NOVAPLUS®

EKLA CORPORATION

NOVAPLUS® hCG Pregnancy Serum/Urine

Combo Test Cassette

CLIA Waived - Urine CLIA Moderate - Serum

INTENDED USE

The hCG Pregnancy Serum/Urine Combo Test Cassette is a rapid chromatographic immunoassay for the visual, qualitative detection of human chorionic gonadotropin in human urine or serum specimens to aid in early detection of pregnancy. This device is intended for prescription use in clinical laboratories and point-of-care use settings.

INTRODUCTION

Human chorionic gonadotropin (hCG), a glycoprotein hormone secreted by viable placental tissue during pregnancy, is excreted. hCG levels rise rapidly, reaching peak levels after 60-80 days[1-5]. The appearance of hCG in urine after implantation and its rapid rise in concentration makes it an ideal marker for the early detection of pregnancy[6].

PRINCIPLE

The hCG Pregnancy Combo Test Cassette detects human chorionic gonadotropin through visual interpretation of color development in the internal strip. Anti-hCG antibodies (goat anti HCG polyclonal antibody)are immobilized on the test region of the membrane, and goat anti-mouse IgG antibodies immobilized on the control region[7-8]. During testing, the specimen reacts with anti-hCG antibodies (mouse anti-hCG monoclonal antibodies) conjugated to colored particles and precoated onto the sample pad of the strip. The mixture then migrates through the membrane by capillary action and interacts with reagents on the membrane. If there is sufficient hCG in the specimen, a colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

MATERIALS

MATERIALS PROVIDE

- Individually packed test devices
- Disposable pipettes
- Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Specimen collection container
- Centrifuge
- Timer
- External control materials (see Quality Control section)

PRECAUTIONS

- For *in vitro* diagnostic use only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions.
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded according to local regulations.

STORAGE AND STABILITY

- The kit should be stored at 39-86°F until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- Do not freeze.
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results[9].

SPECIMEN COLLECTION AND STORAGE

- The hCG Pregnancy Combo Test Cassette is intended for use with human urine or serum specimens only.
- Although urine specimens from any time of day can be used, first morning urine specimens are preferable as they contain the highest concentration of hCG.
- Only clear specimens are recommended for use with this test. Serum should be separated as soon as possible to avoid hemolysis.
- Turbid specimens should be centrifuged, filtered or allowed to settle and only the clear supernatant should be used for testing.
- Collected urine/serum specimens must be put in clean, dry containers.
- Perform testing after specimen collection. Do not leave specimens at room temperature for prolonged periods. Specimens may be stored at 36-46°F for up to 48 hours. For long term storage, specimens should be kept below-4°F.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
- Icteric, lipemic, hemolysed, heat treated and contaminated sera may cause erroneous results[10].

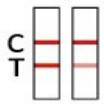
PROCEDURE

Bring tests, specimens, and/or controls to room temperature (59-86°F) before use.

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. For best results the assay should be performed within one hour.
2. Add 3 drops of specimen (approximately 120 µL) directly into the specimen well (S) and start the timer.
   Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result area. As the test begins to work, color will migrate across the result area in the center of the device.
3. Wait for the colored band(s) to appear. Read the result at 3 minutes when testing a urine specimen, or at 5 minutes when testing a serum specimen. Do not interpret the result after 10 minutes.

NOTE: Low hCG concentrations may produce very weak T lines after a prolonged period of time. Therefore, do not interpret the result after 10 minutes.

INTERPRETATION OF RESULTS



POSITIVE: Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE: Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.
3. Negative results are expected in healthy non-pregnant women. The amount of hCG in a sample can vary greatly with gestational age and between individuals.

QUALITY CONTROL

Control standards are not supplied with this kit. Though there is an internal procedural control line in the test device of control region, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance. Quality control testing should be performed with each new lot, each new shipment and every thirty days to check storage. Please contact our Technical Support at 1-800-328-4215 (9-5 CST M-F) for controls that work with the device.

LIMITATIONS OF THE TEST

1. Very dilute urine specimens, exhibiting low specific gravity, may not contain representative levels of hCG. If pregnancy is suspected after a negative result, a first morning urine sample should be obtained 48-72 hours later and tested.
2. Very low levels of hCG are present in urine or serum 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 interpreted in conjunction with other clinical and laboratory data.
3. When hCG levels are below the minimum detection level of the test, a false negative result may be obtained. If pregnancy is suspected after a negative result, a first morning urine specimen should be collected 48-72 hours later and tested.
4. A positive result may be obtained with β-core fragment hCG at concentrations over 200 pmol/L.
   A negative result may be obtained with β-core fragment hCG at concentrations below 200 pmol/L.
   A false negative result may obtained with β-core fragment hCG at concentrations above 2 x 10^6 pmol/L.
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. A confirmed pregnancy diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

120 positive and negative clinical serum samples and 120 positive and negative clinical urine samples were analyzed at 3 sites (including 2 POL sites).

Table: hCG Pregnancy Combo Test Cassette vs. QuickVue+ One-Step hCG Combo test

1) Urine Sample

		QuickVue+ device	
		+	-
NOVAPLUS	+	60	0
	-	0	60

2) Serum Sample

		QuickVue+ device	
		+	-
NOVAPLUS	+	60	0
	-	0	60

The comparison agreements are >99.9%.

SENSITIVITYAND PRECISION

The hCG Pregnancy Combo Test Cassette will display positive results with specimens containing HCG at levels of 10 and 20mIU/ml or greater for serum and urine respectively. 3 lots devices were evaluated at 3 physician's offices by 9 operators.

Typical Serum format Data

hCG Concentration (mIU/mL)	Site 1		Site 2		Site 3		Total result		% Negative	% Positive
	-	+	-	+	-	+	-	+		
0	50	0	50	0	50	0	150	0	100%	0
4	50	0	50	0	50	0	150	0	100%	0
6	50	0	50	0	50	0	150	0	100%	0
8	24	26	25	25	25	25	74	76	49.3%	50.7%
10	0	50	0	50	0	50	0	150	0	100%
12	0	50	0	50	0	50	0	150	0	100%
14	0	50	0	50	0	50	0	150	0	100%
16	0	50	0	50	0	50	0	150	0	100%
20	0	50	0	50	0	50	0	150	0	100%
50	0	50	0	50	0	50	0	150	0	100%

Typical Urine format Data

hCG Concentration (mIU/mL)	Site 1		Site 2		Site 3		Total result		% Negative	% Positive
	-	+	-	+	-	+	-	+		
0	50	0	50	0	50	0	150	0	100%	0
5	50	0	50	0	50	0	150	0	100%	0
10	50	0	50	0	50	0	150	0	100%	0
12	50	0	50	0	50	0	150	0	100%	0
16	25	25	24	26	22	28	71	79	47.3%	52.7%
20	0	50	0	50	0	50	0	150	0	100%
24	0	50	0	50	0	50	0	150	0	100%
30	0	50	0	50	0	50	0	150	0	100%
50	0	50	0	50	0	50	0	150	0	100%
100	0	50	0	50	0	50	0	150	0	100%

SPECIFICITY

The specificity of hCG Pregnancy Combo Test Cassette was determined in cross reactivity studies with known amounts of Luteinizing Hormone (hLH), Follicle Stimulating Hormone (hFSH) and Thyroid Stimulating Hormone (hTSH). 300 mIU/mL hLH, 1000 mIU/mL hFSH and 1000 µIU/mL hTSH all gave negative results.

Tests were performed for samples with 10 and 20 mIU/mL hCG in urine, and 5 and 10 mIU/mL hCG in serum. No interference was found for the following substances at the giving concentrations.

Acetaminophen (20mg/dL)	Phenylpropanolamine(20 mg/dL)	Cholesterol (250mg/dL)	(1400ug/dL)
Acetoacetic Acid (2000mg/dL)	Salicylic Acid (20mg/dL)	Triglyceride (500mg/dL)	Hemoglobin (2000mg/dL)
Asorbic Acid (20mg/dL)	Phenothiazine (20mg/dL)	Codeine (6ug/dL)	Pregnanediol (1500ug/dL)
B-hydroxybutyrate (2000mg/dL)	EDTA (80mg/dL)	Ethanol (1.0%)	Thiophene (20mg/dl)
Caffeine (20mg/dL)	Acetylsalicylic Acid (20mg/dL)	Methanol (10%)	Ampicillin (20mg/dl)
Ephedrine (20mg/dL)	Benzoylecgonine (10mg/dL)	Albumin (2000mg/dL)	Tetracycline(20mg/dl)
Gentisic Acid (20mg/dL)	Cannabinol (10mg/dL)	Glucose (2000mg/dL)	Ketone(20mg/dl)
		Bilirubin (2mg/dL)	
		Atropine (20mg/dL)	
		Estriol-17-beta	

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GLOSSARY OF SYMBOLS

REF	Catalog number
	Consult instructions for use
IVD	*In vitro* diagnostic medical device
	Manufacturer
	Temperature limitation
LOT	Batch code
	Use by
	Do not reuse

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