OnSite® hCG Combo Rapid Test

REF R1001S CE

Instructions for Use

INTENDED USE

The *OnSite* hCG Combo Rapid Test is a lateral flow chromatographic immunoassay for the early detection of pregnancy, by providing a quick direct visual test for the placental hormone, human chorionic gonadotropin (hCG) in human serum, plasma and urine. The hCG detection limit of *OnSite* hCG Combo Rapid Test is 12.5 mIU/mL in serum/plasma specimen and 25 mIU/mL in urine specimen.

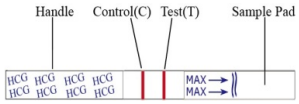
The *OnSite* hCG Combo Rapid Test is not intended for quantitative results, nor for over the counter (OTC) sales. It is designed for professional use only, and provides only preliminary analytical data. For a final diagnosis of pregnancy, a more specific alternative clinical method must be used to obtain a confirmed analytical result.

SUMMARY AND EXPLANATION OF THE TEST

The hCG is produced by human trophoblastic tissue and it appears around the 8-9th day after ovulation where fertilization has occurred, or around the 4th day after conception. In a 28 day cycle with ovulation occurring at day 14, hCG can be detected in urine or serum in minute quantities around day 23, or 5 days before the expected menstruation. Its function includes facilitation of implantation as well as maintenance and development of the corpus luteum. The hormone concentration doubles approximately every 2 days and peaks between 7-12 weeks after the first day of the last menstrual period with a mean concentration of 50,000 mIU/mL. Concentrations as high as 100,000 mIU/mL have been reported in normal pregnancies during the first trimester. In normal condition, hCG in urine provides an early indication of pregnancy. Since elevated hCG levels are also associated with trophoblastic disease and certain nontrophoblastic neoplasms, the possibility of having these diseases must be eliminated before a diagnosis of pregnancy can be made[1,2].

The *OnSite* hCG Combo Rapid Test is intended to meet all requirements for yielding rapid, easily read, qualitative results for the purpose of early pregnancy detection via assay of hCG, a placental hormone that may be present in human serum, plasma or urine. The test can be performed within 5 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* hCG Combo Rapid Test is a lateral flow chromatographic immunoassay. The test strip consists of: 1) a colored conjugate pad containing monoclonal anti-hCG antibody conjugated with colloid gold (hCG Ab conjugate), 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with another anti-hCG antibody, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample pad of the test device, the specimen migrates by capillary action across the strip. The hCG if present at the level equal or higher than 12.5 mIU/mL in serum or plasma specimen or 25 mIU/mL in urine specimen will bind to the hCG Ab conjugate. The immunocomplex is then captured on the membrane by the pre-coated anti-hCG Ab, forming a colored T line, indicating an hCG positive test result.

Absence of the T line suggests a negative result. The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of the control antibodies regardless of the color development on the T line. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed pouches containing:
   a. One dip strip device
   b. One desiccant
2. Instructions for Use

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

3. Positive Control
4. Negative Control
5. Saline or Phosphate-Saline buffer (common buffers used in clinic)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. A container to collect urine or serum/plasma specimen

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The test result should be read 5-10 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 5-10 minute window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. electric fan or strong air conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Urine

First morning urine usually contains the highest concentration of hCG and is therefore the best sample when performing the urine test. However, randomly collected urine specimens may be used. Collect a urine specimen in a clean glass, plastic, or wax coated container.

Test specimens as soon as possible after collecting. If not tested immediately, store specimens at 2-8°C for up to 48 hours. If refrigerating or freezing specimens please allow the specimen to equilibrate to room temperature before testing.

Plasma/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C for up to 5 days, if not tested immediately. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.

Step 2: Collect at least 150-200 µL of serum, plasma or urine specimen in a sample container.

Step 3: Take the desired quantity of sealed pouches from the box. When ready to test, open the pouch at the notch and remove the test strip.

Step 4: Dip the strip vertically, immersing the end of the strip marked with arrows into the specimen for at least 10 seconds until sample migrates to test window. Do not immerse past the MAX line.

Step 5: Set up the timer.

Step 6: Result should be read at 5 minutes. Positive results may be visible in as soon as 1 minute. Negative results must be confirmed at the end of 10 minutes only. ***Any results interpreted outside of the 5-10 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.***

QUALITY CONTROL

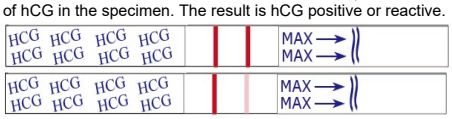
1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen. Otherwise, review the whole procedure and repeat test with a new device.

2. **External Control:** Good Laboratory Practice recommends using the external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit is used.
   c. A new shipment of kits is used.
   d. The temperature during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

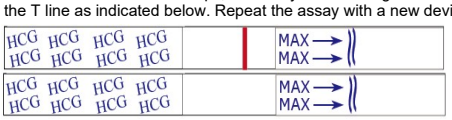
1. **NEGATIVE RESULT:** If only the C line develops, the test indicates that no detectable hCG is present in the specimen. The result is hCG negative or non-reactive.

2. **POSITIVE RESULT:** If both C and T lines develop, the test indicates for the presence of hCG in the specimen. The result is hCG positive or reactive.



Specimens with reactive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. Clinical Performance

1.1 For Urine Specimens

A total of 319 fresh urine specimens were randomly collected from two groups of patients (pregnant and non-pregnant) at two geographically dispersed centers, and tested by *OnSite* hCG Combo Rapid Test and by hCG chemiluminecsence or ELISA. Comparison for all subjects is shown in the follow table 1:

Table 1

	OnSite hCG Combo Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	187	0	187
Negative	0	132	132
Total	187	132	319

Relative Sensitivity: 100% (95% CI: 98.5% - 100%)
Relative Specificity: 100% (95% CI: 97.9% - 100%)
Overall Agreement: 100% (95% CI: 99.1% - 100%)

1.2 For Serum Specimens

A total of 276 fresh serum specimens were randomly collected from two groups of patients (pregnant and non-pregnant) at two geographically dispersed centers, and tested by *OnSite* hCG Combo Rapid Test and by hCG chemiluminecsence or ELISA. Comparison for all subjects is shown in the follow table 2:

Table 2

	OnSite hCG Combo Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	62	0	62
Negative	1	213	214
Total	63	213	276

Relative Sensitivity: 100% (95% CI: 95.8% - 100%)
Relative Specificity: 99.5% (95% CI: 98.0% - 100%)
Overall Agreement: 99.6% (95% CI: 98.4% - 100%)

2. Analytical Sensitivity

The detection limit of hCG for the *OnSite* hCG Combo Rapid Test is 12.5 mIU/mL in serum or plasma specimen and 25 mIU/mL in urine specimen.

The following experiments were done to validate the sensitivity of the *OnSite* hCG Combo Rapid Test: Five groups of urine and serum specimens from 20 normal non-pregnant healthy individuals (confirmed hCG negative using CE/FDA cleared hCG test) were spiked with hCG to the standard (5^{th} IS) concentrations of 0, 6.25, 12.5, 25, 50 mIU/mL. The specimens were run on the *OnSite* hCG Combo Rapid Test. Results were observed at 5 minutes and tabulated in table 3 & 4.

Table 3

hCG mIU/mL in serum specimens	**0**	**6.25**	**12.5**	**25**	**50**
Number of positive	0	0	20	20	20
Number of negative	20	20	0	0	0
Detection Rate %	0%	0%	100%	100%	100%

n=20; Positive Detection Rate at 12.5 mIU/mL is 100%

Table 4

hCG mIU/mL in urine specimens	**0**	**6.25**	**12.5**	**25**	**50**
Number of positive	0	0	11	20	20
Number of negative	20	20	9	0	0
Detection Rate %	0%	0%	55%	100%	100%

n=20; Positive Detection Rate at 25 mIU/mL is 100%

3. Analytical Specificity

Specificity of the *OnSite* hCG Combo Rapid Test was determined from studies on specimens with the following standard obtained from SIGMA. Specimens containing these structurally related hormones at tested concentrations were found not to significantly cross-react with the hCG *OnSite* rapid test.

human luteinizing hormone (hLH)	1,000 mIU/mL
human follicle stimulating hormone (hFSH)	1,000 mIU/mL
human thyroid stimulating Hormone (hTSH)	1,000 mIU/mL

4. Dose Hook Effect

No false negative results due to the dose hook effect were observed for urine specimens containing hCG at concentrations up to 500,000 mIU/mL.

5. Interference

Potentially interfering biological and chemical analytes commonly found in OTC, prescriptions, or abuse drugs were tested in two confirmed negative urine and serum specimen spiked with hCG standard at 50 mIU/mL and 25 mIU/mL. The presence of the following substances at the indicated concentrations (and pH range of 4-9) did not interfere with the results obtained with the *OnSite* hCG Combo Rapid Test.

Biological analytes and concentrations tested:

1. Albumin	300 mg/dL	6. Glucose	12,000 mg/dL
2. Ampicillin	20 mg/dL	7. Bilirubin	2,000 µg/dL
3. Tetracycline	20 mg/dL	8. Hemoglobin	1,000 µg/dL
4. Uric acid	100 mg/dL		
5. Urea	100 mg/dL		

Chemical analytes and concentrations tested:

1. Acetaminophen	20 mg/dL	15. Phendimetrazine	20 mg/dL
2. Acetylsalicylic acid	20 mg/dL	16. Penicillin G	20 mg/dL
3. Amikacin	20 mg/dL	17. Quinine	20 mg/dL
4. Ascorbic acid	20 mg/dL	18. Ranitidine	20 mg/dL
5. Aspartame	20 mg/dL	19. Sodium Salicylate	20 mg/dL
6. Atropine Sulfate	20 mg/dL	20. Tryptophan	20 mg/dL
7. Benzoic Acid	20 mg/dL	21. Tetracycline	20 mg/dL
8. Caffeine	20 mg/dL	22. Tetrahydrozoline	20 mg/dL
9. Deoxyephedrine	20 mg/dL	23. Atropine	20 mg/dL
10. Dextromethorphan	20 mg/dL	24. Ethanol	1%
11. EDTA	80 mg/dL	25. Methanol	1%
12. Gentisic acid	20 mg/dL	26. Heparin	1%
13. Histamine	20 mg/dL	27. Citrate	3.20%
14. Methaqualone	20 mg/dL		

LIMITATIONS OF PROCEDURE

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of hCG in urine or serum from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. If a urine specimen is too diluted, it may not contain representative levels of hCG. If pregnancy is still suspected, a first morning urine should be obtained from the person and the test repeated. The hCG concentration less than 25 mIU/mL may be detected as negative.
3. A number of disease conditions other than pregnancy such as trophoblastic disease, proteinuria, hematuria, choriocarcinoma, ovarian and testicular teratomas can cause elevated levels of hCG. The diagnosis should be considered if appropriate to the clinical evidence.
4. Immunologically interfering substances such as those used in antibody therapy treatments may invalidate this assay.
5. Samples containing very high levels of hCG ≥ 500,000 mIU/mL may yield a test line with color intensity lighter than that, which is expected. When high dose "hook effect" is suspected, it is recommended the test be repeated with a 1:10 dilution of the specimen with DI H_2O.
6. Ectopic pregnancy cannot be distinguished from normal pregnancy from hCG measurements alone.
7. Samples from patients on chemotherapy for cancer should be ruled out before running the assay.
8. Positive hCG levels may be detectable for several weeks following delivery or abortion.
9. Specimens testing positive during the initial days after conception may be negative later due to natural termination of the pregnancy.
10. Low hCG levels may be found in the specimens from highly suspected pregnant women. Some abnormal conditions should be ruled out, such as miscalculation of pregnancy dating, possible miscarriage or blighted ovum, ectopic pregnancy, or cryptic pregnancy.
11. A single hCG measurement may not provide enough information for most diagnoses as the change in hCG concentration is more important than the concentration detected. When obtaining negative test results during a suspected pregnancy, it is recommended to retest a few day later, or test with a specific alternative test method, such as ultrasonography.
12. Results obtained with the *OnSite* hCG Combo Rapid Test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

EXPECTED VALUES

Healthy men and healthy non-pregnant women do not have detectable hCG by the *OnSite* hCG Combo Rapid Test. The hCG levels of 100 mIU/mL can be reached on the day of the first missed menstrual period. The hCG levels peak about 7-12 weeks after the last menstrual period and then decline to lower values for the remainder of the pregnancy. Following delivery, hCG levels rapidly decrease and usually return to normal shortly after parturition.

STANDARDIZATION

The *OnSite* hCG Combo Rapid Test has been calibrated against World Health Organization the Fifth International Standard (5^{th} IS): 179 IU/ampoule NIBSC code: 07/364.

REFERENCES

1. Cart KJ, Dufau ML, Vaitukaitis JL. Appearance of hCG in pregnancy plasma following the initiation of implantation of the blastocyst. J Clin Endocrinol Metab. 1975 40:537-40.
2. Braunstein GD, Rasor J, Danzer H, et al.Serum human chorionic gonadotropin levels throughout normal pregnancy. Am J Obstet Gynecol. 1976, 126:678-81.

Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

PI-R1001S-25mIU Rev. D3.0
Date released: 2020-12-11
English Version

For Export Only, Not for Re-sale in the USA