Original Article

Comparison of the performance of serum hCG (ELISA method) and Urinary pregnancy test (ICT method) in the early detection of pregnancy

https://doi.org/10.47648/zhswmcj.2026.v0802.01

*Khan NZ,[1] Ahmed SM,[2] Sultana GS,[3] Akter K[4]

ABSTRACT

Background: Early identification of pregnancy enables timely antenatal care. Both serum β-hCG (ELISA) and urinary pregnancy tests (ICT) are commonly used, but their performance in early pregnancy varies. **Objective:** To compare the diagnostic performance of serum β-hCG (ELISA) and urinary pregnancy test (ICT) in the early detection of pregnancy, using ultrasonography (USG) as the reference standard. **Methods:** A cross-sectional study was conducted over six months among women aged 18–40 years who presented with suspicion of early pregnancy. A total of 100 participants were recruited through convenience sampling. Each participant underwent serum β-hCG (ELISA), an ICT test for urinary β-hCG, and USG for confirmation. The sensitivity, specificity, and overall accuracy of both tests were compared against USG findings. **Results:** Of 100 participants, 57 pregnancies and 43 non-pregnancies were confirmed by USG. Serum β-hCG was more sensitive (96.5% vs. 82.5%) and accurate (98% vs. 90%) than the urinary pregnancy test; both had 100% specificity. Quantitative β-hCG had limited discriminatory power (AUC = 0.601). **Conclusion:** Serum β-hCG performs better than urine ICT in early pregnancy detection. However, variability in hormone levels limits its diagnostic value alone. USG is the definitive standard. Combining β-hCG with USG improves the reliability of confirmation.

Keywords: Serum β-hCG, ELISA, Urinary pregnancy test, Immunochromatographic technique, Early pregnancy detection, Diagnostic accuracy.

Received on: 13th February 2026 **Accepted on:1st March 2026**

Introduction

Accurate detection of pregnancy in its very early phases has substantial implications for obstetric management, maternal health, and the prevention of complications. The biomarker human chorionic gonadotropin (hCG) is central to early pregnancy diagnostics. Variants of hCG (intact, nicked, hyperglycosylated forms) appear soon after implantation.[1] Quantitative serum assays (e.g., ELISA) can detect hCG at lower concentrations and tend to rise earlier than many urine-based tests,

Author's Affiliation

1. *Nadia Zebin Khan, Associate Professor, Department of Biochemistry, BIRDEM General Hospital, Shahbag, Dhaka. Contact No: +88 01717352457E-mail: nadiazebin@yahoo.com
2. Sk. Murad Ahmed, Consultant, Department of Orthopedic Surgery, Bangabandhu Sheikh Mujib Medical University.Contact no: +8801717454232,-mail: drskmurad@gmail.com
3. Gazi Sharmin Sultana, Professor and Head, Department of Laboratory Medicine, BIRDEM General Hospital, Shahbag, Dhaka.Contact No: +88 01712131617,E-mail: gsharmin@gmail.com
4. Khaleda Akter, Assistant Professor, Department of Biochemistry, BIRDEM General Hospital, Shahbag, Dhaka.Contact No: +88 01710483835, E-mail: khaledapia@gmail.com.

Address of Correspondence: *Dr. Nadia Zebin Khan, Associate Professor, Department of Biochemistry, BIRDEM General Hospital, Shahbag, Dhaka. Contact No: +88 01717352457E-mail: nadiazebin@yahoo.com.

making them more sensitive for early detection.[2] Urinary immunochromatographic (ICT) or qualitative tests, while slower to become positive and possibly less sensitive, offer advantages of low cost, rapid results, ease of sampling, and wider accessibility, especially in low-resource settings.[3,4]

Sensitivity thresholds among urinary tests vary widely. A study of eight commonly used qualitative urine hCG tests in Belgium found that although many claimed detection limits around 25 mIU/mL, few actually achieved this sensitivity; performance fell short in many home-use and professional settings.[5] Another study comparing five early detection urinary tests demonstrated that while specificity was generally high (often ≥ 97%), true sensitivity in detecting pregnancy at very early hCG levels was inferior to serum assays.[6] In Mexico, a semi-quantitative urine pregnancy test (threshold around 1,000 IU/L) had a sensitivity of 88.6% (95% CI: 74.6-95.7) and a specificity of 71.7% (95% CI: 57.4-82.8) when compared to serum quantitative beta-hCG in identifying ongoing pregnancy after medical abortion.[7]

The kinetics of the rise in serum versus urine hCG have been explored in longitudinal studies. For example, serial quantitative serum and urine immunoassays in early pregnancy show that although patterns of rise tend to be parallel, serum measurements detect pregnancies slightly earlier and more reliably during very low hCG phases.[8] Differences in "doubling times," baseline levels detectable, and the minimum hCG concentration for positivity contribute to discrepancies between the two methods.[1,8]

Immunoassays (ELISA, radioimmunoassay, immunometric assays) used for serum hCG offer high analytical sensitivity and precision. A novel Ultra Micro ELISA (UMELISA) method was shown to detect intact hCG, β-hCG, nicked forms, etc., with good correlation ($r \geq 0.95$) to established assays (e.g., Elecsys) for both serum and urine, and demonstrated sensitivity and specificity near 100% in early pregnancy detection when compared to transvaginal ultrasound in one large study. Such precision is harder to find in rapid urinary ICT tests.[5,9]

The clinical context matters: early pregnancy loss, ectopic pregnancy, or pregnancies of unknown location benefit from more sensitive detection. In one study of gynecologic patients in an emergency department (ED), serum β-hCG was positive in 100% of ectopic pregnancies, whereas urine tests were only positive in 60%, underscoring that urine ICT may fail in clinically critical cases despite high overall accuracy.[10] Similarly, studies in IVF settings have compared hyperglycosylated hCG (hHCG) versus standard β-hCG assays, showing that the former may detect pregnancy somewhat earlier post embryo transfer, though the differences are modest and often only clinically useful if assays are very sensitive. [11]

Home pregnancy self-testing and qualitative urine tests are widely used and generally reliable, but meta-analyses reveal variability in sensitivity depending on user training, timing relative to missed period or implantation, and the specific test brand.[12,13] More broadly, WHO and other public health bodies emphasize that in low-resource or remote settings, urine tests (including over-the-counter ICT) remain essential tools, but that their limitations (lower sensitivity in very early pregnancy and the possibility of false negatives) must be recognized.[14,15]

Given all this, there is still a need for direct head-to-head comparison of serum β-hCG (ELISA) and urinary ICT tests for early pregnancy detection under real clinical conditions- particularly with respect to sensitivity, specificity, PPV, NPV, and diagnostic accuracy- using a reference standard such as ultrasonography. The present study addresses this gap by enrolling women aged 18-40 years over six months to compare these two methods in early pregnancy.

Materials and Methods

This cross-sectional comparative study was conducted over six months at the Department of Biochemistry, Zainul Haque Sikder Medical Hospital, Bangladesh, among 100 women aged 18–40 years presenting with suspected early pregnancy. Participants were selected by convenience sampling after applying inclusion and exclusion criteria and providing informed consent. Each underwent serum β-hCG testing by ELISA, urinary pregnancy testing using an immunochromatographic (ICT) kit (sensitivity 25 mIU/mL), and ultrasonography, which served as the reference standard. Diagnostic

performance of both tests was evaluated against USG findings, with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated using 2×2 contingency tables. Data were analyzed using SPSS (version 27), and statistical significance was set at $p<0.05$. Ethical approval was obtained from the Institutional Review Board of [Institute], and written informed consent was collected from all participants.

Inclusion Criteria

- Women aged 18–40 years.
- History of missed menstrual period (up to 8 weeks).
- Willingness to undergo serum β-hCG (ELISA), urinary ICT test, and ultrasonography.
- Provided informed written consent.

Exclusion Criteria

- Women with known gynecological disorders.
- Severely ill patients.
- History of recent abortion or miscarriage.
- Currently receiving fertility treatment or hormonal therapy.
- Refusal to participate or inability to provide consent.

Result

The study population consisted of 100 individuals whose pregnancy status was confirmed using Ultrasonography (USG) as the gold standard. A total of 57 cases were confirmed as True Positive (Pregnant) by USG, while 43 cases were confirmed as True Negative (Not Pregnant).

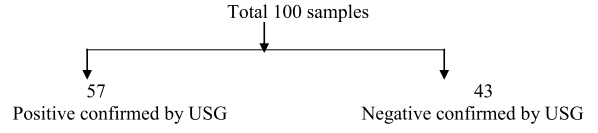

Figure 1: Result of pregnancy test methods USG

Figure 1 presents the results of pregnancy detection using ultrasonography (USG), which served as the gold standard reference method. USG directly confirmed the presence or absence of pregnancy with high accuracy, providing the benchmark against which all other diagnostic tests were compared in this study.

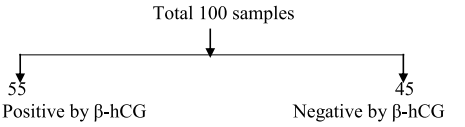

Figure 2: Result of pregnancy test methods βhCG (ELISA)

Figure 2 illustrates the outcomes of serum β-hCG testing by ELISA in comparison to the gold standard USG. The figure demonstrates that the majority of USG-confirmed positive cases were correctly identified by the β-hCG assay, reflecting its strong sensitivity.

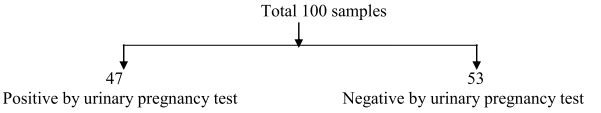

Figure 3: Result of pregnancy test methods βhCG (ELISA)

Figure 3 provides a detailed breakdown of serum β-hCG ELISA results, reinforcing its superior diagnostic performance relative to urine-based methods. The visualization emphasizes both true positive and true negative outcomes, while also highlighting the limited number of misclassifications. This supports the conclusion that serum β-hCG testing is a highly accurate method for pregnancy detection, particularly when evaluated against USG findings

New test	Gold standard test		Total
	Disease Positive	Disease Negative	
Positive	a (TP)	b (FP)	a+b
Negative	c (FN)	d (TN)	c+d
Total	**a+c**	b+d	N(a+b+c+d)

Table 1: Gold standard test method

Table 1 outlines the classic 2×2 contingency framework used for diagnostic test evaluation, where USG serves as the gold standard. The table defines the four diagnostic categories: true positives (a), false positives (b), false negatives (c), and true negatives (d). These values form the basis for calculating performance metrics such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy.

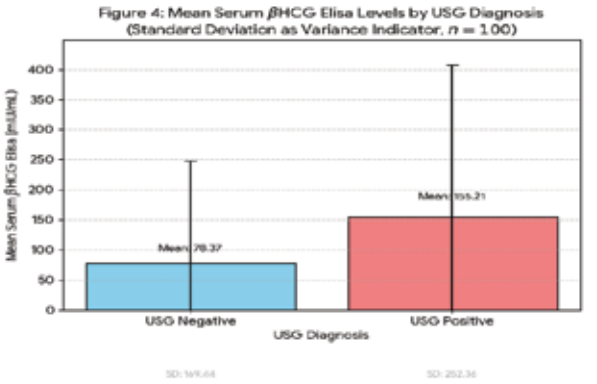

Figure 4: Bar diagram showing the test methods variance (n=100)

Figure 4 illustrates the mean Serum β-hCG Elisa levels, with standard deviation as an indicator of variance, stratified by the USG diagnosis. The bar chart clearly shows that the mean β-hCG level is significantly higher in the USG Positive group (≈155.21 mIU/mL) than in the USG Negative group (≈78.37 mIU/mL), confirming the expected association between higher β-hCG and pregnancy. However, the large standard deviation (SD) error bars, particularly in the USG Positive group (≈252.36 mIU/mL), signify a high degree of variance and a substantial overlap in the distribution of β-hCG values between the two diagnostic categories. This high level of dispersion and overlap indicates that while the mean difference exists, any single β-hCG measurement is unreliable for definitive diagnosis, as many non-pregnant individuals have values that overlap with those who are pregnant, a finding consistent with the low overall discriminatory power reflected by the low Area Under the Curve (AUC) of 0.6014.

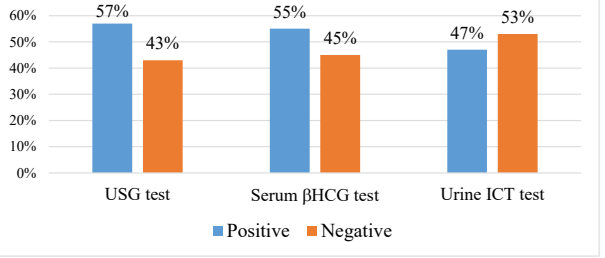

Figure 5: Difference results of test methods (n=100)

Figure 5 highlights the discrepancies between the two testing methods. It shows the number of cases where the results of Serum β-hCG and Urinary ICT differed, emphasizing that some pregnancies were detected by one test but missed by the other. The visual representation underscores the complementary nature of these tests, suggesting that combining methods could improve overall detection rates.

Statistic	USG Negative	USG Positive
N	43.00	57.00
Mean	78.37	155.21
Standard Deviation	169.44	252.36
Minimum	0.50	0.60
Maximum	805.00	950.80

Table 2: Descriptive Statistics for Serum β-hCG ELISA (mIU/mL) by USG Diagnosis

Table 2 shows the descriptive statistics of serum β-hCG levels measured by ELISA, stratified by USG-confirmed pregnancy status. The mean β-hCG concentration was substantially higher in the USG-positive group (155.21 mIU/mL) compared to the USG-negative group (78.37 mIU/mL). However, the wide standard deviations in both groups (252.36 vs. 169.44) indicate considerable variability among individuals, with overlapping ranges between positive and negative cases. This suggests that while mean serum β-hCG values tend to be higher in pregnant women, the variability reduces the discriminatory ability when relying on serum values alone.

Metric	Urine Test (ICT)	Serum β-hCG (Qualitative)
Sensitivity	0.8246	0.9649
Specificity	1.0000	1.0000
PPV	1.0000	1.0000
NPV	0.8113	0.9556
Accuracy	0.9000	0.9800

Table 3: Comparative Diagnostic Metrics for Qualitative Tests (vs. USG)

Table 3 compares the diagnostic performance of the urine pregnancy test (ICT) and the qualitative serum β-hCG test against USG. Both tests demonstrated excellent specificity (100%) and perfect PPV (100%), indicating no false positives. However, the serum β-hCG test outperformed the urine test in sensitivity (96.5% vs. 82.5%), NPV (95.6% vs.

81.1%), and overall accuracy (98% vs. 90%). This suggests that serum β-hCG is more reliable for early pregnancy detection, particularly in ruling out false negatives.

USG Diagnosis	Test Negative	Test Positive
USG Negative	43 (TN)	0 (FP)
USG Positive	10 (FN)	47 (TP)

Table 4: Confusion Matrix for Urine Test (ICT) vs. USG

Table 4 presents the confusion matrix for the urine pregnancy test compared with USG. Out of 100 cases, the urine test correctly identified all 43 non-pregnant cases (true negatives) and 47 true positives. However, it failed to detect 10 pregnancies (false negatives), resulting in reduced sensitivity. This demonstrates that while the urine test has excellent specificity, it may miss some early pregnancies, limiting its reliability as a standalone diagnostic tool.

Metric	Value
AUC	0.6014
Optimal Cutoff (mIU/mL)	2.10
Sensitivity	0.8772
Specificity	0.3023
PPV	0.6250
NPV	0.6500
Accuracy	0.6300

Table 5: ROC and Optimal Cutoff Metrics for Serum β-HCG ELISA

Table 5 shows the ROC–AUC analysis for serum β-hCG as a continuous variable. The AUC of 0.6014 indicates only modest discriminative ability. At the optimal cutoff of ≥2.10 mIU/mL, the test achieved high sensitivity (87.7%) but poor specificity (30.2%), resulting in a considerable number of false positives. The PPV (62.5%) and NPV (65.0%) also reflect moderate diagnostic accuracy. This finding suggests that while lowering the cutoff improves sensitivity, it compromises specificity and overall balance in diagnostic performance.

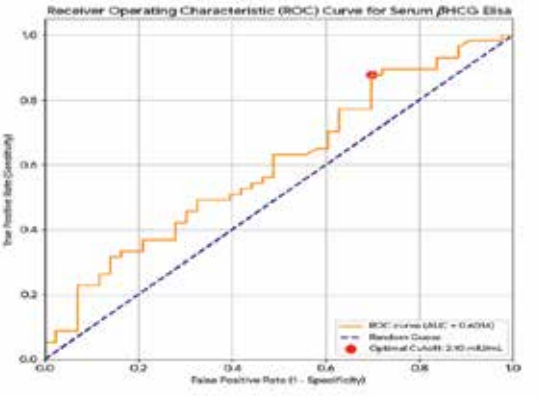

Figure 6: ROC curve

The ROC curve illustrates the diagnostic performance of serum β-hCG levels across different cutoff thresholds. The curve is positioned close to the diagonal line, which corresponds to an AUC of approximately 0.6, confirming limited discriminatory power. While sensitivity is high at lower thresholds, the curve shows a trade-off with specificity, highlighting that serum β-hCG concentration alone, without qualitative interpretation, may not be a reliable predictor of pregnancy status.

USG Diagnosis	Test Negative	Test Positive
USG Negative	13 (TN)	30 (FP)
USG Positive	7 (FN)	50 (TP)

Table 6: Confusion Matrix for Serum βHCG ELISA (Optimal Cutoff ≥2.10 mIU/mL) vs. USG

Table 6 displays the confusion matrix for serum β-hCG using the optimal cutoff value (≥2.10 mIU/mL). Of the 43 USG-negative cases, only 13 were correctly classified as true negatives, while 30 were incorrectly classified as false positives. On the other hand, 50 pregnancies were correctly identified, with 7 missed as false negatives. These results demonstrate that although sensitivity remains high, the low specificity and large number of false positives substantially reduce the clinical usefulness of this cutoff.

Discussion

This study evaluated the diagnostic performance of serum β-hCG (ELISA) and urine-based immunochromatographic (ICT) tests for early pregnancy detection, using ultrasonography

(USG) as the gold standard. Of 100 participants, 57 were confirmed pregnant by USG, and 43 were non-pregnant. The findings highlight the higher diagnostic accuracy of serum β-hCG testing compared with urine ICT, although both methods demonstrated high specificity.

The qualitative serum β-hCG test demonstrated superior performance, with a sensitivity of 96.5%, a specificity of 100%, and an overall accuracy of 98%. These results align with previous studies that report serum β-hCG as a reliable biomarker for early pregnancy detection, particularly when urine-based tests may yield false negatives.[16,17] In comparison, the urine ICT test, while showing excellent specificity (100%) and PPV (100%), had lower sensitivity (82.5%), missing 10 pregnancies confirmed by USG. Similar findings have been reported in other studies, in which urine-based assays often underperform in very early pregnancy because urine hCG concentrations are lower than those in serum.[18,19]

Our ROC–AUC analysis of serum β-hCG levels as a continuous variable showed modest discriminatory power (AUC 0.6014), with high sensitivity but poor specificity at the optimal cutoff of 2.10 mIU/mL. This finding indicates that lowering the cutoff threshold may detect more pregnancies but also increases the number of false positives. Previous studies have also noted the challenge of defining an optimal quantitative cutoff, as serum β-hCG levels vary widely with gestational age and individual physiology.[20,21] Thus, qualitative interpretation of β-hCG appears more clinically useful than relying solely on numerical thresholds, especially in early gestation.

The substantial variability in β-hCG levels observed in our study, with overlapping distributions between pregnant and non-pregnant groups, supports the conclusion that a single serum β-hCG value cannot definitively confirm pregnancy without correlation with clinical or imaging findings. This aligns with earlier reports emphasizing the importance of combining biochemical assays with imaging modalities for accurate pregnancy diagnosis. [22]

In contrast to our findings, some studies have reported that quantitative β-hCG has higher discriminatory ability, particularly when serial measurements are used to track trends rather than relying on a single cutoff. [23,24] Serial doubling of β-hCG in early pregnancy is considered a more reliable indicator, whereas plateauing or declining levels suggest abnormal outcomes.[25] Our study did not evaluate serial values, which may explain the relatively low AUC compared with those studies.

Overall, the results suggest that although both serum β-hCG and urine ICT tests are useful screening tools, serum β-hCG offers superior sensitivity and reliability for early detection. However, USG remains the definitive diagnostic tool, particularly for confirming intrauterine pregnancy and excluding ectopic gestation. A combined approach—using β-hCG testing for initial screening followed by USG for confirmation—may offer the most effective diagnostic pathway.

Conclusion

Serum β-hCG testing demonstrated superior sensitivity and overall accuracy compared with urine ICT testing for early pregnancy detection, though both methods showed perfect specificity. However, wide variability in quantitative β-hCG levels and modest AUC values highlight the limitations of relying on a single cutoff for diagnosis. Ultrasonography remains the gold standard, but combining β-hCG testing with USG provides a more reliable and efficient diagnostic pathway for confirming early pregnancy.

References

1. Padoan A. Laboratory tests for monitoring physiological pregnancy. Journal of Laboratory and Precision Medicine. 2020 Jan 20;5.
2. Nichols JH, Ali M, Anetor JI, et al. AACC guidance document on the use of point-of-care testing in fertility and reproduction. The Journal of Applied Laboratory Medicine. 2022 Sep 1;7(5):1202-36.
3. Betz D, Fane K. Human chorionic gonadotropin. InStatPearls [internet] 2025 Apr 27. StatPearls Publishing.
4. Van Mello NM, Mol F, Opmeer BC, et al. Diagnostic value of serum hCG on the outcome of pregnancy of unknown location: a systematic review and meta-analysis. Human reproduction update. 2012 Nov 1;18(6):603-17.
5. Nickmans S, Vermeersch P, Van Eldere J, Billen J. Performance of qualitative urinary hCG assays. Acta Clinica Belgica. 2014 Aug 1;69(4):277-9.

6. Ryder KW, Munsick RA, Oei TO, et al. Five recent urinary tests for early pregnancy were evaluated. Clinical chemistry. 1983 Oct 1;29(10):1812-4.

7. Grossman D, Berdichevsky K, Larrea F, Beltran J. Accuracy of a semi-quantitative urine pregnancy test compared to serum beta-hCG measurement: a possible screening tool for ongoing pregnancy after medication abortion. Contraception. 2007 Aug 1;76(2):101-4.

8. Sutton-Riley JM, Khanlian SA, Byrn FW, Cole LA. A single serum test for measuring early pregnancy outcome has a high predictive value. Clinical biochemistry. 2006 Jul 1;39(7):682-7.

9. Del Valle García R, Mora JM, San Martín NL, et al. An enzyme immunoassay to determine human chorionic gonadotropin (HCG) in serum and urine samples using an ultra-microanalytical system. Journal of Pharmaceutical and Biomedical Analysis. 2021 Sep 10;204:114239.

10. Naryshkin S, Aw TC, Filstein M, et al. Comparison of the performance of serum and urine hCG immunoassays in the evaluation of gynecologic patients. Annals of emergency medicine. 1985 Nov 1;14(11):1074-6.

11. Ng KW, Chaturvedi N, Coté GL, et al. Biomarkers and point-of-care screening approaches for the management of preeclampsia. Communications Medicine. 2024 Oct 22;4(1):208.

12. Kennedy CE, Yeh PT, Gholbzouri K, Narasimhan M. Self-testing for pregnancy: a systematic review and meta-analysis. BMJ Open. 2022 Feb 1;12(2):e054120.

13. Bastian LA, Nanda K, Hasselblad V, Simel DL. Diagnostic Efficiency of Home Pregnancy Test Kits: A Meta-Analysis. Obstetrical & Gynecological Survey. 1999 Apr 1;54(4):220-1.

14. Yeh PT, Rhee DK, Kennedy CE, et al. Self-testing for proteinuria in pregnancy: a systematic review and meta-analysis. medRxiv. 2022 Aug 16:2022-08.