

WITNESS® LH

For the Detection of
Serum Luteinizing Hormone
DIRECTION INSERT

I. INTRODUCTION

Test Description

The WITNESS® LH test provides an accurate, semi-quantitative measurement of canine and feline luteinizing hormone (LH). The test is simple to perform and rapidly provides information for the veterinary clinician. This assay, when used in conjunction with progesterone testing, identifies the pre-ovulatory LH surge, and thus, the time of ovulation. WITNESS® LH is also used to distinguish ovariectomized from sexually intact bitches or queens.

Indications

Identification of the LH surge provides the most accurate means of canine ovulation timing. It may be used for routine breeding situations and is especially recommended in those instances where there are factors present that could adversely affect conception rates. These include:

- chilled/extended semen breedings
- frozen semen breedings
- bitches with a history of infertility
- breedings with stud dogs with low semen quality
- limited access to the stud dog

Determining bitches and queens that have had their ovaries removed is possible through the detection of luteinizing hormone. Serum concentrations of LH increase after gonadectomy in dogs and cats. Because LH concentrations in serum are < 1ng/mL in sexually intact bitches and queens, except during the LH peak at estrus, it may be assumed that high values in bitches and queens not in estrus are indicative that the ovaries have been removed.

Ovulation Timing in the Bitch

Since the LH surge may occur within a 24 hour period, it is crucial that daily serum samples are tested. Blood should be drawn every day at approximately the same time beginning the 4th or 5th day of proestrus, or when vaginal cytology approaches 50% cornification (Cornification is defined as cells with a roughened, angulated border with or without a nucleus). A progesterone test should also be performed on the first day of testing to confirm a baseline progesterone level. If testing is started after the onset of estrus, it is likely that the LH surge will have already occurred and cannot be identified in retrospect.

Once an increase in LH is identified, Synbiotics recommends that daily serum samples should be tested for progesterone levels. If the detected increase in LH was indeed the actual pre-ovulatory surge, progesterone levels should rise, usually reaching a value above 2 ng/ml within 3 days, and then stay elevated. If progesterone remains low, it indicates that a proestrus fluctuation in LH was identified. Continue daily LH testing until the true LH surge occurs. *Note: The WITNESS® LH test and test interpretation is referenced with the OUV-CHECK® Premate® progesterone assay. Use of other progesterone tests may require different interpretations to explain test results.*

II. TEST PRINCIPLES

WITNESS® LH is a luteinizing hormone assay that provides a convenient semi-quantitative measurement of luteinizing hormone levels without special equipment. The test is an immunochromatographic assay that uses gold-conjugated antibodies to give a visual line in the presence of luteinizing hormone. One test device is used for each serum sample to be tested. A control line is included on each test device. Color development of the control line is indicative of proper testing technique. The WITNESS® LH luteinizing hormone assay allows identification of the LH surge. A positive result occurs when a line appears in the test area which is of similar or greater intensity than the control line. When this occurs, the LH level in the serum sample is greater than 1 nanogram per milliliter.

III. REPRODUCTIVE PHYSIOLOGY IN THE BITCH

Unsuccessful breedings result more often from improper ovulation timing than any other cause. While the estrous cycle of the bitch typically lasts for several weeks, the true fertile period is short (48-72 hours) and is difficult to identify without the use of hormonal assays.

The common indicators of estrus and breeding time, such as vaginal cytology and receptive behavior, are primarily controlled by changes in the hormone estrogen. Unfortunately, these changes are only an approximation of ovulation. Using only these parameters, ovulation could be "missed" by more than a week. Precise identification of the fertile period is made by measurement of the LH surge which actually triggers ovulation. This

surge, which consists of a rapid increase in LH level, occurs in many cases within a 24 hour period. Ovulation occurs 2 days after the LH surge; the oocytes then require an additional 2-3 days to mature, and will live for about 48-72 hours. Thus, the fertile period of the bitch falls between days 4-7 after the LH surge with the most fertile days being on days 5 and 6 post-LH surge.

Progesterone assays are useful for ovulation timing. Before the LH surge, serum progesterone remains low, generally between 0 and 1.0 ng/ml. At about the time of the LH surge, progesterone levels will begin to rise, usually changing from a baseline of 0-1.0 ng/ml into a range of 1.5-2.0 ng/ml. Progesterone will then continue to rise as the cycle progresses and will remain elevated for 2-3 months in non-pregnant as well as pregnant bitches. It is important to appreciate that the absolute progesterone values discussed above may vary by individual. The key event progesterone assays seek to identify is the initial rise of progesterone above the particular individual's baseline level. Once identified, this initial rise in progesterone may be used as an estimate of the LH surge. However, this first rise in progesterone may vary from the day of the actual LH surge in many bitches. While progesterone is elevated during the fertile period, the rate of rise varies from bitch to bitch, as does the absolute value of progesterone which coincides with the LH surge or with the optimal time to breed. As a result, progesterone measurements alone are, at best, only an approximation of the LH surge. Given the fact that good breeding management often plans for multiple breedings over a period of several days relying on the information provided by progesterone assays alone may be sufficient. Some breedings, however, will benefit from a more exact identification of the bitch's fertile period. Identification of the LH surge itself allows the most precise ovulation timing.

During proestrus, small, pulsatile fluctuations in LH may occur, while progesterone remains at low, baseline levels. Progesterone rises, however, after the pre-ovulatory surge in LH. By the third day post-LH peak, the majority of bitches will evidence a rise in progesterone levels above the 2 ng/ml level. Daily progesterone testing after a positive WITNESS® LH test result allows simple differentiation between small fluctuating increases in LH during proestrus and the true, pre-ovulatory LH surge. If there is no confirmation of progesterone rise within 3 days of a positive WITNESS® LH test, it may be assumed that the positive test result was due to a baseline LH fluctuation.

IV. WHEN TO CONDUCT INSEMINATIONS IN THE BITCH

The positive WITNESS® LH test identifies the day of the LH surge. This day is designated as day 0. Count forward to determine the fertile period. Days 4-7 post-LH surge encompass the true fertile period, with peak fertility on days 5 and 6. Properly planned breedings or inseminations coinciding with this window of fertility will optimize the probability of success.

Natural Breeding or Fresh Artificial Insemination

Fresh semen of a normal healthy stud may live up to 5 or more days in the bitch. Therefore, semen inseminated a day or two before the fertile period should be viable at the time of peak fertility. Synbiotics recommends breeding on days 2, 4 and 6 post-LH surge to completely cover the fertile period, and to maximize viable sperm numbers on the bitch's most fertile days. If only two breedings are being performed, they should be accomplished between days 3 and 6 post-LH surge.

Natural Breeding with Compromised Semen Quality

At times, stud dogs produce semen of compromised quality due to age, stress or disease. Breeding during the true fertile period will increase the likelihood of limited sperm numbers encountering mature eggs and will increase the chance of conception. Synbiotics recommends breeding on days 4, 5, 6, and 7 post-LH surge.

Surgical Insemination with Fresh Semen

Occasionally, it is desirable to perform surgical insemination with fresh semen due to low fertility of either the stud dog or the bitch. Synbiotics recommends that this procedure be performed on either days 5 or 6 post-LH surge.

Chilled Extended Semen Breeding

Chilled extended semen will usually live for 2 to 4 days after collection. One day of this time is typically used in shipping. This reduced survival time requires the breeding to be conducted closer to the short fertile period of the bitch. Synbiotics recommends that 2 inseminations be conducted on days 4 and 6 or 3 and 5 post-LH surge. If a surgical insemination is desired, it should be performed on day 5 or 6 post-LH surge.

Frozen Semen Breeding

Frozen semen survives less than 24 hours after thawing,

Luteinizing Hormone Test

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For the Detection of Serum
Luteinizing Hormone

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so timing is crucial. Inseminations must be performed during the true fertile period. If one surgical insemination is planned, it should be performed on day 5 or 6 post-LH surge. If multiple vaginal or trans-cervical inseminations are to be conducted, they are recommended on days 4, 5 and 6.

V. DISTINGUISHING BETWEEN OVARIECTOMIZED AND SEXUALLY INTACT BITCHES OR QUEENS

A single low LH test result (i.e., lighter than the control line) confirms that the bitch or queen is sexually intact. Because brief (20 to 60 minutes) episodic surges in serum LH concentrations may occur in sexually intact bitches or queens, any positive test (i.e. test line equal to or darker than the control line) should be repeated in 2 hours with a new sample. If estrus is suspected and a positive result was obtained, repeat the test in 24 hours with a fresh sample.

VI. SAMPLE INFORMATION

WITNESS® LH requires only three drops of serum to run each test. Collect the blood sample in a plain (red top) vacutainer or serum separator tube. Allow the blood to clot and separate the serum by centrifugation. The sample should not be hemolyzed or lipemic. Testing should be performed the same day as sample collection. If this is not possible, refrigerate the serum for up to 24 hours. Serum may be frozen for prolonged storage. Do not thaw and refreeze.

VII. STORAGE AND STABILITY

Store kit at 2° to 25°C or 35° to 77°F. Do Not Freeze. The foil pouch containing the test device and pipette should not be opened prior to running the test. WITNESS® LH will remain stable until the expiration date provided that the kit has been stored properly.

FOR TECHNICAL ASSISTANCE CALL
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WITNESS® LH Test Procedure

NOTE: Only use SERUM as a sample.

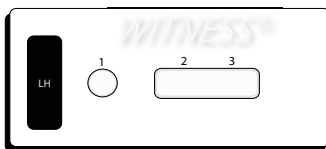
Prior to use, allow SAMPLE to come to room temperature (20° to 25°C; 70° to 77°F)

SAMPLE COLLECTION AND PREPARATION

- Collect the blood sample into a plain (red top) vacutainer or serum separator tube.
- Allow to clot and centrifuge sample.
- Transfer serum to a clean glass or polypropylene tube. Serum must be free of red cells, clots and visible debris.
- **DO NOT USE SEVERELY HEMOLYZED OR GROSSLY LIPEMIC SAMPLES.** When in doubt, collect a better quality sample.
- If serum sample will not be tested immediately, store in refrigerator for up to 24 hours or freeze for longer storage.
REMEMBER: Sample must be at room temperature prior to testing.

TEST PROCEDURE

The WITNESS® LH Test includes a pipet and a test device. The device has a Serum Well “1” and a window with a Test Area “2” and a Control Area “3.”



1. Remove test device from foil pouch by tearing at the notch. Place the device on a flat level surface at room temperature.
2. Draw some serum up into the provided pipet. Hold the pipet perpendicular to the device and add **3 drops** of serum to the Serum Well “1”.
3. Allow the test device to sit undisturbed for **20 minutes**.
4. Read results at **20 minutes**.

Reading results prior to or beyond the 20 minute test interval will invalidate results.

INTERPRETING RESULTS

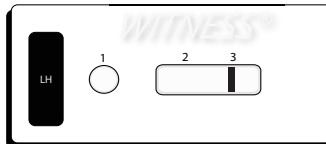
At the end of the test, a pink line should always appear in the area marked “3”. This line assures that the test is complete.

FOR OVULATION TIMING

NEGATIVE RESULT:

If no line appears in the area marked “2”, or if the line is less intense than the control line “3”, the LH value is less than 1 nanogram per milliliter.

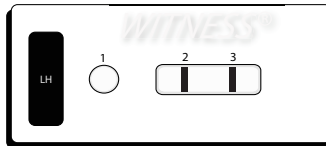
Continue LH testing on a daily basis to determine the optimal time to breed.



POSITIVE RESULT :

If a line appears at the area marked “2” which is of similar or greater intensity than the control line “3”, the LH value is greater than 1 nanogram per milliliter.

For ovulation timing purposes, in the bitch, the first time a positive result is observed is the day of the LH peak. This day is counted as Day 0. Follow the instructions under Section IV. WHEN TO CONDUCT INSEMINATIONS (reverse side of page). Note: It is recommended that you perform a progesterone test in 3 to 4 days to confirm a rise in progesterone.



FOR DISTINGUISHING OVARIECTOMIZED BITCHES/QUEENS

NEGATIVE RESULT:

If no line appears in the area marked “2”, or if the line is less intense than the control line “3”, this indicates that the bitch or queen is intact.

POSITIVE RESULT:

A line appears at the area marked “2” which is of similar or greater intensity than the control line “3”. This positive result in a bitch or queen that is not in estrus indicates that the ovaries have been removed. Repeat the test in 2 hours with a fresh sample to confirm. However, **if estrus is suspected**, repeat the test in 24 hours with a fresh sample.

COMMONLY ASKED QUESTIONS REGARDING OVULATION TIMING

Q: How do I use WITNESS® LH with OVU-CHECK® Premate®?

A: Utilizing both LH and progesterone assays improves ovulation timing accuracy. Perform an initial progesterone test to establish a baseline level during the first 5 days of the bitch’s season. When vaginal cytology indicates 50 percent cornification, begin daily WITNESS® LH testing. When LH testing indicates a positive result, make preparations to breed. Synbiotics recommends an additional progesterone test be run 72 hours post-LH surge to confirm a rise in progesterone.

Q: Why do I need a “confirming” progesterone test after a positive WITNESS® LH test result?

A: Due to the pulsatile nature of LH activity, there is a small chance that a positive WITNESS® LH test result can be obtained due to identification of a baseline fluctuation rather than the true LH surge. Confirming a rise in progesterone before actually performing the insemination will easily identify those few instances where a positive WITNESS® LH test result is due to identification of LH baseline fluctuation.

Q: When should I start LH testing?

A: Blood should be drawn the fourth or fifth day of proestrus or when vaginal cytology approaches 50 percent cornification. Sampling should be performed daily until the LH surge is detected. If sampling begins after the onset of estrus, the LH surge may have been missed. Remember to confirm a baseline progesterone level on the first day of testing.

Q: How long will I have to test?

A: Testing on 200 dogs has shown that, with daily testing beginning as recommended, the LH surge will be detected in most dogs within 6 days. There are six tests to a kit. Because the bitch may require additional testing, you may wish to have another kit on hand to avoid missing a day of testing.

Q: What if I miss a day of testing?

A: The duration of the LH peak varies from dog to dog and lasts only 1 day in approximately 40 percent of bitches. Samples should be drawn and tested on a daily basis. Failure to do so may result in improper timing. If an LH test day is missed, continue to test but supplement LH testing with progesterone assays in the event the LH peak occurred on the missed day.

Perform a progesterone assay if it is suspected that the LH surge was missed. If progesterone is low (below 2 ng/ml), continue testing. If progesterone is high (above 2 ng/ml), call Synbiotics for technical assistance.

**FOR TECHNICAL ASSISTANCE CALL
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GOOD TECHNIQUE = ACCURATE RESULTS!

Lipemic or grossly hemolyzed samples may interfere with the rate at which the sample flows in the device or create a background color which will make interpretation of the results difficult.

If samples cannot be tested the same day, refrigerate for up to 24 hours or freeze for longer storage. Do not thaw and refreeze.

Do not use the kit beyond its expiration date.

Do not open the foil pouch until ready to perform the test.

Do not touch any of the surfaces in the windows of the device.

Keep device flat during testing. Do not disturb while test is being conducted.