



One Step hCG Pregnancy Serum Test Cassette

Cat. No: AC-FHC-202

A rapid one step test for the qualitative detection of human chorionic gonadotropin (hCG) in serum. For professional in vitro diagnostic use only.

INTENDED USE

One Step hCG Pregnancy Test is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in serum to aid in the early detection of pregnancy.

SUMMARY AND EXPLANATION

hCG is a glycoprotein hormone produced by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected in serum as early as 7 to 10 days after conception. hCG levels continues to rise very rapidly, frequently exceeding 100mIU/mL by the first missed menstrual period, and peaking in the 100,000-200,000mIU/mL range about 10-12 weeks into pregnancy.^{7,8,9,10} The appearance of hCG in serum soon after conception, and its subsequent rapid rise in concentration during early gestational growth, make it an excellent marker for the early detection of pregnancy.

One Step hCG Pregnancy Test is a rapid test that qualitatively detects the presence of hCG in serum specimen at the sensitivity of 25mIU/mL. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of hCG in serum. At the level of claimed sensitivity, One Step hCG Pregnancy Test shows no cross-reactivity interference from the structurally related glycoprotein hormones hFSH, hLH and hTSH at high physiological levels.

PRINCIPLE OF TEST

One Step hCG Pregnancy Test is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (hCG) in serum to aid in the early detection of pregnancy. The test utilizes a combination of antibodies including a monoclonal hCG antibody to selectively detect elevated levels of hCG. The assay is conducted by immersing the test in a serum specimen and observing the formation of pink coloured lines. The specimen migrates via capillary action along the membrane to react with the coloured conjugate.

Positive specimens react with the specific antibody-hCG-coloured conjugate and form a pink coloured line at the test line region of the membrane. Absence of this pink coloured line suggests a negative result. To serve as a procedural control, a pink coloured line will always appear at the control line region if the test has been performed properly.

REAGENTS

Coated Antibodies:

Control region: Goat anti-mouse (IgG) polyclonal antibody

Test region: Mouse monoclonal anti-hCG antibody A

Labelled Antibodies: Colloidal gold conjugate of monoclonal anti-hCG antibody B

WARNINGS & PRECAUTION

- 1) In vitro diagnostic use for professional use only.
- 2) Do not use test kit beyond the expiry date.
- 3) The test device must never be reused.
- 4) Serum specimens may be infectious; insure proper handling and dispose of all used reaction devices into a biohazard container.

MATERIALS PROVIDED

- 1) One Step hCG Pregnancy Test
- 2) Disposable pipette
- 3) Instructions for use

MATERIALS NEEDED BUT NOT PROVIDED

- 1) Clean glass or plastic container for specimens collection
- 2) Timer

SPECIMEN COLLECTION

Serum Assay

A serum specimen must be collected into a container without anticoagulant. Allow the blood to clot and separate the serum from the clot. Use the serum for testing.

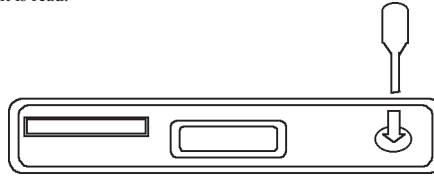
Specimen Storage

The specimen may be refrigerated (2-8°C) and stored up to 2 days. For longer storage, freeze samples at -20°C or below. Refrigerated samples should be allowed to come to room temperature and mixed thoroughly before assaying. Frozen samples should be thawed completely allowed to come to room temperature, and mixed thoroughly before assaying.

DIRECTIONS FOR USE

Allow the test and the specimen to equilibrate to room temperature (15-30°C) prior to testing.

1. To begin testing, open the sealed pouch by tearing along the notch. Remove the test kit from the pouch and use it as soon as possible.
2. Using the pipette provided dispense 3 drops of the serum sample (approx. 0.2 ml) onto the sample well of the cassette (see diagram).
3. Wait for the pink coloured bands to appear. Read the results at 3 minutes. Do not read the results after 10 minutes. It is important that the background is clear before the result is read.



Note: A low hCG concentration might result in a weak line appearing in the test region (T) after an extended period of time; therefore, do not interpret the result after 10 minutes.

INTERPRETATION OF RESULTS

Negative: Only one pink coloured band appears on the control region. No apparent band on the test region.

Positive: Distinct pink coloured bands appear on the control and test regions. The colour intensity of the test bands may vary since different stages of pregnancy have different concentrations of hCG hormone.

Invalid: No line appears in the control zone "C"; the test should be voided since an improper test procedure may have been performed or deterioration of reagents may have occurred. This is due to the internal control built in which a distinct control region (C) line should always appears. Repeat the test using a new device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



STORAGE AND STABILITY

The test kit can be stored at temperatures between 2 to 30°C in the sealed pouch to the date of expiration. The test kit should be kept away from direct sunlight, moisture and heat. The expiration dating was established under these storage conditions.

QUALITY CONTROL

A pink line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. A clear background is an internal negative background control. If the test is working properly, the background in the result area should be white to light pink and not interfere with the ability to read the test result.

External controls should 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 at regular intervals as good laboratory testing process.

Users should follow the appropriate federal, state, and local guidelines concerning the running of external quality controls.

LIMITATIONS

1. False negative results may occur when the levels of hCG are below the sensitivity level of the test. When pregnancy is still suspected, another specimen should be collected 48 hours later and tested.
2. This test has been developed for testing serum samples only. The performance of this test using other specimens has not been substantiated.
3. Moreover, false positives may occur in very low pH samples, as well as high specific gravity samples.
4. Very low levels of hCG (less than 50mIU/mL) are present in serum specimen shortly after implantation. However, because a significant number of first trimester pregnancies terminate for natural reasons, a test result that is weakly positive should be confirmed by retesting with another specimen collected 48 hours later.
5. As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the specimen. Specimens from patients who have received preparations of monoclonal antibodies for diagnosis or therapy may contain HAMA. Such specimens may cause false positive or false negative results.
6. This test provides a presumptive diagnosis for pregnancy. A confirmed pregnancy diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

HIGH DOSE EFFECT

Normal serum that was spiked with hCG concentrations of 62,500, 125,000, 250,000, 500,000, 1,000,000, and 2,000,000 mIU/ml and was used to study the high dose hook effect on One Step hCG Pregnancy Test. It was noticed that both colour bands at the test band region and the control region were visible. However, when hCG levels were over 500,000 mIU/mL, the higher the hCG concentration became, the lighter the band at the rest region became.

ACCURACY

An external clinical evaluation was conducted comparing the results obtained using One Step hCG Pregnancy Test to another commercially available One Step hCG Pregnancy Test. The study included 100 positive or negative serum samples. The results demonstrated 99% agreement when trained technicians performed comparison testing on the urine tests, and 98% agreement for serum samples. The results are shown in Table 1.

Table 1: IND Serum cassette Format– Serum Samples

Cassette Format Serum	Predicate Device		Subtotal
	+	-	
IND	+	42	44
	-	0	56
Subtotal		42	100

SENSITIVITY

One Step hCG Pregnancy test detects serum hCG concentrations greater than 25 mIU/ml as indicated by the appearance of a colour band at the test region. Additionally, samples containing less than 25 mIU/mL hCG may also produce a positive result. The test has been standardized against the 4th W.H.O International Standard (WHO std.ref.75/589)

To evaluate the sensitivity of One Step hCG Pregnancy test at low levels of hCG the following experiments were carried out. Serum samples from 120 known non-pregnant subjects were spiked with hCG to the concentrations of 0, 10, 15, 20, 40, 100 mIU/ml. A total of twenty samples at each concentration were performed and blindly labeled and tested. The results are summarized in Table 3.

Table 3: Sensitivity of One Step hCG Combo Pregnancy Test – Serum Samples

hCG added	0	10	15	20	40	100
# Samples	20	20	20	20	20	20
Negative	20	20	17	0	0	0
Positive	0	0	3	20	20	20

SPECIFICITY

Specificity of One Step hCG Pregnancy test was determined from cross reaction studies with known amounts of luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulation hormone. Samples of serum with different hCG concentrations were mixed individually with 300 mIU LH/mL, 1000 mIU FSH/mL, and 1000 µIU TSH/mL and gave expected results. The results were done in-house by trained technicians in a two day process. The results, which have been pooled together due to little variance are shown in Table 4.

Table 4: Specificity of One Step hCG Pregnancy Test

hCG conc. in sample (mIU/mL)	Unspiked serum samples	Serum samples spiked with homologous hormones		
		FSH	LH	TSH
0	-	-	-	-
	-	-	-	-
	-	-	-	-
20	+	+	+	+
	+	+	+	+
	+	+	+	+
100	+	+	+	+
	+	+	+	+
	+	+	+	+

INTERFERING SUBSTANCES

The One-Step hCG Pregnancy test was checked for possible interference from visibly haemolysed, lipaemic and icteric samples. Human haemoglobin, bilirubin or albumin was spiked into serum samples with different concentration of hCG and tested using un-spiked samples as controls. No significant interference was observed in 20 sample testing results that were either positive or negative for hCG. The results, which have been pooled together due to little variance are shown in Table 5.

Table 5: Non-Specific Interference on hCG Pregnancy Test

Sample No	Unspiked Samples	Serum samples spiked with (mg/mL)			
		Haemoglobin	Bilirubin	Album	
		10	1	0.06	100
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	+	+	+	+	+
12	+	+	+	+	+
13	+	+	+	+	+
14	+	+	+	+	+
15	+	+	+	+	+
16	+	+	+	+	+
17	+	+	+	+	+
18	+	+	+	+	+
19	+	+	+	+	+
20	+	+	+	+	+

The following substances were also added in negative hCG, 20 mIU HCG/mL and 50 mIU hCG/mL spiked serum samples. None of the substances at the concentrations tested interference in this assay when tested with serum cassette based test kits. The substances and their concentration are shown in Table 6.









Table 6: Interfering substances on hCG Pregnancy Test

Substance	Concentration
Acetaminophen	20 mg/ml
Acetylsalicylic acid	20 mg/ml
Ascorbic Acid	20 mg/ml
Albumin	100 mg/ml
Atropine	20 mg/ml
Bilirubin (serum)	40 mg/ml
Bilirubin (urine)	2 mg/dl
Caffeine	20 mg/ml
Gentestic Acid	20 mg/ml
Glucose	2 mg/dl
Haemoglobin	1 mg/dl
Triglycerides (serum)	1.20 mg/dl

BIBLIOGRAPHY

- 1) Chez RA: Fetal and placental endocrinology. Clin Obstet Gynecol 1980; 23:719.
- 2) Goebelsmann U: Protein and steroid hormones in pregnancy. J Reprod Med 1979; 23:166.
- 3) Jaffe RB: Endocrine-metabolic alterations induced by pregnancy. Reproductive Endocrinology, 2nd ed. Saunders, 1986.
- 4) Derman R, Edelman DA, Berger GS: Current status of immunologic pregnancy tests. Int J Gynaecol Obstet 1979; 17:190.
- 5) Horne CHW, Nisbet AD: Pregnancy proteins: A review. Invest Cell Pathol 1979; 2: 217.
- 6) Lind T: Clinical chemistry of pregnancy. Advance Clinical Chem 1980; 21:1.
- 7) Batzer FR. "Hormonal evaluation of early pregnancy" Fertil. Steril. 1980; 34(1): 1-13.
- 8) Catt KJ, ML Dufau, JL Vaitukaitis "Appearance of hCG in pregnancy plasma following the initiation of implantation of the blastocyte", J. Clin. Endocrinol. Metab. 1975;40(3): 537-540.
- 9) Braunstein GD, J Rasor, H. Danzer, D Adler, ME Wade "Serum human chorionic gonadotropin levels throughout normal pregnancy", Am. J. Obstet. Gynecol. 1976; 126(6): 678-681.
- 10) Lenton EA, L Neal, R Sulaiman "Plasma concentration of human chorionic gonadotropin from the time of impantation until the second week of pregnancy", Fertil. Steril. 1982;37(6): 773-778.
- 11) Steier JA, P Bergsjö, OL Myking "Human Chorionic gonadotropin in maternal plasma after induced abortion, spontaneous abortion and removed ectopic pregnancy", Obstet, Gynecol. 1984;64(3): 391-394.
- 12) Dawood MY, BB Sazena, R Landesman "Human chorionic gonadotropin and its subunits in hydatidiform mole and choriocarcinoma", Obstet. Gynecol. 1977;50(2): 172-181
- 13) Braunstein GD, JL Vaitukaitis, PP Carbone, GT Ross "Ectopic production of human chorionic gonadotropin by neoplasms", Ann. Intern Med. 1973; 78(1) : 39-45

GRAPHICAL SYMBOLS USED

	Storage Temperature		Lot Number
	In vitro diagnostic device		Expiry Date
	Read instruction before use		Manufacturer
			Do not use

