CE

CTK Biotech, Inc.
The New Wave of Diagnosis

REF

Catalog Number R1001S

IVD

In vitro Diagnostic

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, hCG, at the cut-off level of 20 mIU hCG/mL of human urine or serum.

The *OnSite* HCG Combo Rapid Test are 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.

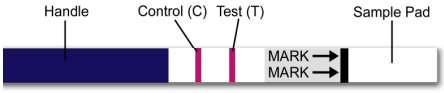
SUMMARY AND EXPLANATION OF THE TEST

Human chorionic gonadotropin (hCG) is produced by 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 subjects, 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 plasma or urine

TEST PRINCIPLE

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



When an adequate volume of test specimen is dispensed into the sample pad of the test strip, the specimen migrates by capillary action across the strip. HCG if present in the specimen at the level equal or higher than 20 mIU/ml will bind to the HCG Ab conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-HCG Ab, forming a burgundy colored T band, indicating a HCG positive test result.

Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti-mouse IgG/mouse IgG-gold conjugate regardless of the color development on the T band. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 50 test devices, each sealed in a foil pouch with two items inside:
   a. One dip strip device.
   b. One desiccant.
2. One package insert (instruction for use).

MATERIALS REQUIRED AND AVAILABLE FOR PURCHASE

1. Positive Control (1 vial, red cap, 1 mL, Cat # R1001-P)
2. Negative Control (1 vial, green cap, 1 mL, Cat # R1001-N)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. A container to collect urine specimen or serum specimen

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives 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℃) 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 hemolized 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 biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 10 minutes after a specimen is applied to the sample well or sample pad of the device. Reading result after 10 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. 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℃. The positive and negative controls should be kept at 2℃-8℃. If stored at 2℃-8℃, 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 over 30℃.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety 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.

If the test is not run immediately following collection of the sample specimen, but is to be run within 48 hours following collection, the specimen should be refrigerated (2℃-8℃).

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2℃ to 8℃ if not tested immediately.

Store specimens at 2℃ to 8℃ up to 5 days. The specimens should be frozen at -20℃ for longer storage.

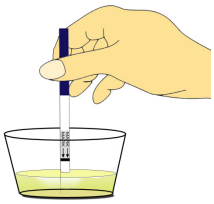
ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: Collect at least 150-200 µLor 3-4 drops of serum or plasma in a sample container.

Step 3: When ready to test, open the pouch at the notch and remove the test strip.

Step 4. Dip the strip into the specimen for at least 15 seconds. Don't allow the specimen reach above the level indicated by the arrows on the strip.
At meanwhile, set up the timer.



Step 5: Remove the strip from the specimen, and place it on a flat, dry surface.

Step 6: Read the test result in 10 minutes. Positive result could be visible as short as 1 minute.

Don't read results after 10 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

Using individual *OnSite* HCG Combo Rapid Test strips as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control (provided upon request) under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit fall outside of 2℃-30℃.
5. The temperature of the test area falls outside of 15℃-30℃.

Expected results are as follows:

Negative Control

Only the C band shows color development. The T band shows no color development.

Positive Control

Both C and T bands show color development.

The appearance of any burgundy color in the T band, regardless of intensity, must be considered as presence of the band.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C band is developed, the test indicates that no detectable HCG is present in the specimen. The result is negative.

2. **POSITIVE RESULT**: If both C and T bands are developed, the test indicates for the presence of HCG in the specimen. The result is positive.

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

PERFORMANCE CHARACTERISTICS

Sensitivity:

The detection limit for the *OnSite* HCG Combo Rapid Test -is 25 mIU/mL. The urinary or serum hCG levels equal to or greater than 25 mIU/mL routinely test positive. Samples containing hCG less than 25 mIU/mL may also produce a very faint positive line, especially with extended assay time from 10 to 30 minutes.

The following experiments were done to validate the sensitivity of the *OnSite* HCG Combo Rapid Test -strip

Seven groups of urine specimens from 20 normal nonpregnant individuals were spiked with hCG to the standard (3rd IS) concentrations of 0, 10, 16, 20, 25, 50, and 100 mIU/mL. The specimens were run on the *OnSite* HCG Combo Rapid Test -. Results are tabulated in table 1 below.

Table 1

HCG mIU/ml	0	10	16	20	25	50	100
Number of positive	0	11	14	18	20	20	20
Number of negative	20	9	6	2	0	0	0

n=20 relative sensitivity at 25 mIU/mL = 20/20 x 100% = 100%

SPECIFICITY:

Specificity of the *OnSite* HCG Combo Rapid Test was determined from studies on specimens with 500 mIU/mL of human luteinizing hormone (hLH), 1,000 mIU/mL of human follicle stimulating hormone (hFSH), and 1,000 µIU/ml of human thyroid stimulating Hormone (hTSH), each standard obtained from SIGMA. Specimens containing these structurally related hormones at tested concentrations were found not to significantly cross-react with hCG antibodies as to yield false positive or false negative results.

ACCURACY:

The accuracy of the *OnSite* HCG Combo Rapid Test was determined by a comparison study with a currently marketed hCG pregnancy test device, and was conducted at an external clinical site. A total of 172 fresh urine specimens, including 91 hCG positive and 81 hCG negative were randomly collected from the patients who visited an OB-GYN office. The two assays gave a complete agreement as shown in Table 2 below:

Table 2

	Reference hCG device (+)	Reference hCG device (-)	Total
The *OnSite* HCG Combo Rapid Test +	91	0	91
The *OnSite* HCG Combo Rapid Test -	0	81	81
Total	91	81	172

INTERFERENCE TESTING:

The Chemicals commonly found in OTC, prescription, or abuse drugs were spiked into both hCG negative and 25 mIU hCG/mL urine specimens. Spiked samples were tested against following substances or pHs at the indicated concentrations. There was no interference observed.

List of potentially interfering chemical analytics and concentrations tested:

1. Acetaminophen	20 mg/dL
2. Acetylsalicylic acid	20 mg/dL
3. Ascorbic acid	20 mg/dL
4. Caffeine	20 mg/dL
5. Gentesic acid	20 mg/dL
6. Phenylpropanoamine	20 mg/dL
7. Salicylic acid	20 mg/dL
8. EDTA	80 mg/dL
9. Acetylsalicylic acid	20 mg/dL
10. Benzoylecgonine	10 mg/dL
11. Atropine	20 mg/dL
12. Cannalbinol	10 mg/dL
13. Ethanol	1%
14. Methanol	1%

Biological Analytics

1.Albumin	2,000 mg/dL
2.Glucose	2,000 mg/dL
3.Bilirubin	1,000 µg/dL
4.Hemoglobin	1,000 µg/dL

pH

1. pH 5
2. pH 9
3. pH 6.8

LIMITATIONS OF PROCEDURE

1. 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 will be detected as negative.
2. 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. These diagnosis should be considered if appropriate to the clinical evidence.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Immunologically interfering substances such as those used in antibody therapy treatments may invalidate this assay.
5. Samples containing very high levels of hCG ≥600,000 mIU/mL may yield a test band 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. Grossly hemolyzed or lipemic samples should not be used since they may give inaccurately lower or erratic results.
7. Ectopic pregnancy cannot be distinguished from normal pregnancy from hCG measurements alone.
8. Samples from patients on chemotherapy for cancer should be ruled out before running the assay.
9. Positive hCG levels may be detectable for several weeks following delivery or abortion.
10. Specimens testing positive during the initial days after conception may be negative later due to natural termination of the pregnancy.

EXPECTED VALUES

Healthy men and healthy non-pregnant women do not have detectable hCG by the *OnSite* HCG Combo Rapid Test. HCG levels of 100 mIU/mL can be reached on the day of the first missed menstrual period. 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 Third International Standard (3rd IS).

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English Version

For Export Only, Not For Re-sale In the USA

Index of CE Symbol

	Attention, see instructions for use
IVD	For *in vitro* diagnostic use only
REF	Catalog #
LOT	Lot Number
	Use by
Σ	Tests per kit
2°C 30°C	Store between 2-30℃
	Do not reuse
	Manufacturer
	Date of manufacture