RapidTech Plus LH
One Step Urine Ovulation Prediction Test

INTENDED USE
The RapidTech Plus LH Ovulation test is an in-vitro immunochromatographic one step assay designed for qualitative determination of human luteinizing hormone (hLH) in urine to predict the time of ovulation.

SUMMARY AND EXPLANATION OF PROCEDURE
Human luteinizing hormone (hLH) is a glycoprotein hormone secreted by the anterior pituitary. This hormone has a molecular weight of approximately 30,000 and is composed of alpha and beta subunits. The amino acid sequence of alpha-hLH is essentially identical to that of other human hormones including follicle stimulating hormone (hFSH), thyroid stimulating hormone (hTSH) and human chorionic gonadotropin (hCG).

It is the beta subunit of hLH that confers the biological and immunochemical specificity of the hormone (1). hLH, hFSH together with other steroid hormones are known to play important roles in regulating ovulation and ovarian functions during the menstrual cycle. Maturation of an ovarian follicle and its oocyte begins during the end of the preceding menstrual cycle. In response to hFSH released by the pituitary, the follicle undergoes rapid growth. As follicles develop, estradiol secretion begins to rise slowly and is followed by a rapid increase. This increase of estradiol level is generally believed as the trigger for the rapid rise and peaking of hLH activity at the mid-cycle (hLH surge). Approximately 12-24 hours after the hLH surge, the wall of the enlarged follicle ruptures at ovulation and the mature ovum is extruded.

After ovulation, hLH returns to its base line level within two days with the concomitant increase of progesterone level to initiate luteal phase. The luteal phase lasts predictably about 14 days. Unless pregnancy occurs, a new follicle begins the selection procedure for maturation in the next menstrual cycle.

In view of the characteristic variation of hLH during the menstrual cycle, rapid and sensitive measurement of hLH is an important tool in the diagnosis and management of infertility in females (2, 3). Detection of the hLH surge can aid in predicting the time of ovulation. The onset of the hLH surge precedes ovulation by approximately 30 hours (4). The analysis of hLH has been used successfully to time oocyte retrieval for in vitro fertilization (5) and would similarly assist timing of artificial insemination.

PRINCIPLE
The RapidTech Plus LH test is a qualitative, two site sandwich immunooassay (6, 7) for the determination or human luteinizing hormone (hLH) in urine. The membrane was pre-coated with anti-hLH capture antibody on the test band region and goat anti-rabbit antibody on the control band region. During testing, the patient urine is allowed to react with a colored conjugate (anti-hLH monoclonal antibody-colloidal gold conjugate) which was pre-dried on test strip. The mixture then moves upward on the membrane chromatographically by a capillary action. When hLH is present in sample, a color band with a specific antibody, hLH, colored conjugate complex will form at the test region of the membrane. 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 approximately 20 mIU/ml hLH. When the intensity of the test band is equal or higher than that of control band the test is positive, indicating the hLH surge is likely in process.

REAGENTS AND MATERIALS SUPPLIED
1. Test Device.
2. Disposable urine dispenser.

MATERIALS REQUIRED BUT NOT SUPPLIED
1. Specimen collection containers.
2. Timer

STORAGE AND STABILITY
The test kit is to be stored at refrigeration (2º to 8ºC) or room temperature (up to 30ºC) in the sealed pouch for the duration of the shelf life.

PRECAUTION
1. For in-vitro diagnostic use only.
2. Do not use test kit beyond expiration date.

WHEN TO START TESTING
First, determine the length of your menstrual cycle. This is the number of days from the first day of menstrual bleeding to the day before the next bleeding begins again. Refer to the following chart to determine when one should start testing. The first day of bleeding is day 1.

Perform at least 5 tests five days in a row or until the LH surge has been detected.

SPECIMEN COLLECTION
Urine sample must be collected in a clean, dry container, either plastic or glass, without preservative. Samples may be refrigerated (2º to 8ºC) and stored up to 72 hours prior to assaying. If samples are refrigerated, they must be equilibrated to room temperature before testing. Urine samples exhibiting visible precipitates should be filtered, centrifuged, or allowed to settle and clear aliquots obtained for testing.

Choose a convenient time of the day to collect urine. Try to collect urine at about the same time each day for the entire cycle.

TEST PROCEDURE
Test device together with patient's samples, controls or reference materials should be brought to room temperature (18º to 25ºC) prior to testing.

1. Remove the test device from its protective pouch (bring the device to the room temperature before opening of the pouch to avoid condensation of moisture on the membrane). Label the device with patient or control identifications.
2. Dispense 5 drops (approximately 0.2 ml) of specimen into the sample well. Wait for 3 minutes for both colored bands to appear.
3. Read results in 5 minutes.

INTERPRETATION OF RESULTS
- If the color intensity of the test band is less than that of the control band, the specimen is likely at its basal hLH levels. On the other hand, if the test band is more intensive than or equivalent to that of the control band, the specimen is likely, at or around the surge level.
When neither test band nor control band appears on the membrane the test should be voided since improper test procedure or deterioration of reagents probably occurred.

It is recommended that a basal hLH urine specimen (7 days after the beginning of menstrual cycle, see below) is obtained and tested to establish test band intensity at basal level.

QUALITY CONTROL
The procedural control is included in the test. A colored band appearing on the control region of the membrane indicates proper performance and reactive reagents.

Good laboratory practices include the use of control specimens to ensure proper kit performance. Negative and positive controls containing hLH at various concentrations are available commercially.

EXPECTED VALUES AND LIMITATIONS
- hLH is normally detectable at low levels in urine or serum of healthy men (2-15 mIU/ml), or premenopausal women not during the hLH surge. Prior to the tests the following charts should be consulted.

<table>
<thead>
<tr>
<th>hLH level</th>
<th>mIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmenopausal Women</td>
<td>10-200</td>
</tr>
<tr>
<td>Premenopausal Women</td>
<td></td>
</tr>
<tr>
<td>a. baseline level</td>
<td>5-20</td>
</tr>
<tr>
<td>b. surge level</td>
<td>40-200</td>
</tr>
<tr>
<td>Men</td>
<td>2-15</td>
</tr>
</tbody>
</table>

Each laboratory should establish their own criteria for interpretation of results as baseline hLH levels and patterns of hLH secretion can vary among individuals. When the baseline hLH level of a patient is in doubt, a urine sample collected 7 days after the beginning of the menstrual cycle can be used as the negative reference. If a sample produced more intense test band than the day 7 sample, a hLH surge is indicated.

- Women suffering from polycystic ovary syndrome may have elevated hLH concentration (8).
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

Sensitivity arid Specificity
This LH ovulation test has been designed to produce a definitive color band at test region when tested with 35 mIU/ml or higher of hLH (WHO 2nd IS for human luteinizing hormone) at room temperature. During the evaluation of the One Step LH ovulation test, samples containing 35 mIU/ml of hLH were tested 60 times, and definitive color bands at test region detected were more intensive than that of the control band 100% of times. Specificity of the One Step LH ovulation test was determined from cross reaction studies with known amounts of follicle stimulating hormone (hFSH), thyroid stimulating hormone (hTSH) and chorionic gonadotropin (hCG). Samples containing 200 mIU/ml hCG, 200 mIU/ml hFSH and 200 µIU/ml hTSH yielded color less intensive than that of 20 mIU/ml hLH Reference.

Accuracy
Correlation with a Qualitative Visual Test: 350 urine specimens from 70 menstrual cycles were analyzed by this LH test procedure in parallel with a commercial available visual methods. The surge dates of all of these cycles, were identified and agreed by both RapidTech Plus LH test and the competitor assay,

Interference Testing
The following substances were added in hLH free and hLH spiked urine samples. None of the substances at concentration tested interfered in the assay.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Acetylsaticylic Acid</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Atropin</td>
<td>20mg/ml</td>
</tr>
<tr>
<td>Caffeine</td>
<td>20mg/dl</td>
</tr>
<tr>
<td>Gentamic Acid</td>
<td>20mg/dl</td>
</tr>
<tr>
<td>Glucose</td>
<td>2 g/dl</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1 mg/dl</td>
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</tbody>
</table>

STANDARDIZATION
This LH test has been standardized to World Health Organization Second International Standard (IS 80/552).

REFERENCES

Genix Technology
Vancouver, Canada. Revision A: June 1997