White Paper

CLINITEST hCG testing on the CLINITEK Status Analyzer achieves clinical performance and sensitivity criteria

An internal validation study

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Summary

Human chorionic gonadotropin (hCG) is a hormone released by the developing placenta during pregnancy. The hormone reaches a detectable level in the urine of pregnant women very soon after conception, and levels initially rise very rapidly thereafter. This rapid rise in concentration makes hCG a reliable marker for the early diagnosis of pregnancy.

The CLINITEST® hCG Test from Siemens Healthineers is a pregnancy test for in vitro diagnostic use that employs chromatographic immunoassay for the qualitative determination of the hCG hormone in urine. The test runs on a cassette that is used with the CLINITEK Status® family of analyzers, which report hCG concentrations as positive, negative, or borderline for pregnancy. An internal validation study assessed the clinical performance and sensitivity of the test on three lots of CLINITEST hCG pregnancy test cassettes manufactured under a new quality control system introduced by Siemens Healthineers in 2012.

Study results from 13,500 individual tests demonstrated an acceptably low incidence of borderline and false-positive results with urine samples negative for hCG. Results from more than 4700 tests showed an excellent rate of agreement of positive results with hCG-positive samples. Further testing also demonstrated concordance with all sensitivity acceptance criteria. The acceptable clinical performance and sensitivity of the CLINITEST hCG Test reaffirm its utility as a rapid and reliable method for detection of pregnancy at an early stage.

Introduction

hCG and pregnancy

hCG is a glycoprotein hormone secreted by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected in serum as early as 7 days following conception. Urine hCG concentrations are approximately one-half (or less than one-half) of corresponding serum hCG concentrations. Therefore, hCG may be detected in urine as early as 14 days after conception (approximately 28 days since the last menstrual cycle). The hormone doubles in concentration about every 2 days until it peaks at approximately 8–10 weeks after the last menstrual period. Over the next 10 or more weeks, the level of hCG decreases. The appearance of hCG soon after conception and its subsequent rise in concentration during early gestational growth makes it an excellent marker for the early detection of pregnancy.

The CLINITEST hCG Test

The CLINITEST hCG Test employs an easy-to-use cassette that is utilized with the CLINITEK Status family of analyzers. The cassette mounts on a test table. The cassette is automatically drawn into the analyzer after a urine sample is added to the cassette's sample well. Qualitative hCG results are reported by the analyzer as positive, negative, or borderline:

- **A positive** result is reported when the urine hCG level is ≥25 mIU/mL.
- A **negative** result is reported when the urine hCG level is ≤5 mIU/mL.
- A **borderline** result is indicated when the urine hCG level is between 5–25 mIU/mL.

Borderline results are considered indeterminate for pregnancy, and the test should be repeated within 48–72 hours. Negative test results in patients suspected to be pregnant should also be followed up with a retest on a urine sample obtained 48–72 hours later. Similarly, to exclude pregnancy with the highest degree of certainty, repeat testing should be conducted on a sample obtained 48–72 hours later after an initial negative result. As true with any diagnostic test, a clinical diagnosis should never be made based solely on a single CLINITEST hCG test result.

If a positive test result is obtained and non-pregnancy is suspected, it is standard laboratory and clinical practice to have the test repeated with another urine sample obtained 48 hours later. In acute situations, it is recommended that any result be confirmed with a quantitative serum hCG test. As a general rule, any CLINITEST hCG test result not fitting a patient's clinical picture should prompt repeat testing on a new sample and a quantitative serum hCG test.

False-negative, false-positive, and borderline hCG results

Inaccurate (false-negative and false-positive) pregnancy test results may arise even when proper technique is used and testing procedures are followed correctly. All qualitative pregnancy tests will produce a small number of false-positive results. A number of conditions other than pregnancy, including trophoblastic disease and certain non-trophoblastic neoplasms, can cause borderline levels of hCG. Borderline CLINITEST hCG results are in no sense diagnostic, but in the absence of procedural error may, when results do not fit a clinical picture, indicate to clinicians that further investigation is called for. Table 1 lists causative factors (excluding procedural error) that can generate false-negative, false-positive, and borderline hCG results with the CLINITEST hCG Test.

The advantages of analyzer-read CLINITEST hCG testing

Qualitative visual "dip-and-read" pregnancy tests are increasingly being replaced by analyzer-read chromatographic immunoassay tests that determine hCG in urine samples. Accurate and consistent analyzer-read pregnancy test results offer significant advantages over traditional dipstick methodology and reporting. Since no visual interpretation or manual documentation is required, the chance for error is greatly reduced. Pregnancy test analyzers connected to a laboratory or hospital information system (LIS/HIS) (for example, the CLINITEK Status Connect System) automatically report hCG results that can be acted upon immediately and facilitate the trend toward testing and healthcare provision in decentralized, ambulatory settings.

Potential Causes of a False-negative Result	Potential Causes of a False-positive Result	Potential Causes of a Borderline Result
• Very early pregnancy • Pre-embryo implantation • Concentrated urines containing low levels of hCG • Urine contaminated with proteolytic enzymes • Degradation in old samples • Interfering antibodies in the medications of patients on antibody therapies	• Undetected early spontaneous abortion (around 22% of pregnancies abort spontaneously, without being detected) • The presence of interfering substances such as endogenous proteins, drugs, microorganisms, erythrocytes, and/or leukocytes • The detection of low concentrations of hyperglycosylated hCG and beta core fragments (seen very early in pregnancy) that other manufacturers' tests may not detect	• Undetected early spontaneous abortion • Ectopic pregnancy, when hCG levels rise at a significantly slower rate than in normal pregnancy • Trophoblastic diseases, including a variety of cancers • Increased hCG levels in healthy, non-pregnant post-menopausal women • Passively acquired hCG from a blood transfusion • hCG levels may remain detectable for up to several weeks following delivery, miscarriage, or hCG injections (from IVF treatment) • Interfering antibodies in the medications of patients on antibody therapies

Table 1 Potential causes of false-negative, false-positive, and borderline hCG results.

CLINITEST hCG testing on the CLINITEK Status family of analyzers is CLIA-waived in the United States.

- Accurate and consistent analyzer-read and -reported results eliminate transcription errors.
- Results are reported as early as 2 minutes if positive, and within 5 minutes if negative.
- The analyzer precisely times the reading of results and verifies that the test has been completed properly.
- A printed record of results includes patient and operator ID.
- CLINITEST hCG testing improves workflow by eliminating hands-on waiting time, so the time for patient care is increased.
- The CLINITEK Status family of analyzers improves QC with automatic quality checks for sufficient sample and proper sample flow. They generate error flags when these conditions are not met.
- CLINITEK Status Connect System analyzers can be programmed to automatically check QC results and initiate a QC lockout when results fail.
- With the CLINITEK Status Connect System, results can be automatically transmitted to a LIS/HIS for improved data capture.

Study purpose

Siemens Healthineers introduced a new quality control system to oversee the manufacture of CLINITEST hCG test cassettes in 2012. The purpose of this study was to reaffirm appropriate clinical performance and sensitivity of the CLINITEST hCG Test subsequent to the introduction of new processes and measures under which CLINITEST hCG test cassettes are manufactured and released. Reaffirmation of accurate, dependable results ensures that healthcare practitioners can confidently employ the test as a rapid and reliable marker for early detection of pregnancy. The study investigated:

- Rates of borderline and false-positive results with hCG negative clinical samples
- The rate of agreement of positive results with positive clinical samples
- Sensitivity of the test with positive, spiked samples and negative urines

Methods

Performance study

Performance of the CLINITEST hCG Test was assessed on three lots of recently manufactured CLINITEST hCG pregnancy test cassettes at the Siemens Healthineers facility in Elkhart, Indiana, over a two-month period in July and August of 2012. 675 hCG-negative and hCG-positive clinical urine samples (obtained both internally and from external clinical sites) were run on six CLINITEK Status+ and three CLINITEK Status Analyzers across the three lots, amounting to a study involving more than 18,000 individual tests. 500 of the samples tested were negative for hCG, and 175 were hCG positive (>25 mIU/mL).

200 µL of each sample was applied to each cassette using a standard laboratory pipette. For each CLINITEST hCG cassette test lot, one replicate of each sample was run on each analyzer. After samples were tested at room temperature on a cassette lot, they were frozen at –20°C until needed for testing with the next cassette lot. Testing was conducted in multiple runs, with both negative and positive samples tested in each run. For QC purposes, Bio-Rad qUAntify® Plus Urinalysis Control, Level 1 and Level 2 was tested on each analyzer at the beginning of every testing day. Test data was collected using validated DCAT data collection software, and the occurrence rates for borderline and positive results were calculated.

Sensitivity study

Clinical sensitivity of the CLINITEST hCG Test was assessed on three lots of recently manufactured CLINITEST hCG pregnancy test cassettes using six CLINITEK Status+ and three CLINITEK Status analyzers. hCG-negative urine samples were obtained from three known non-pregnant individuals and pooled to achieve sufficient volume. The urine pools were then split into nine different samples and spiked to hCG concentrations of 0, 2, 5, 10, 15, 20, 25, 50, and 100 mIU/mL. After verification of spiked sample hCG concentrations, three replicates of each sample were tested on each analyzer by cassette test lot.

200 µL of each sample was applied to each cassette using a standard laboratory pipette. 54 replicates per hCG concentration were tested on CLINITEK Status+ analyzers, and 27 replicates per hCG concentration were tested on CLINITEK Status analyzers. QC was performed using Bio-Rad qUAntify Plus Urinalysis Control, Level 1 and Level 2 at the start of testing with each CLINITEST hCG cassette test lot. Test data was collected using validated DCAT data collection software, and the percent positive results for each spiked sample test were calculated.

CLINITEST hCG Cassette Test Lot #	Number of Tests Run	Number of Borderline Results Reported	Number of False-positive Results Reported	Borderline Result Rate (%)	False-positive Result Rate (%)
35015	4500	10	0	0.22	0.00
35163	4500	20	2	0.44	0.04
35460	4500	7	1	0.16	0.02

Table 2 Negative sample testing results.

Spiked Sample hCG Concentration (mIU/mL)	CLINITEK Status+ Analyzer			CLINITEK Status Analyzer		
	Negative Result	Borderline Result	Positive Result	Negative Result	Borderline Result	Positive Result
0	54	0	0	27	0	0
2	53	1	0	27	0	0
5	42	12	0	23	4	0
10	14	14	26	5	8	14
15	3	1	50	4	0	23
20	2	0	52	1	0	26
25	0	0	54	0	0	27
50	0	0	54	0	0	27
100	0	0	54	0	0	27

Table 3 Results from spiked sample replicate testing across all analyzers and CLINITEST hCG cassette test lots (n = 54 or n = 27).

Spiked Sample hCG Concentration (mIU/mL)	CLINITEK Status+ Analyzer Positive Result (%)	CLINITEK Status Analyzer Positive Result (%)	Total (%)
0	0	0	0
2	0	0	0
5	0	0	0
10	48	52	49
15	93	85	90
20	96	96	96
25	100	100	100
50	100	100	100
100	100	100	100

Table 4 Calculated percent positive results by spiked hCG concentration across analyzers and all CLINITEST hCG cassette test lots.

Results

Performance study

Table 2 summarizes the borderline results reported and borderline rates calculated from negative hCG sample testing across three lots of CLINITEST hCG test cassettes. Positive results reported and false-positive rates calculated are also shown.

One criterion of acceptable clinical performance of the CLINITEST hCG Test on the CLINITEK Status family of analyzers is a rate of borderline results with negative patient urines ≤0.9%.[1] This internal validation study reported borderline rates by CLINITEST hCG cassette test lot of 0.22%, 0.44%, and 0.16% with negative clinical samples. Only three of 13,500 tests reported a false-positive result with a negative sample, representing a rate of ≤0.04% across the three CLINITEST hCG cassette test lots.

175 positive (hCG >25 mIU/mL) clinical samples were tested with each CLINITEST hCG cassette test lot across all nine CLINITEK Status+ and CLINITEK Status Analyzers (a total of 4725 individual tests). Only one sample returned a negative result on one CLINITEK Status+ Analyzer (with lot #35163). This sample reported positive on all other analyzers.

The acceptable rate of agreement for positive results with positive hCG samples is ≥99.1%.[1]

For cassette test lot #35163, the rate of agreement of positive results with positive samples was 99.9%; lots #35015 and #35460 demonstrated a rate of agreement for positive results with positive samples of 100.0%.

Sensitivity study

Spiked sample test results across all analyzers and CLINITEST hCG cassette test lots are shown in Table 3. Table 4 shows calculated percent positive results by hCG concentration across analyzers and all CLINITEST hCG cassette test lots.

The CLINITEST hCG test detects urinary hCG concentrations of at least 25 mIU/mL (calibrated to the World Health Organization 3rd International Reference Preparation). Acceptable sensitivity at hCG concentrations of 25, 50, and 100 mIU/mL on the CLINITEK Status family of analyzers is 100% positive results.[2] Acceptable sensitivity at an hCG concentration of 0 mIU/mL requires that all samples report negative.[2] The present study reported results that met all sensitivity acceptance criteria. At the clinical levels of 25, 50, and 100 mIU/mL, 100% of results across all analyzers and CLINITEST hCG cassette test lots were positive. At the 0 mIU/mL level, all samples gave a negative result.

Discussion and conclusions

Study results from negative hCG samples demonstrated an overall incidence of borderline results ≤0.44%, well within the acceptable test performance criterion of a rate of ≤0.9% with negative urines. The incidence of false-positive results was 3 in 13,500 negative urine samples tested (≤0.04%). The rate of agreement of positive results with positive samples was ≥99.9%, exceeding the performance acceptance criterion of ≥99.1%. Results also demonstrated concordance with all sensitivity acceptance criteria. 100% of tests on all positive spiked samples (at 25, 50, and 100 mIU/mL) reported positive, and 100% of tests on all negative samples (0 mIU/mL) reported negative.

Taken collectively, the validation study results confirmed acceptable clinical performance and sensitivity of the CLINITEST hCG Test after the introduction of new processes and measures to oversee the manufacture of CLINITEST hCG test cassettes. Accurate, dependable results ensure that healthcare practitioners can confidently employ the CLINITEST hCG Test as a rapid and reliable marker for early detection of pregnancy. The study results endorse the increasing uptake of the CLINITEST hCG Test as a replacement for traditional "dip-and-read" pregnancy test methods.

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References:

1. *CLINITEST hCG Pregnancy Test Instructions for Use (IFU). 06878007, 2008-08. See Performance Characteristics: Method Comparison.*
2. *CLINITEST hCG Pregnancy Test Instructions for Use (IFU). 06878007, 2008-08. See Performance Characteristics: Sensitivity.*

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