

USING TARGET TO IDENTIFY OPEN COWS

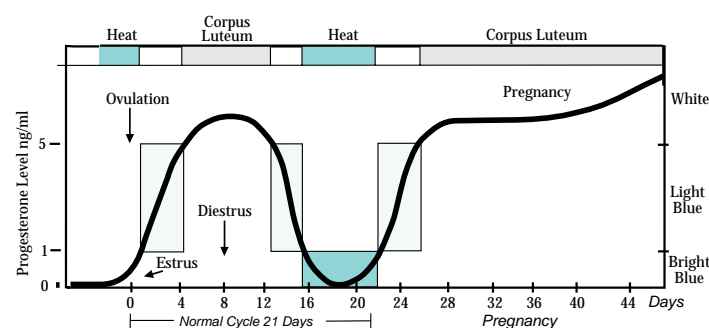
Progesterone, a natural hormone that circulates in the cow's blood and can be found in the milk, is produced by the corpus luteum (CL) and fluctuates during a normal estrous cycle. Milk or blood progesterone levels accurately reflect the different stages of the estrous cycle.

The various stages of the estrous cycle are:

- Estrus (or standing heat) Day 0: The cycle begins. The normal cycle is about 21 days. Each heat (estrus) lasts 6-18 hours. Progesterone levels are lowest during this time (see figure). The test result is bright blue.
- Metestrus (Day 1-7): Ovulation occurs after estrus; the CL forms on the ovary and begins producing progesterone. The progesterone levels begin to rise. The test result is light blue.
- Diestrus (Day 8-16): The progesterone concentration is very high during this time. The test result is white.
- Proestrus (Day 17 to the onset of the next estrus): The CL begins to regress and the progesterone levels begin to drop. The test result is light blue.

Progesterone levels during the reproductive cycle

In normally cycling cows a low progesterone level indicates an



open cow. (Light blue or bright blue test result.) Pregnant cows continue to produce progesterone throughout gestation (white test result).

Two important uses for the TARGET test are when a cow is suspected to be in heat to confirm estrus or about 20 days after insemination to check if the cow is open. At 20 days after insemination progesterone levels will be low (bright blue) if the cow is open, or high (white) if the cow is pregnant. The open cow can then be rebred.

When used in this way TARGET can help improve breeding efficiency.

USING TARGET WITH PROSTAGLANDIN

- Prostaglandin is used when the cow has a functional corpus luteum (progesterone level is high) in order to induce heat (low level of progesterone).
- In order for prostaglandin to be most effective, it is important to know where the cow is in her cycle at the time of the injection in order to predict the result.
- For an unbred cow, a white result indicates that the cow is in diestrus or may have a persist corpus luteum. Prostaglandin may be used at the time of a white result to bring her into heat. About 72 hours after prostaglandin administration, a progesterone test will confirm heat (bright blue result). If the result is not bright blue, the CL has not fully regressed and could recover. Another treatment of prostaglandin is advisable in this case.
- For a cow that has been bred, a white result 20 days after insemination can indicate pregnancy. Prostaglandin should NOT be used at this time, since it lowers progesterone and will cause an abortion of the pregnancy.

USING TARGET TO CONFIRM HEAT

The stress of milk production can effect the cyclic activity. Also, the hormonal activity after calving can reduce the intensity of estrus behavior. Therefore, it is sometimes difficult to observe heat. If a milk sample taken on the suspected day of estrus gives a bright blue result, this confirms that the cow is in heat and ovulation is about to take place.

USING TARGET TO IDENTIFY CYSTS

- TARGET can be useful in diagnosing cysts. A normally cycling cow will show one white and one blue result for two samples taken 7 days apart.
- If the results for two samples taken 7 days apart for a nonpregnant cow are both white, this indicates a luteal cyst. Cows with luteal cysts can be treated with prostaglandin. The treatment was effective, if a TARGET test taken 2-4 days after treatment shows bright blue results. This means the cow is now in heat and can be bred.
- Cows that have consistently low progesterone results (bright blue) for two samples taken 7 days apart are likely to have a follicular cyst. Cows with follicular cysts can be treated with GnRH or hCG and bred when they come into heat. The therapy was effective, if a white result (high progesterone) is obtained for a sample taken 10 days after treatment for a follicular cyst.

USING TARGET FOR EMBRYO TRANSFER

Progesterone testing of the recipient cow is an important step in an embryo transfer protocol. It is recommended that the progesterone level in the recipient be greater than 3 ng/ml for the best success rate.

TEST PREPARATION

Milk samples should be collected into a clean container. Samples of foremilk or from the milk jug are best. The last milk can be used, but it is less preferred. Initial strippings should not be used. Label the samples with the cow number, sampling date, and cycle day.

If a milk sample is not tested within 30 min., it should be stored in the refrigerator- do not freeze. If a milk sample is to be tested more than 6 hrs later, add one drop of milk preservative (ormilk preservative tablet) to each 10 mls of milk immediately after collection.

Allow samples and all kit reagents to come to room temperature before starting test (2-24 hrs). Use a timer while performing the test.

Other Important tips:

- Store this kit in the refrigerator when not in use. Do not freeze.
- Always reseal the plastic bag after removing a test cup.
- Timing during Step 2 (Time of the enzyme) IS IMPORTANT: one (1) minute.
- Do not exchange test cups or reagents between different kits.


PROTOCOL

TEST PREPARATION


Take the kit out of the refrigerator at least 2 hours before using it in order to allow the kit to come to room temperature.
(It is possible to leave the kit at room temperature overnight, if desired.)

1 Shake sample. Add 4 drops of milk or serum or plasma to the center of the cup. WAIT 2 MINUTES.

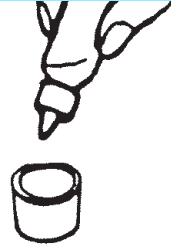
Use a new pipette for each sample.



2 Add 4 drops Sample Wash (WHITE CAP BOTTLE with RED LABEL). Wait for liquid to drain into cup. REPEAT this step 1X or until the cup is white.

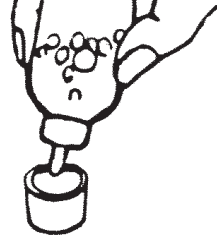


3 Add 3 drops enzyme from the RED CAP BOTTLE to the center of the cup and WAIT ONE MINUTE - timing this step is important.

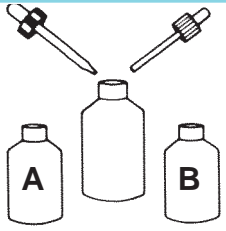


4 Fill cup with wash solution from the WHITE CAP BOTTLE with WHITE LABEL to the top of the inner line.

Wait for liquid to drain completely into cup.

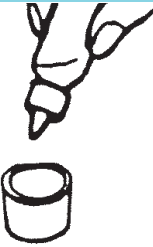


5 Prepare fresh substrate solution in empty BLUE CAP MIXING BOTTLE using one dropper (filled to the mark) EACH of Substrate A and Substrate B. Shake the freshly prepared substrate in the mixing bottle well.

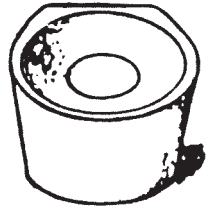


6 Add 4 drops FRESHLY prepared substrate (BLUE CAP) to the center of the cup.*

* Throw out any unused solution in the blue mixing bottle after 30 minutes, since the substrate decomposes. Save blue mixing bottle for future tests.



7 Record color results: Bright Blue, Light Blue, or White in 9 MINUTES.



Rev. 6/03

INTERPRETING THE RESULTS

| Color And Progesterone Level | Interpretation |
|-----------------------------------|--|
| Bright Blue 0 - 1 ng/ml | No progesterone is present and there is NO functional CL. Bright blue means standing heat in normally cycling cows . Ovulation will occur in 6-18 hours. In the prepubertal heifer or postpartum cow, a bright blue result can indicate lack of cycling. A bright blue test result for each of 2 samples taken 7 days apart strongly indicates the presence of a follicular cyst. |
| Light Blue 1 - 5 ng/ml | Progesterone is either rising after heat or falling (approaching heat). See Figure. A second test should be run in 2 days. If a test is repeated 2-3 days later, the results will be either bright blue (heat) or white (high progesterone). In embryo transfer the recipient should show a light blue test result when an embryo is transferred. |
| White > 5 ng/ml | A white result indicates a high progesterone level and a functional CL is present. A) A white result indicates pregnancy if the test was taken 19 to 23 days after breeding or that the cow is in mid cycle. B) A white test result for each of 2 samples taken 7 days apart strongly indicates the presence of a luteal cyst. |



BOVINE CL CHEK

FOR IDENTIFYING THE OPEN COW

Rapid Bovine Progesterone Kit

- This kit is the most accurate method for determining the presence of a corpus luteum (CL) and for determining if it is producing progesterone.
- By identifying open cows earlier this kit will reduce the days open and lower costs.
- Recommended for use in embryo transfer programs to check if there is adequate progesterone level in the recipient to support the embryo.



TEL: 1800-999-1961 FAX: 1-609-275-9485