P-5120-K

Status hCG

One-Step Pregnancy Test

For Professional *In Vitro* Use

Rapid Immunoassay for the Qualitative Detection of Human Chorionic Gonadotropin in Urine

For the Early Detection of Pregnancy

LifeSign, LLC

CLIA Waived

Item No. 35135 35 Test Kit

Intended Use

Status hCG One-Step Pregnancy Test is a simple immunoassay for the Qualitative Detection of Human Chorionic Gonadotropin (hCG) in Urine for the Early Detection of Pregnancy.

Summary and Explanation

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by the placental trophoblastic cells shortly after the fertilized ovum is implanted in the uterine wall.[1-4] The primary function of hCG is to maintain the corpus luteum during early pregnancy. The appearance of hCG in both urine and serum soon after conception, and its rapid rise in concentration make it an excellent marker for pregnancy. The hormone level may become detectable in both urine and serum as early as 7 to 10 days after conception.[1- 4] The concentration of hCG continues to rise rapidly, frequently exceeding 100 mIU/mL by the first missed menstrual period and peaking in the 30,000- 100,000 mIU/mL range by 10 to 12 weeks into pregnancy. The hormone is comprised of two non-covalently bound dissimilar subunits containing approximately 30% carbohydrate by weight.5 The alpha subunit is structurally similar to other human pituitary glycoprotein hormones, whereas the beta subunit confers unique biological and immunological specificity to the molecule.[6,7]

The ***Status hCG*** One-Step Pregnancy Test is a rapid test for detecting pregnancy. The test is a solid-phase, two-site immunometric assay in which a combination of monoclonal and polyclonal antibodies is used to selectively detect hCG in urine with a high degree of sensitivity. In the test procedure, sample is added to the sample well using a dropper and sample is allowed to soak in. If hCG is present in the specimen, it will react with the conjugate dye, which binds to the antibody on the membrane to generate a colored line. Presence of two colored lines, one at the Test Position and the other at the Control Position, indicates a positive result, while the absence of the line at the Test position indicates a negative result.

Reagents and Materials Provided

Status hCG One-Step Pregnancy Test kit contains enough reagents and materials to perform all the tests.

- Status device. (Test device containing the polyclonal anti-hCG coated membrane and a pad with the mouse monoclonal IgG (anti-hCG)-dye conjugate in a protein matrix containing 0.1% sodium azide)
- Disposable dropper
- Package insert

Materials Maybe Required but Not Provided

- Timer
- Specimen cup
- Latex gloves

Warning and Precautions

- For in vitro diagnostic use only.
- Do not interchange materials from different product Lot's and do not use beyond the expiration date.
- The ***Status hCG*** One-Step Pregnancy Test device should remain in its sealed pouch until ready for use.

Storage and Stability

Status hCG One-Step Pregnancy Test kit should be stored at 2–30°C (36–86°F) in the sealed pouch.

Specimen Collection and Preparation

Urine Assay:

- 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.
- Bring specimens to room temperature prior to testing. 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.

Specimen Storage

- If testing will not be performed immediately, the specimens should be refrigerated (2–8°C) for up to 48 hours. Bring specimens to room temperature prior to testing.
- For prolonged storage, specimens may be frozen and stored below –20°C. 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 and ship by the quickest means possible.

Procedures

Procedural summary

The procedure consists of adding the specimen to the sample well in the device and watching for the appearance of colored lines on the membrane.

Procedural notes

The instructions below must be followed to achieve optimal test results

- Before opening the pouch, the ***Status hCG*** One-Step Pregnancy Test device must be allowed to stand at room temperature for at least 30 minutes prior to testing.
- Label the ***Status hCG*** device with the patient name or control number.
- Handle all specimens as if capable of transmitting disease.
- After testing, dispose of the ***Status hCG*** device, and the dropper

following good laboratory practices. Consider each material that comes in contact with specimen to be potentially infectious.

Test Procedure

STEP 1

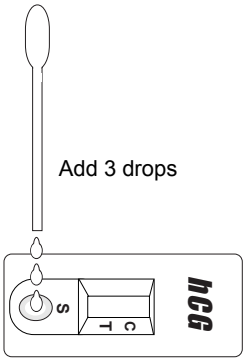
For each test, open one ***Status hCG*** pouch, and label the Status device with the patient ID.

STEP 2

Holding the dropper in a vertical position, add 3 drops of sample into the Sample well (S).

STEP 3

Read the results at 3–5 minutes.
Do not interpret the results after 5 minutes.



Interpretation of Results

Positive

Two pinkish-purple lines, one each in the Test Position (T) and in the Control Position (C). Each of the following indicates a positive test result

(examples of positive results)

NOTE: A specimen containing a detectable level of hCG will generate a pinkish-purple line in the Test Position (T) within 3–5 minutes. The time required to generate the line is dependent on the hCG concentration in the sample. Positive results may be detected in as early as one (1) minute, depending on the hCG concentration. To be interpreted as positive, the pinkish-purple line at the Test position should be clearly distinguishable from the background color of the membrane. In strong positive tests, the color intensity of the Control line (C) may be much lighter than that of the Test line (T). Note: The high dose hook effect has been found to occur at approximately 500,000 mIU/mL. For samples with extremely high concentration of hCG, the higher the hCG concentration, the lighter the color band at the test region may appear.

a. Two strong pinkish-purple lines, one each at the Test (T) and Control (C) Positions
b. One strong pinkish-purple line at the Test Position (T) and one light pinkish-purple line at the Control Position (C).
c. One light pinkish-purple line at the Test Position (T) and one pinkish-purple colored line at the Control Position (C).

Negative

Only one pinkish-purple line, at the Control Position (C).

(examples of negative results)

NOTE: In the absence of hCG, or in the case that the hCG concentration is below the detection limit of the test, there will be no apparent line at the Test Position (T); rather, there may be a uniform background color over the membrane area. The Control line at the Control Position (C) should be clearly visible.

Invalid

A distinctive colored line at the Control Position (C) should always appear. The test is invalid if no Control line forms.

(examples of invalid results)

NOTE: If there is no distinct pinkish-purple line visible at the Control Position, the test is inconclusive. The Control line should always appear. If there is a suspected procedural error, the result should be considered inconclusive. It s recommended that in these cases the test be repeated with a new test device.

Limitations

- Elevated hCG levels have been reported in patients with both gestational and nongestational trophoblastic diseases.[8,9,10] 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.

- 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.[11]

- 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.[12] 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.[13] A suspected ectopic pregnancy may be further evaluated using a quantitative hCG assay.

- 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 test line

- The physician should evaluate data obtained with this kit in light of other clinical information.

- 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.

- 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.

- In rare occasions, persistent low levels of hCG present in men and in nonpregnant women (concentrations 3 to 100 mIU/mL) may result in positive results.[14,15]

User Quality Control

Internal Control: Each ***Status hCG*** One-Step Pregnancy Test device has a built-in control. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should appear at C position indicating an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents at the Control line and the conjugate-color indicator are reactive. In addition, the clearing background in the Result window is considered as an additional procedural control by providing a distinct readable result. This may be considered an internal negative procedural control. If background color appears in the Result window which interferes with your ability to read the test result and obscure the formation of the control band, your result may be invalid. If the problem persists, contact LifeSign for technical assistance at 1-800-526-2125.

External Control: External controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested before using a new lot or a new shipment of kit as good laboratory testing practice and that users follow federal, state, and local guidelines for quality control requirements. For information on how to obtain controls, contact LifeSign's Technical Services.

Expected Values

Status hCG One-Step Pregnancy Test is capable of detecting hCG level of 25 mIU/mL in urine(calibrated against the WHO 3rd International Standard). HCG levels in normal early pregnant women vary and hCG levels often exceed 100 mIU/mL by the first day of the missed menstrual period.1 The test is usually capable of detecting hCG by the first day of the missed menstrual period.

Performance Charateristics

Clinical Evaluation

A total of 247 blind clinical urine samples were studied. These specimens were assayed with ***Status hCG*** One-Step Pregnancy Test and Tandem® Icon™ II according to the package inserts (Table 1). Thirty-six (36*) samples are from menopausal women.

Table 1 (Urine Assay)

Status hCG One-Step Pregnancy Test vs. Tandem®Icon™ II with Urine Specimens

Test Result (# of Samples)

	Tandem®Icon™ II	***Status hCG***
Positive	78	78
Negative	133	133
Menopausal	Not Determined	36 (Negative)

Overall Accuracy: 100%

Relative Sensitivity: 100%

Relative Specificity: 100%

The data demonstrate the excellent correlation between ***Status hCG*** One-Step Pregnancy Test and and Tandem® Icon™ II. The clinical accuracy and sensitivity of the two tests are found comparable.

Physicians' Office Laboratory Evaluation (Proficiency Study)

Reproducibility of Status hCG™ test was evaluated at three physicians' offices using a total of 60 blind control samples. The panels consisted of 5 negative (–), 5 low positive (25 mIU/mL hCG), 5 moderate positive (200 mIU/ mL hCG), and 5 high positive (500 mIU/mL hCG) samples. The results obtained at each site agreed 100% with expected results.

Sensitivity

Standard controls (calibrated to the WHO 3rd International Standard) ranging from 5 mIU/mL to 40 mIU/mL were tested in 5 replicates. The results confirmed the sensitivity of 25 mIU/mL at 3–5 minute assay time.

Specificity

Thirty-six urine specimens collected from menopausal women were studied. Specimens from menopausal women are known to interfere frequently with
pregnancy tests due to cross-reactivity with other gonadotropin hormones such as Leutenizing hormone. These specimens were assayed with ***Status hCG*** One-Step Pregnancy Test . All 36 specimens were found negative.

The assay is free of interference from other commonly known homologous hormones when tested against the levels specified below (Table 2).

Table 2

Homologous Hormones:

	Urine
hFSH	1000 mIU/mL
hLH	500 mIU/mL
hTSH	1000 µIU/mL

Other Interfering Substances

At the level of claimed sensitivity, ***Status hCG*** One-Step Pregnancy Test showed no interference when the following potentially interfering substances, both endogenous and exogenous, were added to urine samples which had hCG levels of 0 and 25 mIU/mL (Table 3).

Table 3. Potentially Interfering Substances added to Urine and Tested with the ***Status hCG*** One Step Pregnancy Test

Table 3

Substance Added	**Concentration Added in Urine**
Drugs:	
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	20 mg/dL
Ampicillin	20 mg/dL
Ascorbic Acid	20 mg/dL
Atropine	20 mg/dL
Caffeine	20 mg/dL
Gentisic Acid	20 mg/dL
Phenothiazine	20 mg/dL
Phenylpropanolamine	20 mg/dL
Salicylic Acid	20 mg/dL
Tetracycline	20 mg/dL
Urinary Analytes:	
Bilirubin	2 mg/dL
Glucose	2000 mg/dL
Hemoglobin	25 mg/dL
Ketones	100 mg/dL
Albumin	2000 mg/dL
Homologous Hormones:	-
hFSH	1000 mIU/mL
hLH	500 mIU/mL
hTSH	1000 µIU/mL

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Symbols Key

	Instructions For Use (Read)
REF	Item Number
	Store At
	Expiration Date
CONT	Contents
DEV	Test Device
IFU	Instructions For Use
PIP	Transfer Pipette
	Do Not Reuse
IVD	For *In Vitro* Diagnostic Use
LOT	Lot Number
	Manufacturer
MF	Manufactured For
EC REP	Authorized Representative
CE	CE Mark

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CE

EC REP

MT Promedt Consulting GmbH
Altenhofstrasse 80
66386 St. Ingbert
Germany
+49-68 94-58 10 20

Manufactured by PBM

Princeton BioMeditech Corporation
4242 U.S. Hwy 1, Monmouth Jct.
New Jersey 08852, U.S.A.
1-732-274–1000 www.pbmc.com

MF Manufactured for:

lifeSign

A PBM Group Company
85 Orchard Road,
Skillman, NJ 08558
800-526-2125, 732-246-3366
www.lifesignmed.com