β-hCG-CHECK-1

Quantitative determination of β-hCG in plasma, serum or urine samples
- FOR EASY READER® AND EASY READER+® USE ONLY -
Ref.: 3291-3L (20 tests) / 3291-3L-10T (10 tests) - PATENTED TEST -

I- PRINCIPLE

The β-hCG-CHECK-1 test is a rapid quantitative assay for the detection of human chorionic gonadotropin beta chain either free or from intact hCG in plasma, serum and urine. The method, which is patented, employs a unique combination of anti β-hCG monoclonal-dye conjugate and polyclonal-solid phase antibodies to selectively identify β-hCG in the test samples with a high degree of sensitivity.

These antibodies recognize specific epitopes which are present either in the free β-hCG subunit or in the β-subunit of the intact hCG.

Depending on the β-hCG concentration, different lines will appear on the test allowing the quantitative measurements of β-hCG level in serum, plasma or urine samples when used in combination with VEDALAB's rapid tests readers.

II- β-hCG-CHECK-1 KIT COMPONENTS

Each kit contains everything needed to perform 10 or 20 tests.

1- β-hCG-CHECK-1 reaction devices:	10	20
2- Disposable plastic pipettes:	10	20
3- Instruction leaflet:	1	1

4- Controls (Optional):

Positive control (ref. V053 for serum and urine, freeze-dried) and Negative control (ref. V054 for serum and urine, liquid): the controls (1 x 0.5 mL) are prepared from non-infectious human chorionic gonadotropin, tested and found negative for anti-HIV, anti-HCV and HBs antigen and contain 0.05 % sodium azide. The concentration range is indicated on the vial label.

III- STORAGE AND STABILITY

1- All β-hCG-CHECK-1 test components should be stored at room temperature (+4°C to +30°C).

2- Do not freeze the test kit.

3- β-hCG-CHECK-1 test is stable until the expiry date stated on the package label.

IV- PRECAUTIONS

1- This test is designed for *in vitro* diagnostic use and professional use only.
2- Read carefully the instructions before using this test.
3- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1% solution of sodium hypochlorite for one hour before disposal.
4- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
5- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
6- Avoid any contact between hands and eyes or nose during specimen collection and testing.
7- Do not use beyond the expiry date which appears on the package label.
8- Do not use a test from a damaged protective wrapper.

V- SPECIMEN COLLECTION AND PREPARATION

1- β-hCG-CHECK-1 rapid test is performed on plasma, serum or urine sample.

a) Plasma, serum

2- The serum specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid haemolysis).
3- If anticoagulant is needed, only citrate, EDTA or heparin should be used.
4- Each specimen should be treated as if potentially infectious.
5- If the test is to be run within 48 hours after collection the specimen should be stored in the refrigerator (+2°C to +8°C). If testing is delayed more than 48 hours, the specimen should be frozen. The frozen specimen must be completely thawed, thoroughly mixed and
brought to room temperature prior to testing. Avoid repeated freezing and thawing.
6- In case of cloudiness, high viscosity or presence of particulate matter into the serum specimen, it should be diluted with equal volume (V/V) of diluting buffer (not provided but available upon request) before testing.

b) Urine

2. For optimal detection of early pregnancy, a first morning urine specimen is preferred since it contains the highest concentration of β-hCG. However, randomly collected urine specimens may be used.
3. Collect the urine specimen in a clean container **without preservatives.**
4. If testing is not immediate, the specimen should be refrigerated (+2°C to +8°C) or kept cool (below +25°C) for up to 24 hours. In such case bring the specimen to room temperature prior to testing.
5. If testing is delayed more than 24 hours, the specimen should be frozen. The frozen specimen must be completely thawed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.
6. A new urine sample should be preferably collected for testing in case of cloudiness, presence of sediments or signs of microbial growth / contamination.

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VI- ASSAY PROCEDURE

IMPORTANT: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

a) Control testing

- Positive control: Wait for 15 minutes after freeze-dried dissolving.
- Negative control: Ready to use.
- Add the requested volume (150µL) with **lab pipette (disposable tips)** into the sample well of the cassette and read the result at 15 minutes.
- The expected concentration levels (**in IU/L**) is indicated on the vial label and obtained result must match with the indicated value. The concentration level can change slightly depending on lot number.

- Positive control: The reconstituted vial should be kept between +2°C and +8°C and should be used within 7 days after reconstitution.

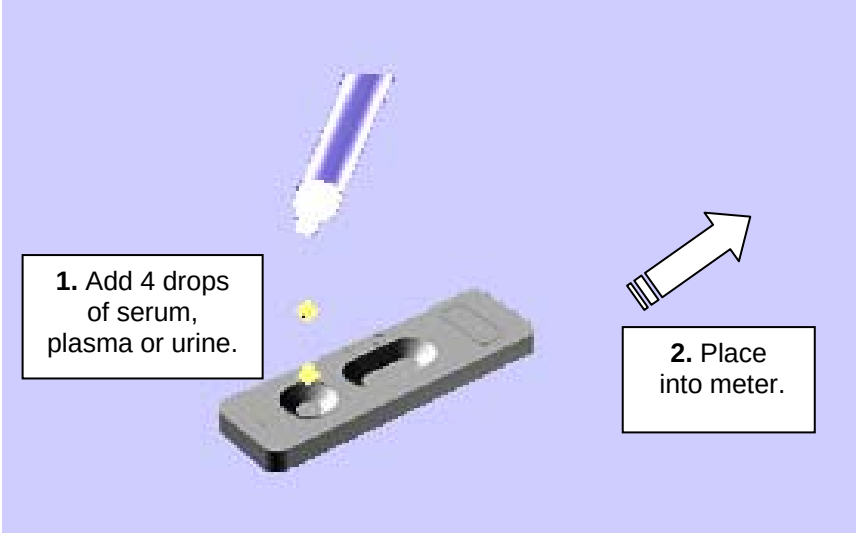
- Negative control: After each use, promptly replace the stopper and keep the vial between +4°C to +30°C.

b) Samples testing

Follow the below instructions or refer to the picture n°1.

1. Allow specimen and β-hCG-CHECK-1 test device to come to room temperature prior to testing.
2. Remove the reaction device from its protective wrapper by tearing along the split.
3. Label device with the patient's name or control number.
4. Fill the serum/urine dropper with specimen and by holding it vertically, dispense drop-wise into sample well (▷). Add exactly 4 drops (150µL without air bubble) of plasma/serum/urine in the sample well (▷).
5. Read the result **(in IU/L**) at 15 minutes exactly either using the immediate or countdown reading mode (see corresponding leaflet). In case the result reading is made at different time, wrong results will be obtained.

For general instructions describing how to use the VEDALAB's rapid tests readers, refer to the corresponding leaflet.



Picture n°1

VII- PERFORMANCES CHARACTERISTICS

a) Linearity

The measuring range is 5 to 500,000 IU/L (500 kIU/L) and results will be given as per the table hereunder:

hCG concentration in sample		Reader Results (Expressed either in IU/L or kIU/L)		Sample dilution factor for quantitative value*
IU/L	kIU/L	IU/L	kIU/L	
0 – 4.9	0- 0.0049	« < 5 IU/L »		None
5 – 999	0.005 – 0.999	Quantitative result		None
1,000 - 4,999	1- 4.999	« 1000 – 5000 IU/L »	1-5 kIU/L	1/20
5,000 - 49,999	5 – 49.999	« 5000 – 50000 IU/L »	5 –50 kIU/L	1/100
50,000 - 249,999	50 – 249.999	« 50000 – 250000 IU/L »	50-250 kIU/L	1/1000 (1/20 + 1/50)
250,000 - 499,999	250 – 499.999	« 250000 – 500000 IU/L »	250-500 kIU/L	1/1000 (1/20 + 1/50)
500,000 and over	500 and over	« > 500000 IU/L »	>500 kIU/L	1/2000 (1/50 + 1/40)

The linear measuring range being 5 to 1,000 UI/L (1 kIU/L), there will be a need to perform a second test with diluted sample (as per the table above) in case an exact β-hCG value is requested for samples having an β-hCG concentration over 1,000 IU/L (1 kIU/L).

*** Please multiply the obtained β-hCG value by using the sample dilution factor, in order to get the final β-hCG concentration in the sample.**

Saline should always be used as diluent for the patient's sample further dilution (whatever the initial matrix, i.e. serum, plasma or urine samples).

For the result reading of diluted samples in saline, select "β-hCG (**U**)" not "β-hCG (**S/P**)" whatever the initial matrix of the diluted sample (ie serum, plasma or urine) as saline buffer matrix is very similar to urine and proceed as usual.

b) Sensitivity

The β-hCG-CHECK-1 test is detecting β-hCG levels of 5 IU/L, according to WHO 4th International Standard n° 75/589.

The β-hCG IRP Standard (N° 75/551) has also been evaluated and the β-hCG-CHECK-1 test is detecting β-hCG levels of 1 IU/L (rendered as < 5IU/L by the reader) according to this IRP Standard.

c) Hook effect

Specimen containing high levels of β-hCG (1,000,000 IU/L or 1,000 kIU/L) when tested consistently showed positive results.

d) Cross reactivity

1) The following hCG components have been tested using β-hCG-CHECK-1 test. Cross reactivity values have been calculated on a weight/weight basis.

- hCG (Boehringer): 100%
- β-hCG (Boehringer): 125%
- αhCG (Boehringer): <1.0%

2) The following concentrations of homologous hormones are found to have no interferences with β-hCG-CHECK-1 test.

- hTSH 250 µIU/mL WHO 81/565
- hLH 250 mIU/mL WHO 2nd IS 80/552
- hFSH 900 mIU/mL WHO 1st IS 83/575

Menopausal urines:

A study was performed using 50 urine specimens from non pregnant or post-menopausal women. The specimens from post-menopausal women were chosen because urine from these patients frequently interferes with pregnancy tests due to cross reactivity with other gonadotropin hormones.

All 50 urine specimen tested over 5 days were negative when tested with β-hCG-CHECK-1.

e) Correlation

A panel of 30 human pre-assayed sera (BECKMAN-COULTER ACCESS analyser) is assayed using the β-hCG-CHECK-1 rapid test. Results are quantified using VEDALAB's rapid tests reader.

Results show a correlation coefficient of 98.3% (CI 95% [96-99] between β-hCG-CHECK-1 rapid test and ACCESS BECKMAN - COULTER.

CI 95%: 95% confidence interval

f) Interferences

Potentially interfering substances were added to urine which had hCG levels of 0 and 25 mIU/mL. The level of interfering substances was determined to be in excess of what would be excreted after 8 hours by the human kidney.

In each case, no interference with the expected results of β-hCG-CHECK-1 test was observed.

Acetaminophen	20 mg/dL
Albumin	1.4 g/dL
Ampicillin	20 mg/mL
Ascorbic acid	20 mg/dL
Atropin	20 mg/dL
Bilirubin	30 mg/mL
Caffeine	20 mg/mL
Gentisic acid	20 mg/mL
Glucose	2 g/dL
Haemoglobin	30 mg/mL
Tetracycline	40 mg/mL

g) Intra-assay reproducibility

1) Urine samples

Within run reproducibility was evaluated by measuring 20 replicates of urine samples containing 10 and 25 IU/L of β-hCG respectively. The obtained CV's (coefficient of variation) are 11.4 % and 12.5 % respectively.

2) Serum samples

Within run reproducibility was evaluated by measuring 20 replicates of two control serum samples (Biorad Lyphochek) containing 16 and 148 IU/L of β-hCG respectively. The obtained CV's (coefficient of variation) are 10.2 % and 13 % respectively.

h) Inter-assay reproducibility

1) Urine samples

Between lots reproducibility was determined by using two specimens containing 10 and 25 IU/L of β-hCG respectively measured using three different lots of β-hCG-CHECK-1 rapid test (3 replicates/lot). The obtained coefficients of variation (CV) are 13.08 % and 10.86% respectively.

2) Serum samples

Between lots reproducibility was determined by using two control serum samples (Biorad Lyphochek) containing 13.5 and 105 IU/L of β-hCG respectively measured using three different lots of β-hCG-CHECK-1 rapid test (3 replicates/lot). The obtained coefficients of variation (CV) are 17.83% and 16.96% respectively.

VIII- LIMITATIONS

1. Urine from healthy men and non-pregnant women will normally show undetectable levels of β-hCG when tested on β-hCG-CHECK-1 test.
2. In addition to pregnancy, β-hCG has been found in patients with both gestational and non-gestational trophoblastic disease. Since the β-hCG of trophoblastic neoplasms is similar to that found in pregnancy, these conditions, which include choriocarcinoma and hydatidiform mole, should be ruled out before a diagnosis of pregnancy is reached.
3. A normal pregnancy can not be distinguished from an ectopic pregnancy based on β-hCG levels alone. Also, spontaneous miscarriage may cause confusion in interpreting test results.
4. A very early pregnancy containing an extremely low concentration of β-hCG can give a negative result. In this case, another specimen should be obtained at least 48 hours later and tested.
5. β-hCG levels may remain detectable for several weeks after normal delivery, delivery by caesarean section, spontaneous abortion, or therapeutic abortion.
6. **Serum/plasma samples only:** Some specimens with high concentration of rheumatoid factor (RF), heterophiles or Forssman antibodies may yield non specific positive results during testing. Such cases should be discriminated before testing.
7. **Serum/plasma samples only:** The test is designed to eliminate the potential interference of human antibodies to murine IgG (HAMA). However, high level of HAMA could give falsely positive results.
8. As for any diagnostic procedure, the physician should evaluate data obtained by the use of this kit in light of other clinical information.
9. The presence of hydroxyethyl-cellulose in the composition of catheter lubricant may give false positive results with β-hCG-CHECK-1 test at a concentration equal or higher than 0.1%.
10. This format of the test is to be only used with VEDALAB's rapid tests readers.
11. If the reading time (15 minutes) is not strictly respected, wrong results will be obtained.
12. This format of test should not be used for visual reading.
13. As for any diagnostic method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/- 25% should be considered for the final value and for the clinical significance of the result.
14. Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.

IX- BIBLIOGRAPHY

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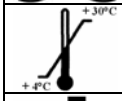
	Read the instructions before use		For *in vitro* diagnostic use
	Temperature limitations		Do not reuse
	Manufacturer		

Manufactured by VEDALAB - France