NG-Test® hCG Whole Blood

Rapid test for the qualitative detection of human chorionic gonadotropin (hCG) in whole blood and serum.
For professional use only.

NG·BIOTECH LABORATOIRES

Ref: ENO411HCG / Rev: 220519EN

Intended use

The NG-Test® hCG Whole Blood is a rapid immunoassay for the qualitative detection of human chorionic gonadotropin[1] (hCG) in human whole blood and serum as an aid for the early detection of pregnancy.

Summary

Human chorionic gonadotropin (hCG) is a glycoprotein secreted by trophoblast cells during pregnancy. This hormone interacts with ovary specific receptors and promotes the maintenance of the corpus luteum for the maternal recognition of pregnancy. This allows the corpus luteum to secrete progesterone which play key roles to prepare uterus for fetus growth. The possible detection of hCG in whole blood and serum as early as 7 to 10 days after conception makes it an excellent marker for confirming rapidly pregnancy[2-4].

The NG-Test® hCG Whole Blood uses highly specific monoclonal antibodies targeting hCG in human whole blood and serum. Its detection at a level above 10 mIU/mL is used to detect early pregnancy.

Test principle

The NG-Test® hCG Whole Blood uses monoclonal hCG antibodies to selectively detect elevated levels of hCG in whole blood and serum. The assay is conducted by dispensing an adequate volume of the blood or serum specimen into the sample well of the cassette using the lancet and the micropipette. A buffer solution is added to the well to facilitate the reagent capillary action across the strip held in the cassette. The sample then migrates across a membrane toward the results window where the labeled hCG complex is captured at a test line region containing immobilized monoclonal anti-hCG. The appearance of two red lines, one at test region (T) and the other at the control region (C) indicates the presence of hCG in the sample. A colored line should always appear in the control line region (marked "C"), indicating that the proper volume of specimen has been added and that the test worked correctly. If hCG is absent or below the detection limit (< 10 mIU/mL), only the control line will appear in the result window

Reagents and material provided

Each kit contains:

- 20 cassettes, each sealed in a foil pouch with desiccant
- 20 calibrated micropipettes capable delivering 20µl accurately
- 20 lancets
- 20 alcohol prep pads
- 1 buffer solution in plastic dropper bottle
- 1 package insert

Material required but not provided

- Timer
- Disposable gloves
- Specimen collection containers for serum
- Calibrated micropipette for 10µL(serum only)

Precautions

- For Professional in vitro diagnostic use only.
- Do not use after the expiration date.
- Do not use test if pouch is torn or damaged.
- The test device should remain in the sealed pouch until use.
- Perform the test quickly after opening the aluminum pouch.
- Wear protective clothing such as laboratory coat, disposable gloves and eye protection when specimens are assayed.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Make sure all collected blood is transferred on test strip. Too much or too little sample size may lead to deviation of results.
- Do not use the buffer of another kit.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- The test must be placed on a flat surface while waiting for the results. The test should never be oriented upwards.
- The test device should not be reused.
- The test device should be discarded in a suitable biohazardous waste container after testing according to local regulations.

Storage and stability

Store as packaged in the sealed pouch between 4 and 30°C. The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE**. Do not use beyond the expiration date. If the kit has been stored at 4-8°C, bring it to room temperature (15-25°C) for at least 10 minutes.

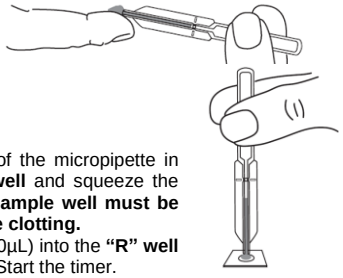
Specimen collection

Fresh capillary blood from finger prick should be used as a test specimen. However, serum is a suitable alternative specimen. The specimen should be collected in a clean glass or plastic container.

Procedure

WHOLE BLOOD – Capillary Collection:

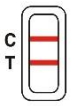
1. Wear protective gloves.
2. Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
3. Clean the patient's finger with the alcohol swab.
4. Open the pouch and remove the device. Once opened, the device must be used immediately.
5. Open the lancet and prick the lateral side of the patient's fingertip to obtain a drop of blood (20µL). Using gentle pressure, massage the finger towards the fingertip to encourage a drop of blood to form.
6. Hold the capillary micropipette **horizontally (do not press the bulb)** and touch with the tip of the micropipette the blood sample. The sampling is realized automaticaly by capillarity until reaching the black mark.
7. To expel the sample, place the tip of the micropipette in contact with the strip into the **"S" well** and squeeze the micropipette bulb. **Transfer to the sample well must be immediate in order to avoid sample clotting.**
8. Add **2 drops of buffer solution** (2x40µL) into the **"R" well while holding the bottle vertically.** Start the timer.
9. Read the results at **5 minutes** and interprete as below. Do not read the test results after 10 minutes.



SERUM (from a prepared sample in a laboratory):

1. Collect 10µL of serum using a calibrated micropipette (not included in the kit).
2. Dispense the sample into the **"S" well**.
3. Add **2 drops of buffer solution** (2x40µL) into the **"R" well while holding the bottle vertically.** Start the timer.
4. Read the results at **5 minutes** and interprete as below. Do not read the test results after 10 minutes.

Interpretation of results



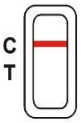
Positive

Positive

Both test line (T) and control line (C) appear in the result window.

NOTE:

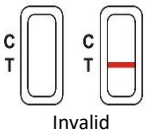
- *The intensity of the color in the test line regions may vary depending on the concentration of the hCG in the sample. A faint line (C or T) should be considered as positive result.*
- *Neither the quantitative value nor the rate of increase in hCG can be determined by this qualitative test.*



Negative

Negative

Only the control line (C) appears in the result window. The absence of a test line (T) indicates a negative result.



Invalid

Invalid

If the control line (C) does not appear, the test result is invalid.

Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

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Quality Control

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this kit. However, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Limitations

1. If a negative result is obtained although pregnancy is still suspected, the hCG level in the specimen may be below the detection limit of the test (10 mIU/mL) It is recommanded to repeat the test 24 to 48 hours later.
2. Very low levels of hCG are present in whole blood specimen shortly after implantation. However, because a significant number of first trimester pregnancies terminate for natural reasons[5], a test result weakly positive should be confirmed by retesting 48 hours later.
3. A number of conditions other than pregnancy, including trophoblastic disease, testicular tumors, prostate cancer, breast cancer and lung cancer cause elevated levels of hCG [6-9]. Therefore, the presence of hCG in whole blood specimen should not be used to diagnose pregnancy unless these conditions have been ruled out.
4. This test provides a presumptive diagnosis for pregnancy.
   A confirmed pregnancy diagnosis should be made by a physician after all clinical and laboratory findings have been evaluated.

Performance characteristics

1. Sensitivity and specificity

A retrospective study was performed on 168 specimens with known hCG concentrations using a reference method: 72 positive specimens at a concentration ≥ 10 mIU/mL and 96 negative specimens. Results are summarized in the table below:

		Reference hCG Method	
		positive	negative
NG-Test® hCG Whole Blood	positive	72	0
	negative	0	96

NG-Test® hCG Whole Blood reached 100% sensitivity (95%CI 95.01%-100%) and 100% specificity (95% CI 96.23%-100%) when compared to the reference method.

2. Clinical evaluation

NG-Test® hCG Whole Blood was evaluated prospectively and independently in early pregnancy units on 200 patients[10]. Considering the claimed limit of detection, the test demonstrated 100% and 99.3% Positive Predictive Value and Negative Predictive Value respectively.

3. Limit of detection

Real human specimens were spiked to reach the following levels of hCG:
Sample 1; [hCG] <2 mIU/mL
Sample 2: [hCG] = 8 mIU/mL
Sample 3: [hCG] = 10 mIU/mL
Sample 4: [hCG] = 11 mIU/mL

The hCG levels were confirmed by an external laboratory using a reference method.
Twenty replicates were performed on 3 lots of tests with sample 1 for a total of 60 repetitions.
Twelve replicates were performed on 3 lots of tests with sample 2, sample 3 and sample 4 .Results obtained are summarized in the table below (all replicates were in agreement for each sample):

	1[hCG] <2 mIU/ml	2[hCG] =8 mIU/ml	3[hCG] =10 mIU/ml	4[hCG] =11 mIU/ml
Lot 1	Negative	Negative	Positive	Positive
Lot 2	Negative	Negative	Positive	Positive
Lot 3	Negative	Negative	Positive	Positive

The limit of detection of NG-Test® hCG Whole Blood is 10 mIU/mL.

4. Hook Effect

No hook effect observed up to 500,000 mIU/mL.
The test bands may appear with a less intensive color than expected for samples containing high levels of hCG> 500,000 mIU/mL.

5. Repeatability and reproductibility

The repeatability and reproducibility of the NG-Test® hCG Whole Blood was evaluated with positive and negative samples. No significant difference was observed between batches, operators and inter-run results.

6. Interfering substances

The following potentially interfering substances were added to hCG negative and positive (10 mIU/mL) specimens.
None of the substances at the concentration tested interfered in the assay.

Interfering substances	Concentration level
LH	300 mIU/mL
FSH	1000 mIU/mL
TSH	1000 mIU/mL
Rheumatoid Factor	174 IU/mL
Human anti-mouse antibodies	715 ng/mL
Antinuclear antibodies (centromere type speckled nucleolar and homogeneous)	Titer > 1280
Caffeine	200 µg/mL

7. Standardization

The test has been standardized to the WHO 5th International Standard (NIBSC - 07/364).

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Symbols

Σ	Content sufficient for		Expiry date
IVD	*In Vitro* Diagnostic Medical Device		Do not reuse
LOT	Batch number	REF	Product reference
	Read Instructions for Use before use	+4°C +30°C	Temperature limits
	Manufacturer		

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