# L-CHECK-1

## Immunochromatographic quantitative rapid test for the detection of Luteinizing Hormone (LH) in whole blood, plasma, or serum samples FOR EASY READER® AND EASY READER+® USE ONLY

**Ref. 5091 (20 tests) / Ref. 5091-10T (10 tests)**

### I- INTENDED PURPOSE

The L-CHECK-1 is a quantitative rapid screening test for the detection of the Luteinizing Hormone (LH) in whole blood, plasma, or serum samples to be used as an aid for medical healthcare professionals assessing primary ovulation but also fertility disorders. The determination of the Luteinizing Hormone only is not sufficient to diagnose certain types of fertility profiles such as peri-menopause, menopause or infertility. Additional examination and assessment of other sexual hormones levels are necessary for final diagnosis.

### II-PRINCIPLE

The Luteinizing hormone (LH) is a glycoprotein produced by the anterior pituitary in response to luteinizing releasing hormone (LH-RH) secreted by the hypothalamus. In women the LH and FSH (follicule stimulating hormone) are subject to the complex ovulation cycle. The increase and release of LH appears 12 to 18 hours before ovulation occurs.
In men, LH stimulates the intersticial cells (Leydig cells) to produce testosterone.
L-CHECK-1 is a rapid quantitative assay for the detection of human luteinizing hormone LH in whole blood, plasma or serum samples.
As the test sample flows through the absorbent device, the labelled antibody-dye conjugate binds to the LH binding site, forming an antibody-antigen (LH) complex. This complex in continuation binds to the anti-LH antibody in the positive reaction zone (T) and produces a strong pink-rose colour band. In the absence of LH, there is no band appearing in the positive reaction zone (T).
Unbound conjugate irrespectively of LH concentration binds to the reagents in the control zone (C), producing a strong pink-rose colour band, demonstrating that the reagents are functioning correctly.

### III- L-CHECK-1 KIT COMPONENTS

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

| | | |
|---|---|---|
| 1- L-CHECK-1 reaction devices: | 10 | 20 |
| 2- Disposable plastic pipettes: | 10 | 20 |
| 3- Diluent in a dropper bottle: | 2.5 mL | 5 mL |
| 4- Instruction leaflet: | 1 | 1 |

5- **Controls** (**Optional):**
**Positive control ref. V7000 and Negative control ref. V7001:** a freeze-dried preparation of a non-infectious compound in diluted human serum, tested and found negative for anti-HIV, anti-HCV and HBs antigen, containing 0.05 % sodium azide is optionally available as a positive and negative control (1x 0.25 mL). The concentration range is indicated on the vial label.

### IV- STORAGE AND STABILITY

1- All L-CHECK-1 kit components, including optional control before reconstitution with distilled water, should be stored at any temperature between +4°C and +30°C in their original package.
2- **Do not freeze the test kit**.
3- L-CHECK-1 is stable until the expiry date stated on the package label.

### V- PRECAUTIONS

1- For *in vitro* diagnostic use and for professional use only.
2- Read the instruction notice carefully before using the test.
3- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimen 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 the test from a damaged protective wrapper.
8- Do not use beyond the expiry date which appears on the package label.

### V- SPECIMEN COLLECTION AND PREPARATION

1- L-CHECK-1 is to be performed on human serum, plasma or whole blood.
2- The 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 (lithium) should be used.**
4- Each specimen should be treated as if potentially infectious.
**5- Whole blood samples should be tested immediately (< 4 hours). Finger prick samples should be assayed just after collection.**
6- 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.
7- 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.

## VII- ASSAY PROCEDURE

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

**a) Controls testing**

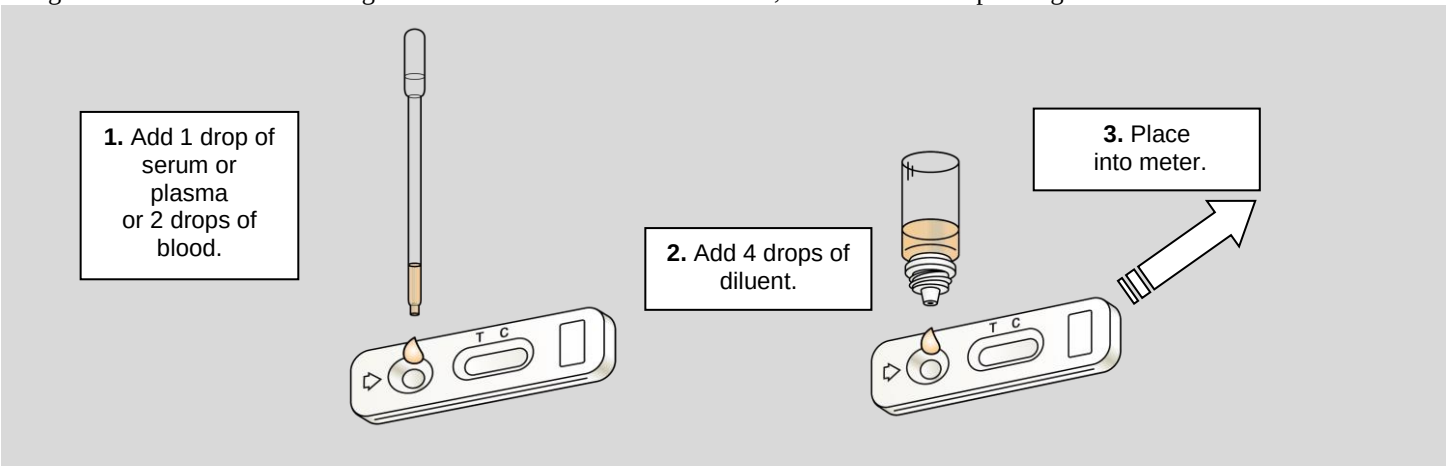
- Wait for 15 minutes after the freeze-dried control has dissolved.
- Add the requested volume (25µL) with **lab pipette (disposable tips)** into the sample well of the cassette and proceed in the same way as for a patient's sample.
- The expected concentration level (**in mIU/mL**) is indicated on the vial label and obtained result must match the indicated value. The concentration level can change slightly depending on lot number.
- **The reconstituted vial should be kept at +2°C to +8°C and should be used within 7 days after reconstitution**.

**b) Samples testing**

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

1- Allow samples and L-CHECK-1 test devices 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 plastic pipette with specimen (serum, plasma or whole blood) and by holding it vertically, dispense one drop (25 µL) into sample well if serum or plasma is used. If whole blood is used, dispense 2 drops (50 µL) into the well (▷) **and wait for the blood sample to be completely absorbed before adding diluent**.
5- Hold the diluent vial vertically and slowly add exactly 4 drops of diluent (150 µL) in the sample well (▷) **with an interval of 2-3 seconds between each drop.**
6- Read the result **(in mIU/mL**) after 10 minutes either using the immediate or countdown reading mode (see corresponding leaflet).

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




**Picture n°1**

## VIII- PERFORMANCES CHARACTERISTICS

**a) Linearity**

A study has been performed using serum samples obtained from dilutions of LH W.H.O reference material (international standard n° 81/535). Covering a range of 0 to 600 mIU/mL. The dose response obtained with the L-CHECK-1 quantitative test fits a linear regression in the range of 5 to 400 mIU/mL:

$$Y = 0.9438x + 1.9676$$

Linear regression coefficient ($R^2$) = 0.9986

The measuring range is 5-400 mIU/mL.
For LH concentration below 5 mIU/mL, the result will be given as "< 5 mIU/mL".
For LH concentration over 400 mIU/mL, the result will be given as "> 400 mIU/mL".
For samples whose concentration is higher than 400 mIU/mL, dilute with saline and repeat the assay as per instructions of Part VII.

**b) Accuracy**

Serial dilution of LH W.H.O reference material (International standard n°81/535) in LH negative serum pool have been tested using L-CHECK-1 quantitative test.
The obtained results are summarized in Table 1.

| | LH concentration (mIU/mL) | | | | | |
|---|---|---|---|---|---|---|
| International standard theorical concentration | 9,4 | 18,8 | 37,5 | 75 | 150 | 300 |
| Mean of L-CHECK-1 results (5 replicates) | 9,0 | 17,9 | 37,8 | 79,9 | 139,0 | 285,7 |
| **CV*** | **12.0 %** | **9.6 %** | **11.9 %** | **9.2 %** | **10.9 %** | **6.4 %** |
| **Bias** | **-4.2 %** | **-4.8 %** | **+0.8 %** | **+ 6.5%** | **-7.3 %** | **-4.8%** |

***Coefficient of variation*

**Table 1: Accuracy**

The bias between nominal and measured values is statistically (95% t-test) non-significant and LH concentrations determined using L-CHECK-1 test are accurately measured when compared to W.H.O reference material.

**c) Analytical sensitivity**

Concentrations close to 3 mIU/mL are detected by L-CHECK-1 test. In these cases, results will be rendered as "< 5 mIU/mL".
Expected LH concentration in male (adults) and female (follicular and luteal phases) are below 25 mIU/mL. For mid-cycle and postmenopausal females, the LH concentration is in the range of 34-90 mIU/mL and 10-65 mIU/mL respectively.

**d) Diagnostic sensitivity and specificity and overall correlation**

A panel of 52 human pre-assayed serum samples (Biomerieux VIDAS analyser) is assayed using the L-CHECK-1 quantitative test.
A summary of obtained results (using VEDALAB reader) is reported in the table 1 (negative samples correspond to samples for which the LH concentration is < 25 mIU/mL and positive samples correspond to samples for which the TSH concentration is ≥ 25 mIU/mL).

| | | VIDAS | | |
|---|---|---|---|---|
| | | Positive | Negative | **Total** |
| L-CHECK-1 | Positive | 28 | 0 | **28** |
| | Negative | 1 | 23 | **24** |
| | **Total** | **29** | **23** | **52** |

**Table 1: Summary of results**

***Diagnostic sensitivity :***

$\frac{28}{29} \times 100 = 96.6\%$ (CI* 95% [81.5 – 100.0])

***Diagnostic specificity :***

$\frac{23}{23} \times 100 = 100\%$ (CI* 95% [84.9 – 100.0])

***Global correlation :***

$\frac{(28+23)}{52} \times 100 = \frac{51}{52} \times 100 = 98.1\%$ (CI* 95% [89.2 – 100.0])

**CI 95%: 95% Confidence interval*

On the other hand, the coefficient of correlation between quantified results of L-CHECK-1 test and VIDAS results is 96.1% (CI 95% [93.0 – 97.8]).

**e) Analytical specificity (cross-reactivity)**

there is no cross reaction when using the L-CHECK-1 quantitative test the following others hormones (W.H.O international standards concentrations):

- hCG up to a concentration of 10 000 IU/L
- TSH up to a concentration of 150 mIU/L
- FSH up to a concentration of 500 IU/L

**f) Interferences**

1- Rheumatoid factor (RF)

A serum sample with RF concentration of 1 633 IU/mL has not shown any false positive results. Therefore, there is no interference of the L-CHECK-1 quantitative rapid test up to a RF concentration of 1 633 IU/mL.

2-HAMA

A HAMA (anti-mouse human antibody) positive serum samples (type 1 or 2) have not shown any false positive results. Therefore, there is no interference of the rapid quantitative test L-CHECK-1 on HAMA type 1 and type 2 positive samples.

3-Anticoagulants

Negative (0 mIU/mL), weak positive (30 mIU/mL) and strong positive (100 mIU/mL) LH samples, spiked with EDTA dipotassium (final concentration: 1.8 mg/mL), citrate trisodic (final concentration : 32 mg/mL) or heparin lithium (final concentration: 17 U/mL) did not show any effect on L-CHECK-1 quantitative test results (negative or positive).

4-Hemoglobin, bilirubin and triglycerides

Negative (0 mIU/mL), weak positive (30 mIU/mL) and strong positive (100 mIU/mL) LH samples, spiked with hemoglobin (final concentration: 5g/L), bilirubin (final concentration : 30 mg/L) or triglycerides (final concentration: 10 g/L) did not show any effect on L-CHECK-1 quantitative test results (negative or positive).

**g) Matrix effect**

Results between serum, plasma and whole blood samples show an excellent correlation. There is no matrix effect on L-CHECK-1 quantitative test when using plasma, serum or whole blood samples.

**h) Hook effect**

No hook effect was observed up to 3 000 mIU/mL LH international standard concentration.

**i) Intra-lot repeatability**
Within run reproducibility was evaluated by performing 20 replicates of four serum samples with different concentrations (0, 10, 30 and 100 mIU/mL) using L-CHECK-1 quantitative test and VEDALAB reader. Negative sample results obtained are all similar. Coefficients of variations obtained for 10, 30 and 100 mIU/mL positive samples are respectively 10.0%, 11.0% and 9.3%.

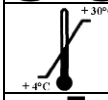
**j) Inter-lot reproducibility**
Between run reproducibility was determined by performing three samples with different concentration (0, 30 and 100 mIU/mL) using three different lots of L-CHECK-1 quantitative test and VEDALAB reader. Negative sample results obtained are all similar. Coefficients of variations obtained for 30 and 100 mIU/mL positive samples are respectively 14.5% and 12.8%.

## IX- LIMITATIONS

1- As for any diagnostic procedure, the physician should confirm the data obtained using this test by other clinical methods.
**2-Whole blood samples should be tested immediately (< 4 hours). Finger prick samples should be assayed just after collection.**
3- Some serum specimens with high rheumatoid factor concentration (RF) or C-reactive protein (CRP) may yield non specific positive results during testing. Such cases should be considered before testing.
4- 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.
5- This format of test is to be only used with VEDALAB rapid tests readers (EASY READER® or EASY READER+®).
6- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.
7- This format of test should not be used for visual reading.
8- As it is true 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.
9- Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.

## X- BIBLIOGRAPHY


**1. Khan S.A., Quasi M.H. and Diczfalusy E.J. -** The significance of in vitro bioassays for the estimation and characterization of human luteinizing hormone (LH)– Endocrinol. Invest.: Volume 7: 1-22. – **(1984).**
**2. Garcia J.E., Jones G.S. and Wright G.L.** Prediction of the time of ovulation– Fertility and Sterility: Volume 36: 308-315. **- (1981).**
**3. Ed GRAY C.H and JAMES W.T.H BUTT W.R**. – Hormones in blood — 3rd edition, Academic Press, London, Gonadotrophins, 7,147-177. **(1983).**
**4. LANDGREN B.M., UNDEN A.L. and DICZFALUSY E. -** Hormonal profile of the cycle in 68 normal menstruating women – Acta Endocrinologica, 94, 89-98. **(1980).**
**5. Vandana Kalia, Atul N. Jadhav and K.K. Bhutani**– Luteinizing hormone estimation –Endocrine Researche, 30:1, 1-17. **(2004).**


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

Manufactured by VEDALAB - France

**CHANGES DESCRIPTION**
Changes type:

| | |
|---|---|
| - N/A | Not Applicable (creation) |
| - Technical change | Addition, revision and/or removal of information related to the product. |
| - Administrative | Implementation of non-technical changes noticeable to the end-user. |

| Changes type | Change description |
|---|---|
| Administrative | -Addition : Chap I, Biblio n° 3, 4 et 5, Changes Description |
| Technical | - Modification: Chap VIII a), b), f), h), i), j)<br>- addition : Chap VIII d), e), g) |

**Note**: Minor typographical, grammar, spelling and formatting changes are not reported in the change details.