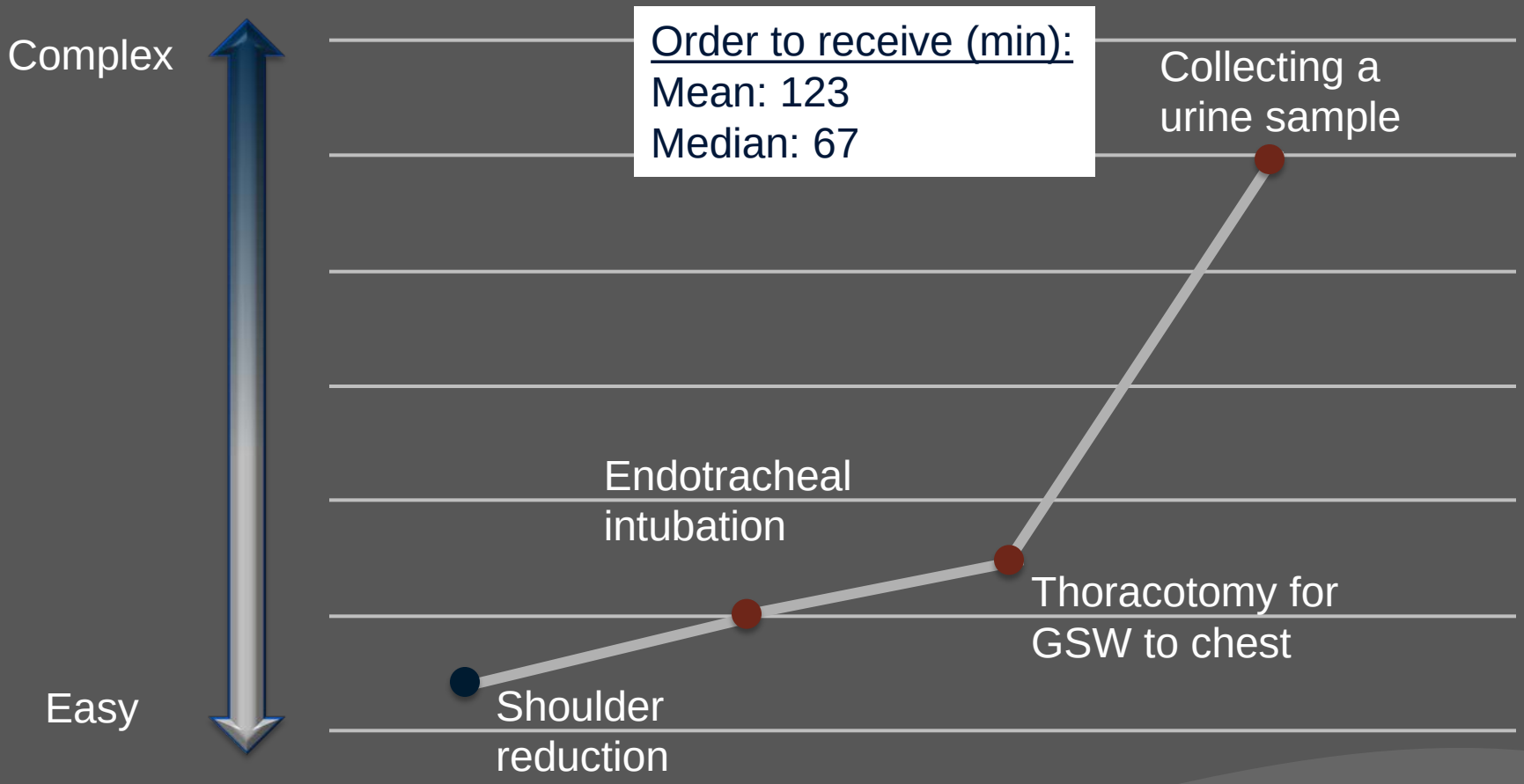
ED staff perspectives on how POCT:	Strongly Agree or Agree (%)
Improved patient care	87
Shortened LOS	96
Improved communication	61
Improved time management	78

- Change in how results obtained (e.g. push not pull)
- Visibility of testing area to ED staff

Plerhoples W, et al. *Am J Emerg Med* 2004;22:460-464

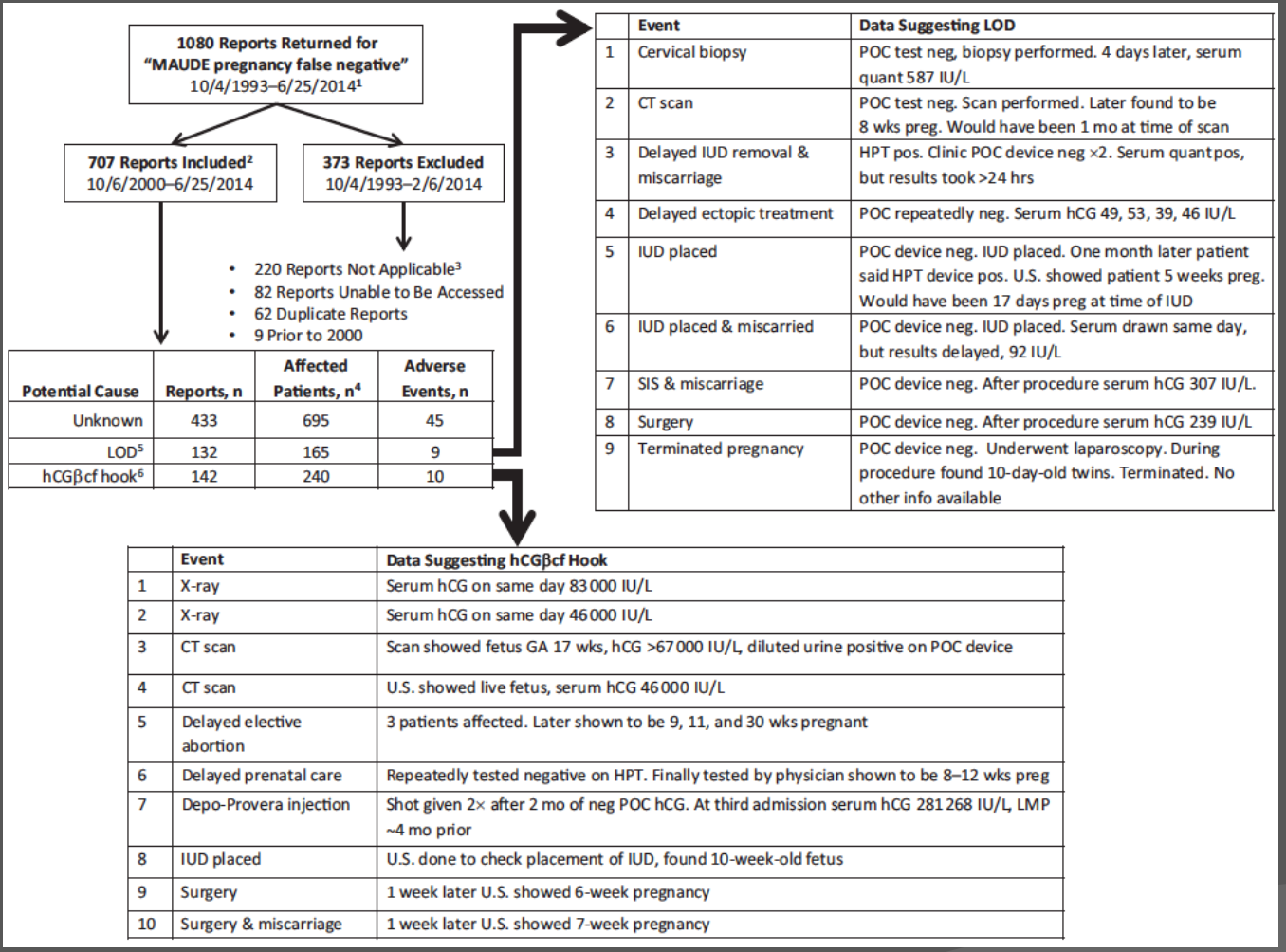
Difficulty of ED Procedures:

Survey of ED Physicians



Schwartz I. Personal communication (2013)

Adverse Outcomes Due to Urine POCT



Potential Cause	Reports, n	Affected Patients, n[4]	Adverse Events, n
Unknown	433	695	45
LOD[5]	132	165	9
hCGβcf hook[6]	142	240	10

	Event	Data Suggesting LOD
1	Cervical biopsy	POC test neg, biopsy performed. 4 days later, serum quant 587 IU/L
2	CT scan	POC test neg. Scan performed. Later found to be 8 wks preg. Would have been 1 mo at time of scan
3	Delayed IUD removal & miscarriage	HPT pos. Clinic POC device neg ×2. Serum quant pos, but results took >24 hrs
4	Delayed ectopic treatment	POC repeatedly neg. Serum hCG 49, 53, 39, 46 IU/L
5	IUD placed	POC device neg. IUD placed. One month later patient said HPT device pos. U.S. showed patient 5 weeks preg. Would have been 17 days preg at time of IUD
6	IUD placed & miscarried	POC device neg. IUD placed. Serum drawn same day, but results delayed, 92 IU/L
7	SIS & miscarriage	POC device neg. After procedure serum hCG 307 IU/L.
8	Surgery	POC device neg. After procedure serum hCG 239 IU/L
9	Terminated pregnancy	POC device neg. Underwent laparoscopy. During procedure found 10-day-old twins. Terminated. No other info available

	Event	Data Suggesting hCGβcf Hook
1	X-ray	Serum hCG on same day 83 000 IU/L
2	X-ray	Serum hCG on same day 46 000 IU/L
3	CT scan	Scan showed fetus GA 17 wks, hCG >67 000 IU/L, diluted urine positive on POC device
4	CT scan	U.S. showed live fetus, serum hCG 46 000 IU/L
5	Delayed elective abortion	3 patients affected. Later shown to be 9, 11, and 30 wks pregnant
6	Delayed prenatal care	Repeatedly tested negative on HPT. Finally tested by physician shown to be 8–12 wks preg
7	Depo-Provera injection	Shot given 2× after 2 mo of neg POC hCG. At third admission serum hCG 281 268 IU/L, LMP ~4 mo prior
8	IUD placed	U.S. done to check placement of IUD, found 10-week-old fetus
9	Surgery	1 week later U.S. showed 6-week pregnancy
10	Surgery & miscarriage	1 week later U.S. showed 7-week pregnancy

Nerenz RD, et al. *Clin Chem* 2015;61:483-486

Cowboy Lab Medicine

- 35-year-old female with several episodes of vomiting followed by syncope
- Breast-feeding since giving birth 8 months earlier
- Denies fever, cough, vaginal bleeding, abdominal pain, or possible pregnancy
- Physical exam
  - Exquisitely tender abdomen in all quadrants (soft and nondistended)
  - Blood pressure: 70/43
  - Heart rate: 85 bpm
- Whole blood mixed with saline and tested for hCG using a qualitative device
- Transvaginal ultrasound identified a ruptured ectopic pregnancy

Habboushe JP, et al. *Am J Emerg Med* 2011;29:840.e3-840.e4

Cowboy Lab Medicine: A Response

- Blood-based hCG tests offer several advantages over urine tests
- Authors modified intended use of the qualitative hCG test device without validation
  - Violated federal and NY state regulations
- Potential for severe consequences
  - Loss of laboratory accreditation
  - Patient harm
- Adapting intended use of a diagnostic test for fit specific clinical needs is tempting
  - Should not do without input and guidance from laboratory professionals

Grenache DG, et al. *Am J Emerg Med* 2013;31:992-993

Summary

- hCG is a molecularly heterogeneous hormone
- hCG variants can influence the performance of qualitative hCG test devices
- The analytical sensitivity of qualitative urine hCG tests is questionable
- Qualitative hCG tests performed at the POC produce rapid results but have no effect on ED length of stay

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