

# Point-of-Care hCG Testing: Optimizing Patient Safety, Knowing Benefits and Limitations

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# Learning Objectives

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At the end of this presentation, participants should be able to:

1. Describe the current state of POC hCG testing
2. Identify potential limitations with POC hCG methods
3. Analyze the ways to optimize pregnancy testing to increase patient safety
4. Differentiate important operating characteristics of various POC hCG testing formats

# Agenda

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- POC TESTING MENU
- HISTORY OF PREGNANCY TESTING
- WHY DO WE DO POC HCG TESTING?
- BENEFITS OF POC HCG TESTING
- HOW EARLY CAN PREGNANCY BE DETECTED?
- SERUM VERSUS URINE
- QUALITATIVE VERSUS QUANTITATIVE
- ANALYTICAL SENSITIVITY: FALSE POSITIVES VERSUS FALSE NEGATIVES
- KNOW YOUR ASSAY AND ITS LIMITATIONS
- VARIANT HOOK EFFECT
- IQCP DOCUMENTS
- FUTURES

# Point of Care Test Menu (Partial)

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- › **Glucose**
- › **hCG**
- › **Occult blood**
- › **Urine dip strip**
- › **Strep A**
- › PT/INR
- › Hemoglobin/HCT
- › H. pylori
- › Infectious mono
- › Influenza
- › HIV
- › RSV
- › Platelet function
- › Lipid panel
- › Creatinine
- › BNP
- › Troponin
- › Myoglobin
- › CKMB
- › hs-CRP
- › HbA1c
- › Blood gases
- › Electrolytes
- › Drugs of abuse
- › CBC
- › WBC
- › Microalbumin
- › D-dimer
- › aPTT
- › ACT
- › Co-oximetry
- › TSH

# History of the Pregnancy Test Kit

1350 BC

An Egyptian document describes a test where woman urinates on wheat and barley seeds over several days

Wheat	Barley	Interpretation
✓	--	Girl
--	✓	Boy
--	--	Not pregnant

# History of the Pregnancy Test Kit

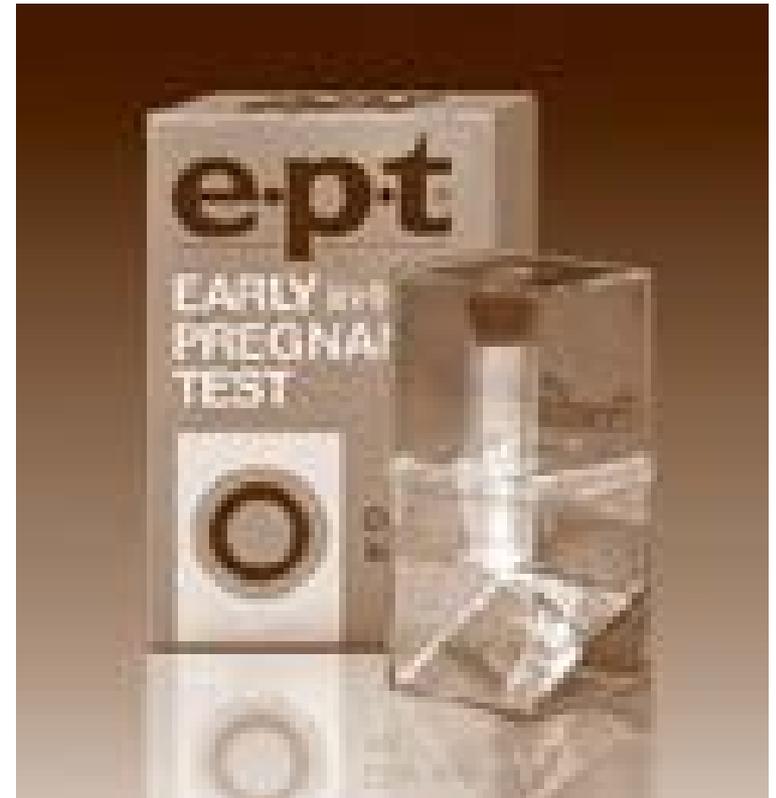
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Middle Ages through the 19<sup>th</sup> century:

- Prophets claimed to be able to diagnose many different conditions and diseases by the color of urine
- Visual aspects of urine used to detect pregnancy
  - “clear, pale-lemon color leaning toward off-white, having a cloud on its surface” indicated pregnancy
- Other tests included mixing wine with urine and observing the results
- Observation of their own physical signs and symptoms (such as morning sickness)
- 1890s – Ernest Starling named the “internal secretions” by certain organs “hormones”

# History of the Pregnancy Test Kit

- › 1920s and 1930s
  - hCG associated with pregnancy
  - Bioassays developed
- › 1960s
  - First immunoassays
    - Hemagglutination
    - Radioimmunoassay
- › 1977
  - The first home pregnancy test kit, e.p.t. is released in the U.S.



# Why POC Urine hCG Testing in Acute Settings?

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To quickly determine whether a woman is pregnant or not

Aids in:

- Determining if symptoms such as abdominal pain, vaginal bleeding and/or vomiting are due to pregnancy
  - Preventing fetal exposure to sources of radiation (x-ray, CT scan, etc.)
  - Preventing the administration of a teratogenic medication
- › Occasionally detects gestational and non-gestational trophoblastic disease or ectopic pregnancy
- › Not normal pregnancies. Values will not increase over time.
- › Can be used to confirm heterophilic antibody interference in serum-based hCG assays

# Why POC Urine hCG Testing in Acute Settings?

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To quickly determine whether a woman is pregnant or not

- Aids in:
  - Commencement of good prenatal care
  - Instituting appropriate precautions early in gestation
    - Avoiding alcohol and tobacco
    - Discontinuing any fetotoxic prescribed and non-prescribed drugs
    - Taking folic acid

# Benefits of POC hCG Testing

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- › Fast
- › Easy to perform
- › Inexpensive
- › Noninvasive
- › No heterophilic antibody interference (urine only)

# When Can Pregnancy Be Detected?

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- hCG is produced by trophoblastic cells after implantation of the fertilized egg in the uterus (usually 6–12 days after ovulation)
- hCG can be detected in the blood or urine several days later
- Varies by:
  - Sample type (serum concentrations tend to be higher than urine)
  - Method (quantitative assays generally have higher sensitivity)
- In some cases, pregnancy can be detected >3 days before expected menstrual period. In all cases by one week after expected menstrual period
- hCG production increases very rapidly, doubling every 1 to 1.5 days in the first 8-10 weeks of pregnancy
- Eventually reaching levels up to 100,000 mIU/mL or more by week 10-12 of pregnancy

# Serum Versus Urine Sample Types

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## Serum:

- › hCG appears in blood before urine, sometimes up to five days earlier
- › Urine assays are not as sensitive as serum assays (even with POC) and may be subject to false-negative results

## Urine:

- › Urine is often easier to obtain and test
- › Serum requires phlebotomy
- › Serum requires centrifugation and possibly transportation to the lab
- › Serum assays are subject to heterophilic antibody interferences, urine assays are generally not

# Qualitative Versus Quantitative

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## Qualitative:

- › Qualitative hCG assays are quicker to perform
- › Qualitative assays can be done at POC
- › Quantitative assays generally need to be done in the laboratory
  - One quantitative POC whole blood/plasma hCG test is on market
- › Quantitative assays require a blood sample
  - There are no FDA-cleared quantitative urine assays
- › Even quantitative assays may miss some hCG variants

# Qualitative Versus Quantitative

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## Quantitative:

- › Quantitative assays have greater sensitivity
  - Most qualitative urine assays have analytical sensitivity of 20-25 IU/L while quantitative tests can be 1-2 IU/L
  - Qualitative serum assays have analytical sensitivity of 10 IU/L
  - Quantitative serum assays are sensitive to 1-2 IU/L, some as low as 0.1 IU/L (5 IU/L is most common cutoff for pregnancy)
- › Qualitative assays are more subject to false negative results

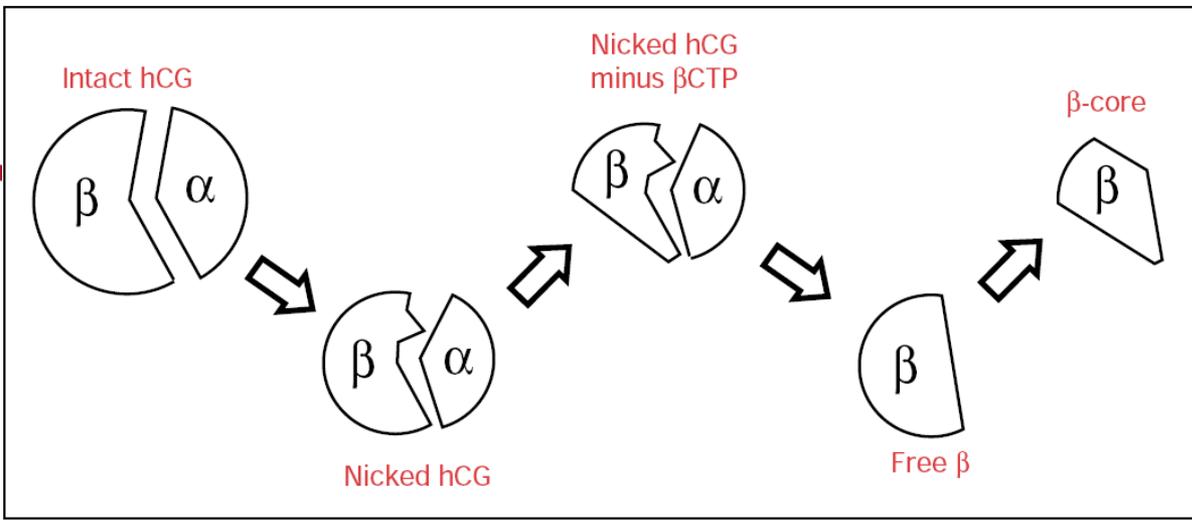


Figure: The metabolism of variant forms of hCG. (Adapted from Cole LA, J. Reproductive Medicine<sup>2</sup>.)

## βhCG Forms

- The typical form of hCG is a mixed dimer of two subunits, α and β.
- In a normal pregnancy, the primary morphologic form of the hormone is the typical α-β dimer, termed intact hCG.
- In certain disease states, hCG variants can become the predominant forms in circulation (see Figure above).
- Various glycosylation states

## Assay Design Can Improve Accuracy

- Different analytic methods for hCG may produce significantly discrepant results. Causes for these differences include:
  - 1) epitope recognition by the antibody(ies) in the assay
  - 2) differential response to variant forms of hCG
  - 3) interferences (e.g., heterophile antibodies)

\*Cole, L.A., Journal Reproductive Medicine, 1994; 39(3) 193-200.

# False Positives Versus False Negatives

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## The Lesser of Two Evils?

- › The 97.5<sup>th</sup> percentile for hCG
  - 1.0 IU/L considering just the menstrual cycle
  - 1.1 IU/L factoring in early pregnancy loss as a source of false positive pregnancy results
  - 1.2 IU/L including perimenopausal women
  
- › The 99<sup>th</sup> percentile yields a 5.0 IU/L cutoff for pregnancy
  - This would detect 98% of pregnancies on the day of missed menses
    - 1 in 50 false-negatives (versus 1 in 7 for a 20 IU/L cutoff)
    - 1 in 400 false-positives due to LH peak hCG production, early pregnancy loss or perimenopause

# False Positives Versus False Negatives

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## The Lesser of Two Evils?

- Lowering the cutoff raises the possibility of false positive results due to LH peak hCG concentrations and pituitary hCG postmenopausal
  - This led to the FDA recommending a **higher cutoff (20-25 IU/L)**
  - Authors argue that:
    - Pregnancy testing is not normally done **at time of LH peak**
    - Pregnancy testing should not be done **post-menopause**
    - **Pituitary hCG** levels in perimenopausal women rarely exceed 5 IU/L
    - Manufacturers recommend **repeat testing 48 hours later** to confirm positive results
    - “The **potential problems of a false-negative test**, fetotoxicity, delay in prenatal care and baby retardation appear much more relevant than the upsets, stress and inconveniences of a **false positive test.**”



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

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

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- In addition to intact hCG, variants are detected during pregnancy:
  - hyperglycosylated hCG (hCG-h)
  - nicked hCG (hCGn)
  - hCG missing the  $\beta$ -subunit C-terminal peptide (CTPhCG)
  - free  $\beta$ -subunit (hCG $\beta$ )
  - hyperglycosylated free  $\beta$ -subunit
  - nicked hCG $\beta$  (hCG $\beta$ n)
  - core fragment of hCG $\beta$  (hCG $\beta$ cf) (predominantly detected in urine)



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

Dina N. Greene <sup>a,\*</sup>, Robert L. Schmidt <sup>b</sup>, Sandy M. Kamer <sup>a</sup>, David G. Grenache <sup>b</sup>,  
Carolyn Hoke <sup>a</sup>, Thomas S. Lorey <sup>a</sup>

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<sup>b</sup> University of Utah School of Medicine, Department of Pathology, Salt Lake City, UT, United States

Most POC hCG assays include the disclaimer, “If a negative result is obtained, but pregnancy is suspected, another sample should be collected and tested 48–72 h following.”

Although it is empirically recognized that false-negative results are possible early in conception, most assume that this corresponds to a period of gestation preceding hCG production



# Know Your Assay's Limitations

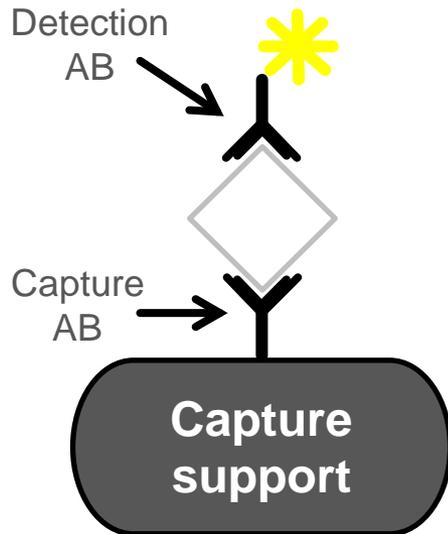
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(Examples from ICON 20 hCG Product Instructions)

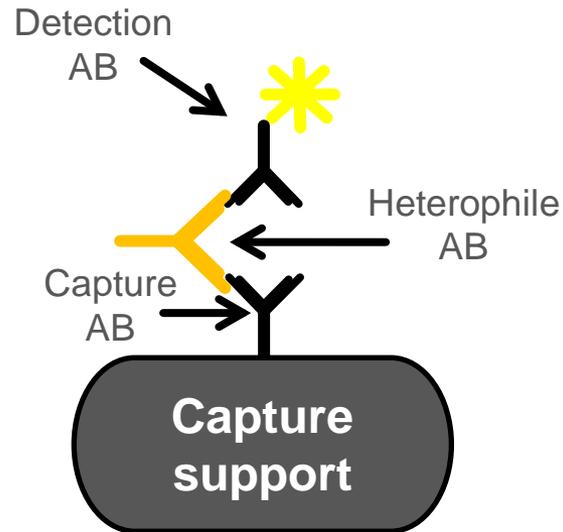
- › As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Similarly, specimens from patients who have been routinely exposed to animals or to animal serum products may contain heterophile antibodies which may cause erroneous results
- › An extremely low concentration of hCG during the early stage of pregnancy can give a negative result. In this case, testing of another specimen obtained at least 48 hours later is recommended
- › The hCG level may remain detectable for several weeks after normal delivery, delivery by caesarean section, spontaneous abortion or therapeutic abortion

# Heterophilic Antibodies

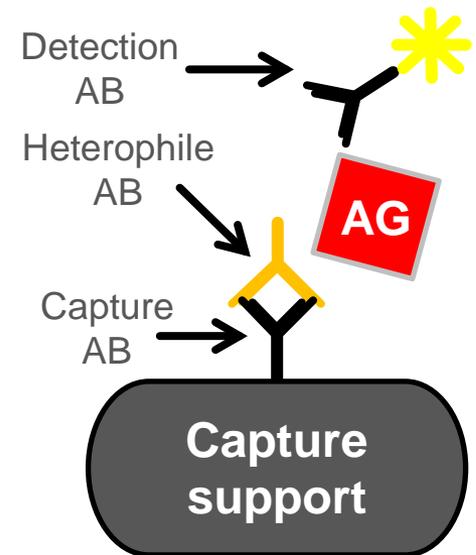
A. No Interference



B. False high/positive



C. False low/negative



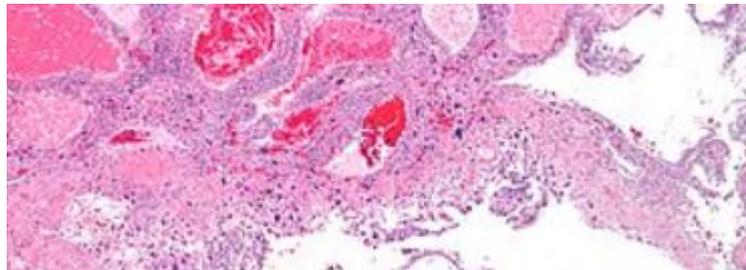
# Know Your Assay's Limitations

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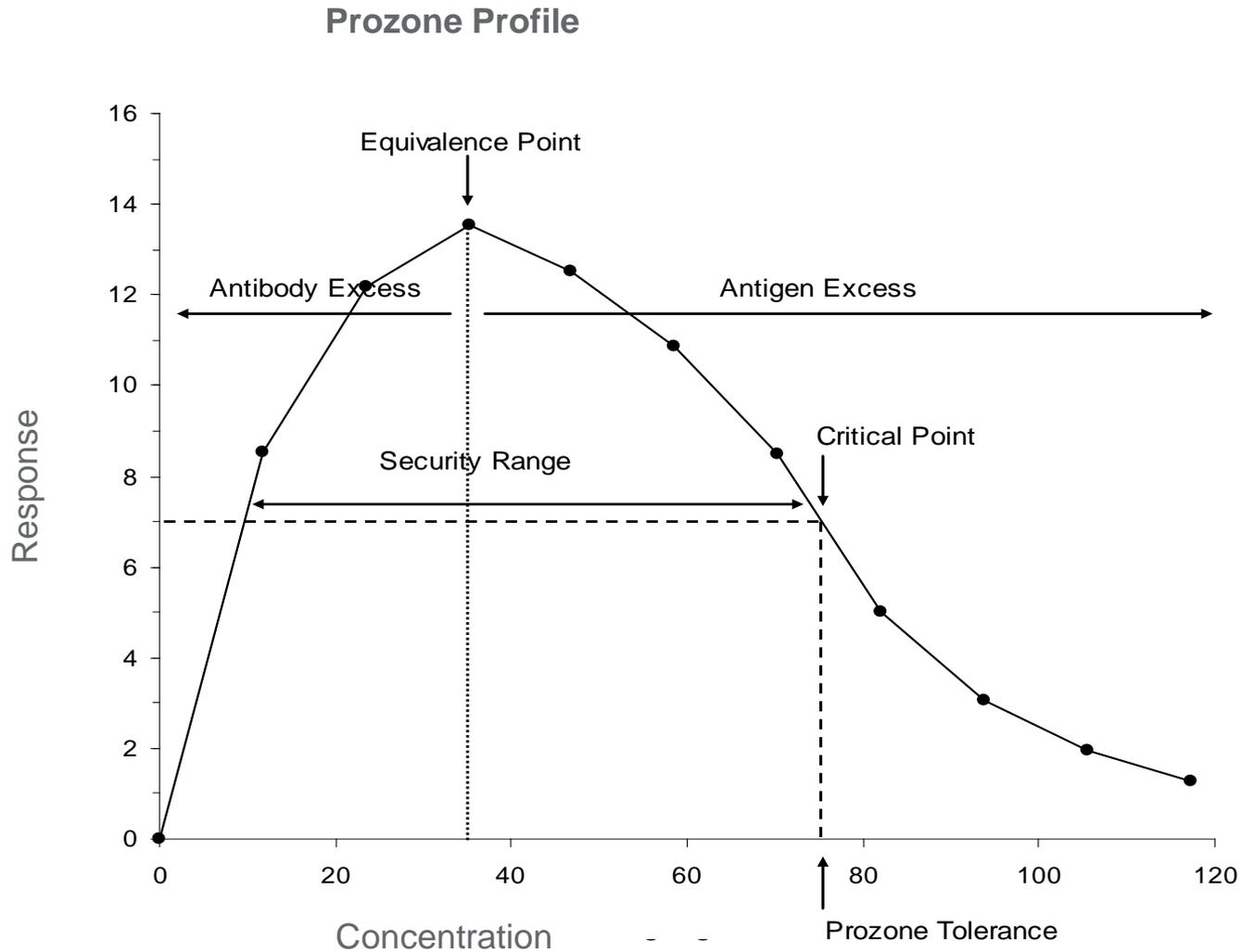
- › **The hCG level in the case of spontaneous abortion may be very low and eventually decrease.** The test is highly sensitive, and specimens which test positive during the initial days after conception may later be negative due to natural termination of the pregnancy. Natural termination occurs in 22% of clinically unrecognized pregnancies and 31% of pregnancies overall. **Subsequent testing of a new urine sample after an additional 48 hours is recommended in order to confirm that the hCG level is rising as indicated in a normal pregnancy**
- › **The concentration of hCG may be very low in the case of ectopic pregnancy.** A suspected ectopic pregnancy may be further evaluated using a serial, quantitative serum  $\beta$ -hCG measurement

# Know Your Assay's Limitations

- › Elevated hCG levels have been reported in patients with **both gestational and nongestational trophoblastic diseases**. The hCG of trophoblastic neoplasms is similar to that found in pregnancy, so these conditions, including choriocarcinoma and hydatidiform mole, should be ruled out before pregnancy is diagnosed.
- › **Very high levels of hCG may exist in certain pregnancies and pathological conditions** (e.g., choriocarcinoma and hydatidiform mole). This may weaken the intensity of the test line.
- › The physician should evaluate data obtained with this kit in light of **other clinical information**.



# The Hook Effect



# Know Your Assay's Limitations

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- › Samples which contain excessive **bacterial contamination** or which have been subjected to **repeated freezing and thawing** should **not be used** because such specimens can give spurious results
- › Urine samples collected after **consumption of a large amount of fluids** may contain a lower hCG concentration. If such a sample is negative, a first morning specimen should be obtained and retested
- › **Degradation of hCG in serum samples** may occur by a certain protease **during prolonged storage even at 4°C** and give a negative test result

# Know Your Assay

## › Analytical sensitivity

Standard controls (calibrated to the **WHO 3 International Standard**) ranging from 5 mIU/mL to 80 mIU/mL in serum or urine were tested in 20 replicates. The results confirm sensitivity of 10 mIU/mL for serum and 20 mIU/mL for urine in three-minute assay times

## › Analytical specificity

The assay is free from interference with other commonly known homologous hormones when tested at the levels specified below

Homologous Hormones	Urine	Serum
hFSH	1000 mIU/mL	1000 mIU/mL
hLH	500 mIU/mL	500 mIU/mL
hTSH	1000 $\mu$ IU/mL	1000 $\mu$ IU/mL

# Know Your Assay

## Other interfering substances

Potentially interfering substances were prepared at the following concentrations in serum which contained either 0 or 10 mIU/mL hCG and urine which contained either 0 or 20 mIU/mL hCG. These samples were tested with the ICON 20 hCG Serum/Urine Test. No interference was found

SUBSTANCE ADDED	CONCENTRATION ADDED	
	<b>in Urine</b>	<b>in Serum</b>
Acetaminophen	20 mg/dL	20 mg/dL
Acetylsalicylic Acid	20 mg/dL	20 mg/dL
Ampicillin	20 mg/dL	20 mg/dL
Ascorbic Acid	20 mg/dL	20 mg/dL
Atropine	20 mg/dL	20 mg/dL
Caffeine	20 mg/dL	20 mg/dL
Gentisic Acid	20 mg/dL	20 mg/dL
Phenothiazine	20 mg/dL	20 mg/dL
Phenylpropanolamine	20 mg/dL	20 mg/dL
Salicylic Acid	20 mg/dL	20 mg/dL
Tetracycline	20 mg/dL	20 mg/dL
URINARY ANALYTES:		
Bilirubin	2 mg/dL	30 mg/dL
Glucose	2000 mg/dL	2000 mg/dL
Hemoglobin	25 mg/dL	250 mg/dL
Ketones	100 mg/dL	-
Albumin	2000 mg/dL	14000 mg/dL
Triglyceride	-	2000 mg/dL

# Know Your Assay

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## › Precautions

For in vitro diagnostic use only

The ICON 20 hCG device should remain in its sealed pouch until ready for use

## › Storage and stability

ICON 20 hCG Serum/Urine Test Kit is to be stored at 2°C to 30°C in the sealed pouch

## › Specimen storage

If testing will not be performed immediately, the specimens **may be refrigerated (2°C to 8°C) for up to 48 hours** prior to assay

For prolonged storage, specimens **may be frozen and stored below -20°C**. Frozen specimens must be completely thawed and thoroughly mixed before using.

**Avoid repeated freezing and thawing**

If specimens are to be shipped, they should be packed in compliance with Federal regulations covering the transportation of etiologic agents. For urine samples, add sodium azide to a concentration of 0.1% as a preservative. Ship sample by the quickest means possible, in a cold pack or frozen

# Know Your Assay

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- › Specimen collection and preparation
  - Approximately 110  $\mu$ l of sample is required for each test
  - Specimens containing particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to assaying
  - Frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing by allowing the specimens to stand at room temperature for at least 30 minutes
- Serum sample
  - Remove serum from the clot as soon as possible to avoid hemolysis. When possible, clear, non-hemolyzed specimens should be used

# Know Your Assay

## › Specimen collection and preparation

- Urine sample

- For optimal early detection of pregnancy, a **first morning urine specimen** is preferred since it generally contains the highest concentration of hCG. However, **randomly collected urine specimens** may be used
- Collect the urine specimen in a clean glass or plastic cup
- Urine containing excessive bacterial contamination should not be used since spurious results may occur with such specimens



## Screening Method to Evaluate Point-of-Care Human Chorionic Gonadotropin (hCG) Devices for Susceptibility to the Hook Effect by hCG $\beta$ Core Fragment: Evaluation of 11 Devices

Robert D. Nerenz,<sup>1</sup> Haowei Song,<sup>2</sup> and Ann M. Gronowski<sup>1\*</sup>

- › The predominant hCG variant in urine, **hCG  $\beta$  core fragment (hCG $\beta$ cf)**, has been demonstrated to cause false-negative results in qualitative point-of-care (POC) hCG devices
- › By about seven weeks of gestation, concentrations of **hCG $\beta$ cf** are approximately **10-fold higher than intact hCG** in urine
- › The authors developed a screening method to evaluate qualitative POC hCG devices for their susceptibility to inhibition by hCG $\beta$ cf and evaluated the performance of 11 commonly used devices

# Variant Hook Effect

- › One-step sandwich immunoassays, including qualitative POC hCG devices, may generate weakly positive or negative signal in the presence of high analyte concentration (**traditional hook effect**)
- › As hCG assays have improved, today the hook effect occurs primarily in the presence of pathologically increased hCG such as gestational trophoblastic disease [i.e., hCG concentrations >500,000 IU/L (1,465,000 pmol/L)]
- › Several POC hCG assays have been shown to be susceptible to a **variant hook effect** when, in addition to intact hCG, one of the antibodies used in the assay recognizes an hCG variant (such as hCG $\beta$ cf) and the other antibody does not recognize it at all
- › The **variant hook effect has been shown to cause false-negative results in some qualitative POC hCG devices** due to the presence of hCG variants at concentrations regularly observed during healthy pregnancy

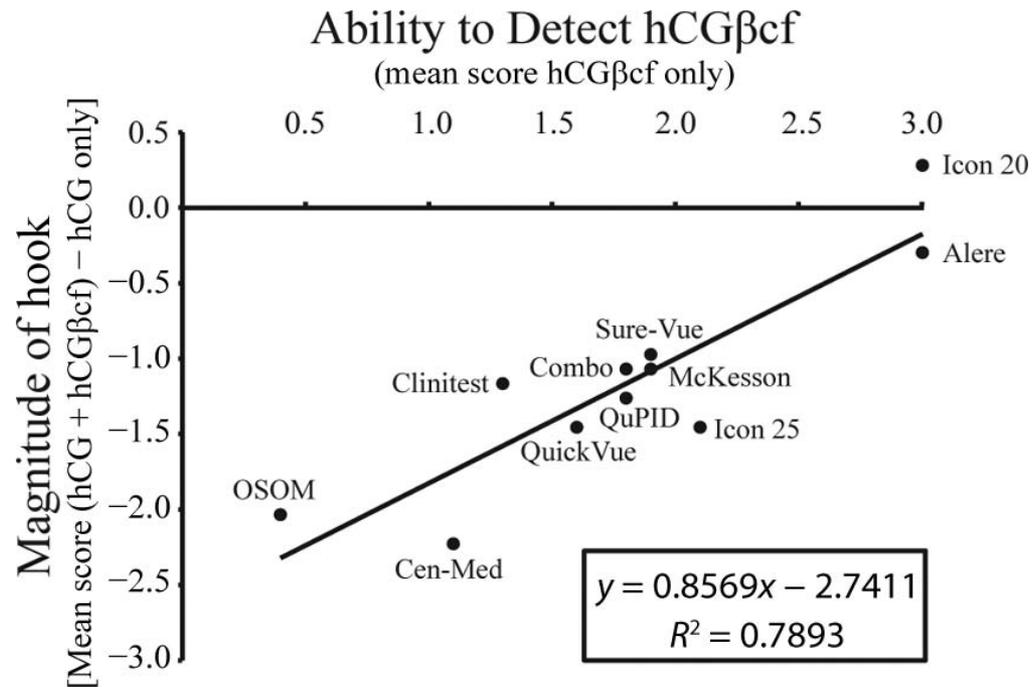


Fig. 3. Correlation between the ability of each device to detect 50,000 pmol/L hCG $\beta$ cf and the magnitude of the hCG $\beta$ cf hook effect [(intact hCG+ hCG $\beta$ cf) – intact hCG only].

The line represents the best-fit correlation. Correlation coefficient was calculated by use of Excel

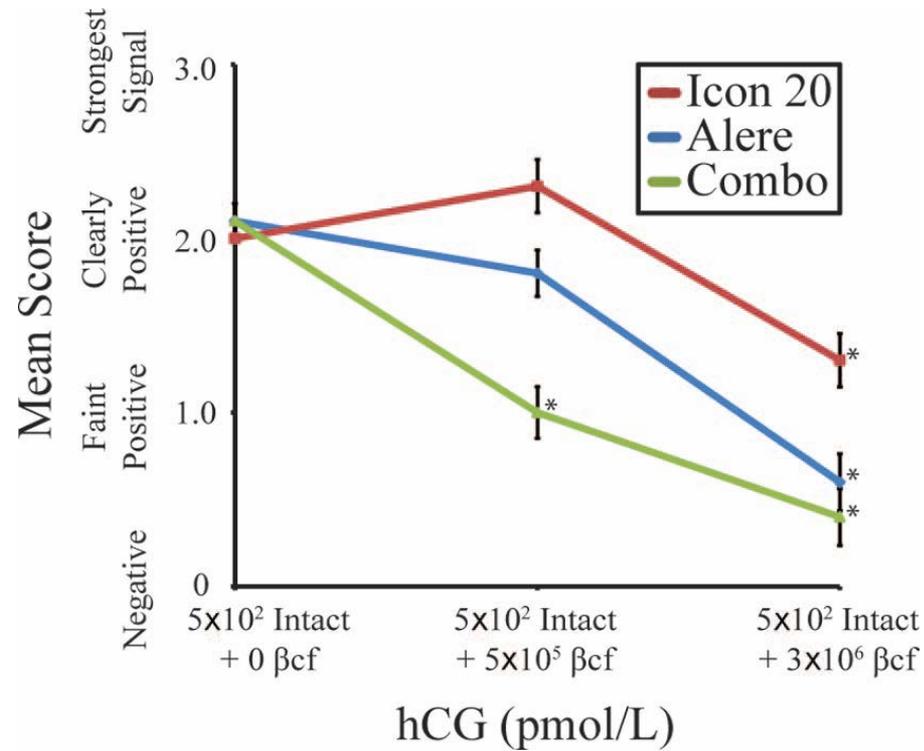


Fig. 4. Performance of the Alere, BC Icon 20, and Cardinal Combo devices with increased concentrations of hCG $\beta$ cf.

# IQCP Template

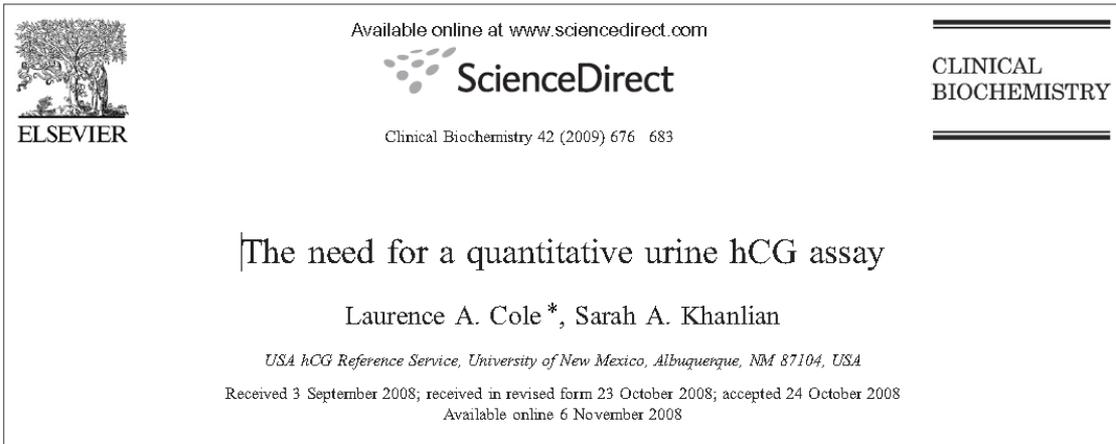
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- › “This guideline is intended for laboratories to follow and develop a customized QC risk assessment using all available resources to assess their entire testing process.”
- › “This template is provided to assist your laboratory with the identification and assessment of potential failures and/or error sources within the test system, and implementation of the resulting quality control procedures to manage these potential failures or errors within the specific laboratory environment.”

# IQCP Template

	Targeted Failure Mode (Hazard)	Measuring System Feature or Recommended Action	Known Limitations of Feature or Recommended Action	Control Process Effective? Yes / No	QCP Actions Required to Address Known Limitations	Residual Risk Acceptable? Yes / No
Step	Failure Mode	Engineering Controls	For Lab Purposes Only			
Training	Intended Use: Risk of erroneous results due to use of test by untrained or non-professional (unauthorized) personnel.	<ul style="list-style-type: none"> <li>› All test operations should be performed by authorized and trained personnel.</li> <li>› Product Instructions (IFU/PI): → "Precautions" - For professional <i>in vitro</i> diagnostic use only.</li> <li>› Laboratory in-service training video provided on Beckman Coulter website.</li> </ul>				
User Safety	Risk of exposure to chemical or biological hazard due to failure to observe Good Laboratory Practices.	<ul style="list-style-type: none"> <li>› Product Instructions (IFU/PI): → "Precautions" - This sections provides details regarding Good Laboratory Practices for safe operation. For example: <ul style="list-style-type: none"> <li>▶ Handle all specimens as if capable of transmitting disease.</li> <li>▶ After testing, dispose of the ICON® 20 hCG device and the disposable dropper following good laboratory practices. Consider each material that comes in contact with specimen to be potentially infectious.</li> </ul> </li> </ul>				
Pre Analytical: Sample	Sample Collection & Sample Integrity: Risk of erroneous results due to pre-analytical variables and when using incorrect specimen collection techniques.	<ul style="list-style-type: none"> <li>› Product Instructions (IFU/PI): → "Sample Collection and Handling" - Serum Sample: Remove serum from the clot as soon as possible to avoid hemolysis. When possible, clear, non-hemolyzed specimens should be used.</li> </ul>				
Pre Analytical: Sample	Sample Collection & Sample Integrity: Risk of erroneous results due to improper sample handling.					
Pre Analytical: Sample	Sample Collection & Sample Integrity: Risk of erroneous results due to presence of human anti-mouse antibodies (HAMA) or heterophile antibodies in specimens.	<ul style="list-style-type: none"> <li>· Product Instructions (IFU/PI): → "Results-Limitations" - As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Similarly, specimens from patients who have been routinely exposed to animals or to animal serum products may contain heterophile antibodies which may cause erroneous results.</li> </ul>				

# The Future of hCG Testing



- › “The USA uniquely does not use quantitative urine hCG tests despite being invaluable in pregnancy testing and in monitoring cancer patients.”
- › In one study, quantitative urinary hCG proved critical in detecting cancer in 3 of 80 cases complicated by false positive serum hCG
- › Analytical sensitivity **may** be increased in both urine and serum POC hCG assays
- › POC hCG testing will continue to improve as assays better detect clinically significant variants of hCG

## Summary

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- › **Both false positives and false negatives** for a pregnancy test are clinically significant concerns for the healthcare profession.
- › Especially in an acute care setting, **false negative results can lead to inappropriate procedures and therapies** for pregnant women, and can create delays in obtaining prenatal care.
- › Similarly, **false positives can delay necessary treatments**. A variety of factors contribute to false negatives including low sensitivities, molecular variants, hook effects and other causes.
- › We have reviewed
  - › 1) The current state of hCG testing in POC settings,
  - › 2) the limitations and advantages of current methods, and
  - › 3) recommendations for optimizing pregnancy testing.

# Learning Objectives

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At the end of this presentation, participants should be able to:

1. Describe the current state of POC hCG testing
2. Identify potential limitations with POC hCG methods
3. Analyze the ways to optimize pregnancy testing to increase patient safety
4. Differentiate important operating characteristics of various POC hCG testing formats

# Thank You!

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