


ADVANCED QUALITY™ One Step Luteinizing Hormone(LH) Test
(Urine)

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

THE ADVANCED QUALITY™ ONE STEP Luteinizing Hormone (LH) TEST IS A RAPID IMMUNOCHROMATOGRAPHIC ASSAY FOR THE SEMI-QUANTITATIVE DETECTION OF HUMAN LUTEINIZING HORMONE (LH) IN WOMEN'S URINE TO PREDICT THE TIME OF OVULATION. THE TEST IS INTENDED FOR HEALTHCARE PROFESSIONAL AND HOME USE.

SUMMARY AND EXPLANATION OF PROCEDURE

Human luteinizing hormone (hLH) is a glycoprotein secreted by anterior pituitary. This hormone has a molecular weight of 30,000 daltons and is composed of alpha- and beta- subunits. The alpha subunit of hLH is essentially identical to that of other glycoprotein hormones including FSH, TSH, and hCG. It is the beta subunit of hLH which confers the biological and immunological specificity of the hormone(1). LH, FSH and steroid hormones are known to be involved in regulating the ovulation and ovarian function during the menstrual cycle. The maturation of ovarian follicles and the oocytes begins at the end of the preceding menstrual cycle. As follicles develop through the stimulation by FSH, estradiol secretion begins to rise slowly and ends with a rapid increase. The rapid rise of estradiol level is generally believed to trigger a rapid rise and peaking of LH secretion at the midcycle (LH surge). Approximately 12 to 24 hours after the LH surge, the rupture of the enlarged follicle will take place to release the mature ovum (ova). Following ovulation, LH returns to its basal level in two days with a concomitant increase of the progesterone level to initiate a luteal phase in the next 14 days. If no pregnancy occurs, a new follicle begins with the same procedure for maturation in the next menstrual cycle. In view of the characteristic changes of LH levels during the menstrual cycle, rapid and sensitive measurement of LH is essential in the diagnosis and management of infertility in woman(2,3). Detection of hLH surge offers a great help in predicting the timing of ovulation. The onset of hLH surge precedes the ovulation by about 12 to 24 hours(4). The assay of hLH has been successfully used to time oocyte retrieval for the in vitro fertilization(5,6) and to assist the timing of artificial insemination.

PRINCIPLE OF ASSAY

The LH One Step Test is a colloidal gold enhanced immunoassay for qualitative or semi-quantitative determination of hLH in urine. The nitrocellulose membrane used as solid phase was precoated with sheep anti-hLH on the test region. During the test, the urine sample is allowed to react with a colored conjugate (mouse anti-hLH monoclonal antibody-colloidal conjugate) which was pre-dried on the test strip. Upon the dropping of the urine sample, the mixture will move along the strip through a capillary action. If hLH is present in the sample, a red color band with a specific antibody-hLH colored immunogold conjugate complex will form on the test band region. On the other hand, a light color band will always appear at the control region. This control band serves as a reference of the color intensity of about 25 mIU/ml hLH. When the intensity of the test band is equal to or stronger than that of the control/reference band, the test result is positive, indicating that the LH surge is likely in progress.

REAGENTS AND MATERIALS SUPPLIED**FOR CARD TEST**

1. Test cards individually foil pouched with a desiccant
2. Plastic dropper.
3. Package insert

FOR STRIP TEST

1. Test strips individually foil pouched with a desiccant.
2. Urine cups
3. Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

1. Urine collection containers (for card test)
2. Clock or Timer
3. Positive and negative urine controls available from commercial distributors.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. Do not use the kit beyond the expiration date imprinted on the outside of the foil pouch.
3. Do not open foil pouch until specimen is collected and ready to be tested.
4. Handle all specimens as potentially infectious.

STORAGE AND STABILITY

The test device can be stored under refrigeration and room temperature (2°-30°C) and will be stable until the expiration date. Do not use after the expiration date.

TEST SCHEDULE

It is recommended that the first day of testing for the LH surge during the menstrual cycle should be the length of one's menstrual cycle minus 17, i.e.,

D=C-17

D: The first day of testing

C: The length of menstrual cycle, which is the number of days from the first day of woman's period(menstrual bleeding) to the last day before her next period begins.

For example, if a woman has a menstrual cycle of 27 days, the first day of testing for the LH surge would be, $D=27-17=10$, the 10th day after the beginning of her current menstrual cycle.

If a woman is unsure about her usual cycle length, she should use her shortest cycle length. In this case, the tests may be needed for more than 5 days. The test can be stopped once the LH surge has been detected.

SPECIMEN COLLECTION

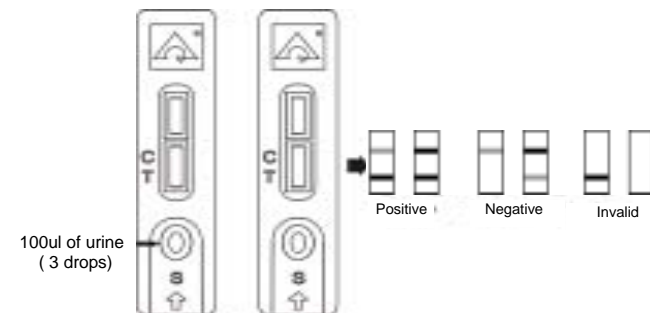
The sample must be collected once each day in one of the urine cups in the kit. Use a new cup each day. For best results, try to collect urine at about the same time each day when conducting the tests. The liquid intake should be reduced for two hours before collecting the urine. The urine can be stored at room temperature for up to 8 hours or in the refrigerator for up to 24 hours. Do not freeze. If sample was refrigerated, it must be equilibrated to room temperature before testing.

BEFORE TESTING

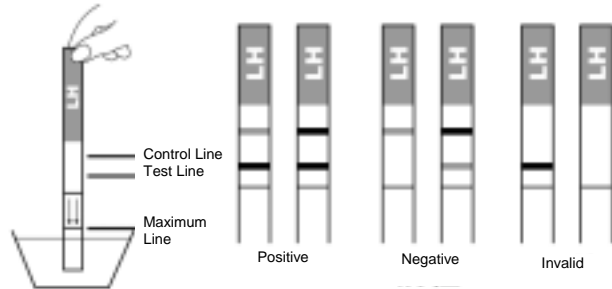
1. Bring all materials and specimens to room temperature.
2. Remove test device from the sealed foil pouch.

ASSAY PROCEDURE**FOR CARD TEST:**

1. Place the test card on a flat dry surface.
2. Using the provided plastic dropper, dispense 100µl of urine sample (3 drops) to the sample well of the test card. Start timing.
3. Read result between 3 and 10 minutes after adding the sample.

**FOR STRIP TEST:**

1. Dip the test strip into the urine sample with the arrows pointing toward the specimen.
2. The urine level should reach the maximum line marked on the strip, but must not exceed the maximum line.
3. Hold the strip in the urine until a reddish color appears at the lower edge of the test membrane (approximately 10 seconds).
4. Withdraw the strip and place it face up on a clean, dry surface.
5. Read the result between 3 and 10 minutes after adding the sample.



READING THE TEST RESULTS

Read results between 3 and 10 minutes. Do NOT interpret results after 10 minutes.

Negative: One (1) pink/purple band forms in the control region; no band is found in the test region, or the color of the test band is less intense than that of the control/reference band. The result indicates basal LH level of the tested specimen.

Positive: Two (2) pink/purple bands form, and the color of the test band is equal to or more intense than that of the control/reference band. This result indicates an LH surge level of the tested specimen.

Invalid: If there is no pink/purple band in the control region, the test result is invalid. Retest the sample using a new device.

EXPECTED VALUES AND LIMITATIONS

1. LH is normally detected at low levels in urine or serum of healthy men (2-15 mIU/ml), or premenopausal women not during the LH surge. Prior to the test, the following charts should be referred:

| | | LH level (mIU/ml) |
|----------------------|----------------|-------------------|
| Postmenopausal Women | | 10-200 |
| Premenopausal Women: | a) basal level | 5-20 |
| | b) surge level | 40-100 |
| Men | | 2-15 |

2. Women suffering from polycystic ovary syndrome may have elevated LH concentration(7).
3. In view of the cross-reactivity with hCG, the test kit is intended only for the analysis of hLH in hCG free samples.
4. A test result read after 10 minutes may not be consistent with the original reading obtained within the 10 minute test period.
5. As with all other diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test. The ultimate decision should be made by the physician following evaluations of all clinical and laboratory findings.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity: The LH One Step Test has been designed to produce a definitive color band at the test region when tested with 25mIU/ml or higher hLH (WHO 1st IS) at room temperature. During the evaluation of this test kit, samples containing 25mIU/ml of hLH was

tested 75 times, and the definitive color bands at the test region were detected and more intense than that of the reference band in every trial.

2. The specificity of the One Step LH Test was determined through the cross-reactivity studies with the known amount of hFSH, hTSH, and hCG. Samples containing 500mIU/ml hFSH or 500µIU/ml hTSH yielded color of intensity less than that of the 25mIU/ml hLH reference.
3. Accuracy: Correlation with a qualitative visual test: 70 urine specimens from 11 menstrual cycles were analyzed by One Step LH Test procedure in parallel with a commercially available visual test kit. The data of LH surge of all of these cycles determined by both kits were consistent.
4. Interference Testing: The following substances were added in LH "free" (<10mIU/ml LH) and 25mIU/ml LH spiked urine. None of the substances at the concentration tested interfered with the assay.

| | | | |
|---------------|----------|----------------------|----------|
| Acetamidophen | 20 mg/dl | Acetylsalicylic Acid | 20 mg/dl |
| Ascorbic Acid | 20 mg/dl | Atropine | 20 mg/dl |
| Caffeine | 20 mg/dl | Glucose | 2 g/dl |
| Hemoglobin | 1 mg/dl | | |

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