

Assessing Sensitivity of hCG Pregnancy Rapid Test in Serum, Plasma, and Urine Samples for Early Pregnancy Diagnosis: A Multi-Center Clinical Study

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Abstract: In this study, the objective was to assess the diagnostic efficacy of the hCG Pregnancy Enhanced Sensitivity Rapid Test, which is a chromatographic immunoassay designed to qualitatively detect human chorionic gonadotropin (hCG) in urine, serum, or plasma, enabling the early detection of pregnancy. To selectively identify increased levels of hCG, the test utilizes a combination of monoclonal and polyclonal antibodies. A clinical evaluation, conducted across multiple centers, aimed to compare the hCG Pregnancy Enhanced Sensitivity Rapid Test with other commercially available rapid tests for the qualitative detection of hCG in urine, serum, or plasma. In the urine study, a total of 608 samples were examined, resulting in 377 negative and 231 positive outcomes. The serum or plasma study encompassed 308 samples, with 240 negative and 68 positive results. The findings revealed that the hCG Pregnancy Enhanced Sensitivity Rapid Test exhibited an overall accuracy exceeding 99% when compared to other rapid tests designed to detect hCG in urine, serum, or plasma. The test displayed no cross-reactivity interference with structurally related glycoprotein hormones hFSH, hLH, and hTSH even at high physiological levels, even when exposed to high physiological levels, at the claimed sensitivity threshold of 10 mIU/mL. These findings highlight the test's efficacy in accurately detecting hCG in urine, serum, or plasma. To conclude, the hCG Pregnancy Enhanced Sensitivity Rapid Test developed by Hangzhou AllTest Biotech Co., Ltd demonstrated excellent performance in detecting hCG in different sample types. The test exhibited high accuracy and reliability, rendering it suitable as an auxiliary tool for pregnancy determination in clinical settings. Additionally, its rapid and accurate results make it particularly valuable in facilitating prompt diagnosis and treatment, particularly in resource-limited settings.

1. Introduction

Accurate and early diagnosis of pregnancy is essential for timely clinical intervention and prenatal care. During pregnancy, the human body produces a hormone called human chorionic gonadotropin (hCG). This hormone is primarily secreted by placental tissue.^[1] It plays a crucial role in maintaining pregnancy and supporting embryonic development.

The concentration of hCG is relatively low in the early stages of pregnancy and gradually increases as pregnancy progresses. Its main function is to maintain the corpus luteum and stimulate the production of progesterone, which is vital for maintaining the thickness and stability of the uterine lining, providing an appropriate environment for embryo implantation.^[2] Additionally, hCG stimulates the production of estrogen in the ovaries, promoting the formation and development of the placenta. The concentration of hCG typically begins to rise in the first week of pregnancy, peaks within the first 12 weeks, and then gradually decreases.^[3]

In addition to early pregnancy diagnosis, hCG measurement is also used to monitor the progress of pregnancy and assess the health of the fetus. Abnormal changes in hCG concentrations may be associated with fetal abnormalities, pregnancy complications (such as ectopic pregnancy or the risk of miscarriage), and pregnancy termination.^[4] The complications related to pregnancy and childbirth kill an estimated 287,000 women. It is noteworthy that nearly 95% of these deaths occurred in developing countries.^[5] Ectopic (extrauterine) pregnancy is a significant contributor to maternal mortality.^[6] Therefore, regular testing and monitoring of hCG concentrations can provide crucial information for healthcare providers to guide pregnancy management and make appropriate decisions.

The measurement of hCG in urine or blood samples remains the primary method for diagnosing early pregnancy. To detect the presence of hCG in urine, serum, or plasma samples, hCG pregnancy rapid tests are commonly employed in these diagnostic procedures. The utilization of high-frequency sound waves in ultrasound examination represents an advanced imaging technique that necessitates specialized equipment.^[7] This procedure is commonly conducted after the initial 6-8 weeks of pregnancy. It is primarily used to determine intrauterine pregnancy, exclude ectopic pregnancy, molar pregnancy, and biochemical pregnancy.

The objective of this study is to assess the practical utility of the hCG Pregnancy Enhanced Sensitivity Rapid Test for qualitatively detecting human chorionic gonadotropin (hCG) in urine, serum, or plasma. The aim is to determine its effectiveness in aiding the early detection of pregnancy. The study aims to compare the performance of the AllTest rapid test, which utilizes biological samples obtained from specific analyzed subjects, with other commercially available high-standard rapid test strips in a multicenter clinical laboratory setting. The objective is to determine the effectiveness of this test.

2. Materials and Methods

2.1 Specimen Collection

The preferred urine specimens for analysis were the first-morning samples, as they tend to exhibit the highest concentration of hCG. However, urine collected at any point during the day was considered acceptable for the diagnostic procedure. Urine specimens had to be collected in clean and dry containers. Any urine specimens containing visible particulates underwent centrifugation, filtration, or were allowed to settle, ensuring clear samples for the required testing procedures. The blood collection process was carried out aseptically, with samples obtained in clean tubes; some without anticoagulants for serum separation, while others contained anticoagulants for plasma extraction. To prevent hemolysis, the serum or plasma was promptly separated from the whole

blood after collection. If possible, transparent non-hemolyzed samples were utilized.

Prior to testing, urine, serum, or plasma samples could be refrigerated at temperatures between 2-8 °C for a maximum duration of 48 hours. Longer preservation periods necessitated freezing the specimens at temperatures below -20 °C. Any frozen samples required complete thawing and thorough mixing before proceeding with the assay procedures.

2.2 Screen Test

Before testing, frozen plasma, serum, or urine samples should be left to thaw in the air for 15-30 minutes. AllTest hCG rapid test cassette with enhanced sensitivity, along with other commercially available high-standard hCG test cassettes, is employed for qualitative hCG analysis of plasma, serum, or urine samples. The qualitative detection of hCG is achieved through chromatographic immunoassay using these devices.

As per the manufacturer's protocol, three drops of plasma, serum, or urine samples should be transferred to the sample well of the testing device using the provided dropper. The appearance of a colored line is anticipated. When testing urine specimens, the results should be read after 3 minutes, while for serum or plasma specimens, the results should be read after 5 minutes. Result interpretation should not be conducted beyond 10 minutes due to the possibility of low hCG concentrations, which may lead to a faint line appearance in the test line region (T) after an extended period. In the case of a positive result, the presence of lines in both the control line region and the test line region confirms the outcome. Conversely, a negative result is determined if a line is observed solely in the control line region. Invalid samples should undergo repeat testing. Results with no line present in the control line region may be classified as invalid.

The presence of a clear background functions as an internal negative procedural control. In the event that a background color emerges in the result window, obstructing the interpretation of the test result, the validity of the result may be compromised. To ensure the accurate performance of the test, it is advised to assess a positive hCG control (containing 10-250mIU/mL hCG) and a negative hCG control (containing 0 mIU/mL hCG) upon receiving a new shipment of tests.

2.3 Cross-reactivity and Interference

To verify cross-reactivity, this study utilized WHO international standards for LH (300mIU/mL), FSH (1,000mIU/mL), and TSH (1,000µIU/mL), along with specimens containing 0mIU/mL hCG and 10mIU/mL hCG. In addition, the study examined the influence of several components, such as Acetaminophen, Caffeine, Acetylsalicylic Acid, and Gentisic Acid at 20 mg/dL, among others, on hCG testing.

3. Results and Discussion

3.1 Results

3.1.1 Accuracy

A multicenter clinical study was conducted to evaluate the performance of the hCG Enhanced Sensitivity Test cassette. The test was compared to another commercially available rapid test for the detection of hCG in urine, serum, or plasma samples.

The results in Table 1 show that a total of 608 urine samples were included, consisting of 377 negative and 231 positive results. The findings revealed that the hCG Pregnancy Enhanced Sensitivity Rapid Test cassette exhibited an overall accuracy of over 99% when compared to other

urine hCG rapid tests.

Table 1: Performance Characteristics for Urine Specimens.

Method		Other hCG Rapid Test		Total Results
hCG Pregnancy Enhanced Sensitivity Rapid Test Cassette	Results	Positive	Negative	
	Positive	231	0	231
	Negative	0	377	377
Total Results		231	377	608

Sensitivity: >99.9% (98.7%~100%)*;

Specificity: >99.9% (99.2%~100%)*;

Accuracy: >99.9 % (99.5%~100%) *;

*: 95% Confidence Intervals.

As shown in Table 2, the serum or plasma study encompassed 308 samples, with 240 negative and 68 positive results. The results demonstrated that the test exhibited an overall accuracy of over 99% when compared to other serum or plasma hCG rapid tests.

Table 2: Performance Characteristics for Serum or Plasma Specimens.

Method		Other hCG Rapid Test		Total Results
hCG Pregnancy Enhanced Sensitivity Rapid Test Cassette	Results	Positive	Negative	
	Positive	68	0	68
	Negative	0	240	240
Total Results		68	240	308

Sensitivity: >99.9% (98.7%~100%)*;

Specificity: >99.9% (99.2%~100%)*;

Accuracy: >99.9 % (99.5%~100%) *;

*: 95% Confidence Intervals.

3.1.2 Sensitivity and Cross-Reactivity

This test is specifically designed to detect hCG at 10mIU/mL or above. It has been standardized against the W.H.O. International Standard. In order to evaluate cross-reactivity, LH (300mIU/mL), FSH (1,000mIU/mL), and TSH (1,000µIU/mL) were introduced to specimens containing negative hCG (0mIU/mL) and positive hCG (10mIU/mL). The findings indicated that there was no evidence of cross-reactivity between these hormones and the hCG test.

3.1.3 Precision

The precision of the assay was evaluated by conducting 10 replicate measurements on three biological samples containing hCG concentrations of 10 mIU/mL, 100 mIU/mL, 250 mIU/mL, and 0 mIU/mL. It was observed that both the negative and positive values were correctly identified with 100% accuracy. To assess the between-run precision of the assay, the same three hCG samples (10 mIU/mL, 100 mIU/mL, 250 mIU/mL, and 0 mIU/mL) were independently tested in 10 separate analyses. The hCG Pregnancy Enhanced Sensitivity Rapid Test Cassettes from three different batches were subjected to testing, and the identification accuracy was 100%. To assess potential interference, various substances including Acetaminophen at 20 mg/dL, Caffeine at 20 mg/dL, Acetylsalicylic Acid at 20 mg/dL, Gentisic Acid at 20 mg/dL, Ascorbic Acid at 20 mg/dL, Glucose at 2 g/dL, Atropine at 20 mg/dL, Hemoglobin at 1 mg/dL, Bilirubin at 2 mg/dL, Bilirubin (serum or

plasma) at 40 mg/dL, and Triglycerides (serum or plasma) at 1,200 mg/dL, were tested at their respective concentrations. No interference from any of the substances was observed at the tested concentrations.

3.2 Discussion

This test Cassette is capable of simultaneously testing three samples in a single window, which includes serum or plasma hCG examination and qualitative urine examination. This capability enhances both the efficiency and cost-effectiveness of the diagnostic process. The data from multi-center clinical comparative experiments with other commercially available high-quality pregnancy tests demonstrates its good performance. The hCG Pregnancy Enhanced Sensitivity Rapid Test demonstrated excellent performance in terms of sensitivity, specificity, and accuracy, all exceeding 99.9% when testing urine or serum/plasma samples. Even at high physiological levels, the hCG Pregnancy Enhanced Sensitivity Rapid Test showed no cross-reactivity interference from structurally related glycoprotein hormones such as hFSH, hLH, and hTSH at the claimed sensitivity level of 10 mIU/mL.

However, this study does have several limitations. Firstly, although clinical comparative experiments were conducted across multiple centers, with a total collection of 608 urine samples and 308 blood samples, more extensive sample testing is still required to obtain more precise data. Secondly, it is important to note that the hCG Pregnancy Enhanced Sensitivity Rapid Test is a preliminary qualitative test. As such, it is not designed to provide the quantitative value of hCG or indicate the rate of hCG elevation. It is unable to detect conditions such as ectopic pregnancy, miscarriage, and the presence of abnormal reproductive cells, placental tissues, and embryonic tissues caused by either high or low levels relative to normal values.^[8] The levels of hCG in urine specimens may not be representative if the volume is very low or if the specific gravity is low. If there are suspicions of pregnancy, it is advisable to collect a first-morning urine specimen and conduct the test after 48 hours. Shortly after implantation, urine, serum, or plasma samples can contain hCG at very low concentrations (below 50 mIU/mL). Considering the spontaneous termination of a substantial proportion of early pregnancies, it is advisable to confirm weakly positive test results by retesting with a specimen collected after 48 hours, either from the first-morning urine or serum or plasma. It is important to be aware that the test can produce inaccurate outcomes, including both false-positive and false-negative results, in specific scenarios. This test may produce false-positive results, as factors other than pregnancy that can lead to elevated hCG levels. These include trophoblastic diseases and certain non-trophoblastic tumors including testicular tumors, prostate cancer, breast cancer, and lung cancer.^[9] Hence, using the presence of hCG in urine, serum, or plasma samples as the exclusive criterion for pregnancy diagnosis should be avoided until these possibilities are excluded. It is essential to consider that this test can also produce inaccurate negative outcomes when hCG levels fall below the test's sensitivity threshold. The hCG variant hook effects can also result in false-negative results.^[10] If there are ongoing suspicions of pregnancy, it is recommended to collect a first-morning urine specimen and perform the test after a 48-hour interval. In cases where there are suspicions of pregnancy and the test consistently produces negative results, it is recommended to seek the guidance of a healthcare professional for further evaluation and diagnosis. There is a possibility of interference from human anti-mouse antibodies (HAMA) in the samples, as with any assay that uses mouse antibodies. Individuals who have received monoclonal antibody preparations for diagnosis or treatment may possess specimens containing human anti-mouse antibodies (HAMA). This presence of HAMA can lead to erroneous outcomes, including both false-positive and false-negative results. Ultimately, the test offers an approximate pregnancy diagnosis, emphasizing that the definitive determination

should be made by healthcare professionals who thoroughly evaluate all pertinent clinical and laboratory findings.

4. Conclusion

In comparison to other commercially available high-standard pregnancy tests, the AllTest hCG Pregnancy Enhanced Sensitivity Rapid Test demonstrates higher sensitivity, specificity, and accuracy in qualitatively detecting hCG in urine, serum, or plasma within a single window. These findings indicate that this rapid test is a valuable diagnostic tool in clinical settings. The AllTest hCG Pregnancy Enhanced Sensitivity Rapid Test demonstrates outstanding performance characteristics, thereby enhancing diagnostic efficiency and reliability. This test is a highly effective and dependable option for early pregnancy diagnosis. However, while these findings bring hope, it must be mentioned that more comprehensive evaluations in different real-world settings may provide further insights. To enhance its capabilities, future advancements may involve the development of quantitative methods to measure antigen concentrations and their rate of change.

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