

**For the Detection of
Serum Luteinizing Hormone
DIRECTION INSERT**

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.

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 $< 1\text{ ng/mL}$ in sexually intact bitches and queens, except during 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.

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 **OVUCHEK® Premate®** progesterone assay. Use of other progesterone tests may require different interpretations to explain test results.*

WITNESS® LH is a luteinizing hormone assay that provides a convenient semiquantitative 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. Based on field observations, the test line may be distinct but of less intensity than the control line in some intact females, shortly before or after the LH surge, as well as in some ovariectomized females.

The common indicators of estrus and breeding time, such as vaginal cytology and receptive behavior, are primarily controlled by changes in the hormone

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

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WITNESS® LH Test Procedure

NOTE: Only use SERUM as a sample.

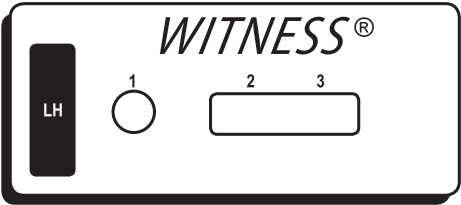
Prior to use, allow SAMPLE to come to room temperature (20° to 25°C; 70° to 77°F)

SAMPLE COLLECTION AND PREPARATION

- Only use SERUM as a sample.
- 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”.



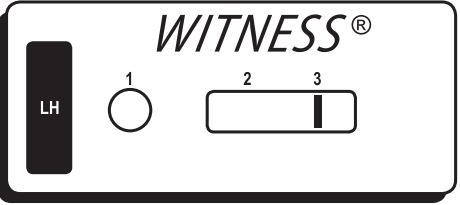
1. Remove test device from foil pouch by tearing at the notch. Place the device on a flat level surface at room temperature.
2. Draw some serum up into the provided pipet. Hold the pipet perpendicular to the device and add **4 drops** of serum to the Serum Well “1”.
3. Allow the test device to sit undisturbed 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”, the LH value is less than 1 nanogram per milliliter. Continue LH testing on a daily basis to determine the optimal time to breed.

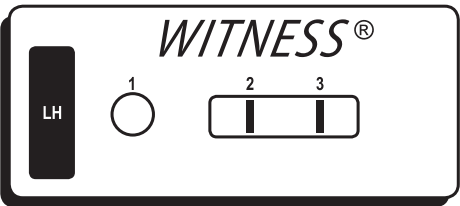


POSITIVE RESULT:

Field observations have shown if a line appears in the area marked “2” which is of similar or greater intensity than the control line “3”, the sample is likely to contain slightly less than 1 nanogram per milliliter.

Field observations have shown if a distinct line appears in the area marked “2” of less intensity than the control line “3”, the sample is likely to contain slightly less than 1 nanogram per milliliter and have been drawn before or after the LH surge (the shoulders of the LH peak). If the daily tests for the LH surge do not reveal a stronger test line, field observations have shown the weaker intensity line should be considered as indicative of the LH surge. Progesterone values may help confirm.

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”, the LH value is less than 1 nanogram per milliliter. This indicates that the bitch or queen is intact.

POSITIVE RESULT:

If a line appears at the area marked “2” which is similar to or greater intensity than control line “3”, the positive result indicates the ovaries have been removed from the bitch or queen. *To rule out that this positive result is due to brief episodic LH pulses in a sexually intact female, the test should be repeated in 2 hours with a fresh sample. If estrus is suspected, a positive result should be confirmed by repeating the test in 24 hours or more with a fresh sample. Subsequent test results from ovariectomized animals should remain positive.*

When a line is present in the area marked “2” which is distinct but of less intensity than the control line “3”, the animal may be intact; however, field observations have shown that some of these animals are ovariectomized.

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 888.ZOETIS1 (888.963.8471)



Manufactured by:
Synbiotics Corporation
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Please contact Zoetis Veterinary Information & Product Support (VMIPS) team at 1-800-366-5288 with questions or comments.

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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.