

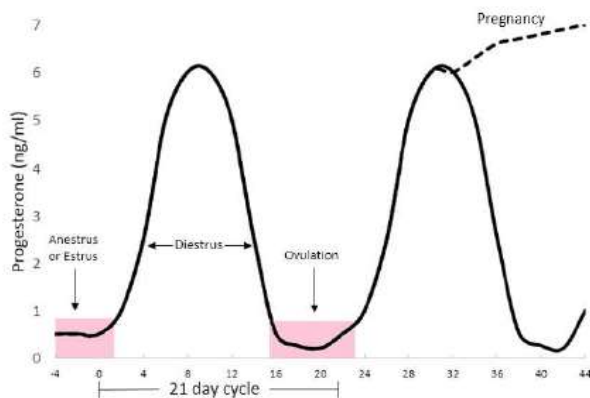
TruStrip™ Equine Progesterone Ovulation Rapid Test

A one step test for the semi-quantitative detection of Progesterone in Equine serum or plasma

Introduction

Progesterone is a hormone that circulates in a mare's blood which is produced by the corpus luteum (CL). Measurement of Progesterone can determine where the mare is in the reproductive cycle. During the winter months (typically October to February) there is no functional CL and no Progesterone is produced. This period is called the winter anestrus. During the spring months, there is a transition period in which the estrus cycle begins. The mare's reproductive cycle begins after the first ovulation. A normal cycle is typically 21 days. During this cycle, heat (estrus) occurs. Estrus lasts 5-7 days. During estrus, Progesterone levels are low (<1 ng/ml). 24-48 hours before the end of estrus, ovulation occurs. Once ovulation occurs, the CL forms on the ovaries and begin producing Progesterone. This brings the period of diestrus. Diestrus is the period in-between the estrus phases where the mare is not receptive to the stallion. During diestrus, the Progesterone levels rise from <1 ng/ml to 5-10 ng/ml. In a pregnant mare, Progesterone levels remain elevated 17 days after breeding. In an unbred mare, the Progesterone levels drop to <1 ng/ml and the estrus cycle begins again. The optimal time to breed is a few days before ovulation and in some cases may be successful a few hours after ovulation.

The Equine Progesterone rapid test can be used to determine where the mare is in the cycle, determine whether there is a functional CL and the need to use prostaglandin, determine if prostaglandin treatment was effective, and monitor if adequate levels of Progesterone are being maintained for pregnancy to avoid late term abortions.



Principle of the test

The progesterone rapid test is a semi-quantitative competitive test. The test contains a nitrocellulose membrane which is pre-coated with Progesterone on the test line region of the strip. A separate pad contains a Progesterone antibody conjugated to gold nanoparticles. As serum is applied to the strip, it migrates upwards by capillary action which causes the Progesterone antibody to be released. The Progesterone conjugated antibody in the absence of Progesterone in serum, will bind to the immobilized Progesterone on the membrane, resulting a pink colored line in the test line region. If Progesterone is present in serum, the antibody will bind to the Progesterone in serum and not to the Progesterone that is immobilized on the test line region. As the concentration of Progesterone in serum increases, the intensity of the test line region decreases. The assay is optimized to result in the disappearance of the test line at serum progesterone concentrations of approximately 10 ng/ml or higher.

Precautions

- Do not use the test after the expiration date
- Do not re-use the test
- Wear protective clothing such as disposable gloves when specimens are being tested
- Handle all specimens as if they contain infectious agents. The cassette should be disposed according to federal, state, and local regulations
- Humidity and temperature can adversely affect results
- Use the test within 20 minutes of opening the foil pouch

Storage and stability

The kit can be stored at room temperature or refrigerated (2-25°C/35-77°C). Do not freeze. The test cassette must remain in the sealed pouch until use. The kit is stable until the date printed on the pouch.

Kit contents

Materials provided: Test cassettes and transfer pipette

Materials required but not provided: Centrifuge and serum or plasma collection tubes

Specimen collection and preparation

Serum (S): Collect whole blood by venipuncture into a red-top Vacutainer® tube. Allow the blood to clot by leaving it at room temperature for 15-30 minutes. Centrifuge the blood for 10 minutes at 1,000xg and collect the supernatant.

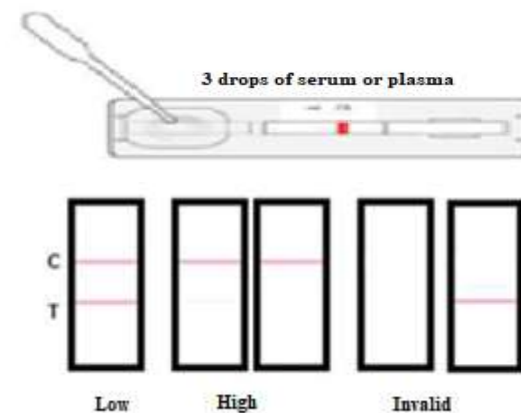
Plasma (P): Collect whole blood by venipuncture into a Blue top (Citrate) or Lavender (EDTA) Vacutainer® tube. Centrifuge the blood for 10 minutes at 1,000xg and collect the supernatant.

Note: Samples must not contain any red blood cells or any significant amount of hemolysis (red color) or lipemia (white color).

Directions for use

Allow the test cassette to come to room temperature (15-25°C/59-77°F) prior to testing.

1. Remove the test cassette and transfer pipette from the foil pouch. Lay the device on a flat and dry surface.
2. Use the transfer pipette to transfer the sample by depressing the bulb of the pipette. Dispense 3 drops to the test cassette
3. Begin a timer for 30 minutes, allow 20-30 minutes for the background to clear. Do not interpret the results after 30 minutes. **Note:** The results will typically be visible within 5 minutes, but 30 minutes is needed for complete development of the test line region.



Interpretation of results

Low: Two lines of similar intensity appear, one in the test region (T) and one in the control region (C). This indicates that the Progesterone is below 1 ng/ml

High: One pink line appears in the control region (C). An extremely faint line or no line appears in the test line region. This indicates that the Progesterone has risen above 5 ng/ml.

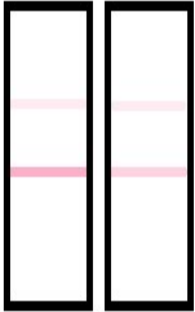
Invalid: A control line fails to appear. Insufficient specimen volume or incorrect procedural technique are the most likely cause. Check the expiration date. Repeat the test with a new cassette. If the problem persists, discontinue using the kit and contact the manufacturer.

Quality Control

A pink line appearing in the control region (C) is the internal procedural control. External controls are not supplied. It is recommended that a positive and negative control (do not use water) be tested as good laboratory practice. If you require external controls, contact ADI to have controls formulated.

Interpretation of results

The Equine Progesterone rapid test is a semi-quantitative competitive test. As the concentration of Progesterone in the sample increases, the intensity of the test line decreases.



0-1 ng/ml: A test line appears that is darker or slightly more intense than the control line. The mare is in winter estrus or may be transitioning to the estrus phase if developing follicles are present upon palpation. If the mare has started her spring cycling, this result indicates that she is in heat. Estrus is typically 5-7 days but may be as short as 3 days. Ovulation occurs 1-2 days before the end of estrus.



2-4 ng/ml: A test line appears that is roughly the same or less intense than the control line. This result can indicate the following;

- 1) If a previous result gave a test line equal to a 0-1 ng/ml result, this indicates that the mare is leaving estrus and entering diestrus, Progesterone is being produced.
- 2) If a previous test showed an extremely faint or no test line, this indicates that the Progesterone is dropping, and the mare is approaching her estrus period again. She should be re-tested in 2 days.
- 3) If a previous test had a similar result, this indicates that the mare is still in diestrus and Progesterone may be increasing or decreasing

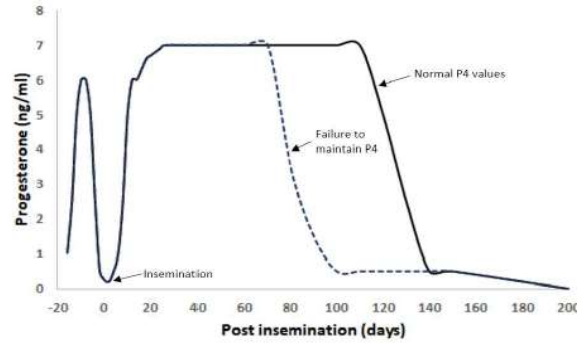


5-9 ng/ml: An extremely faint line appears in the test line region. This result can indicate the following;

- 1) Progesterone is at the peak of diestrus and the mare will be entering ovulation soon.
- 2) If this result is obtained 21 days after breeding, this indicates that pregnancy has occurred. Progesterone above 5 ng/ml is needed to maintain pregnancy.
- 3) If the mare does not exhibit signs of estrus during a breeding season, this result may indicate a persistent CL and prostaglandin treatment may be necessary.

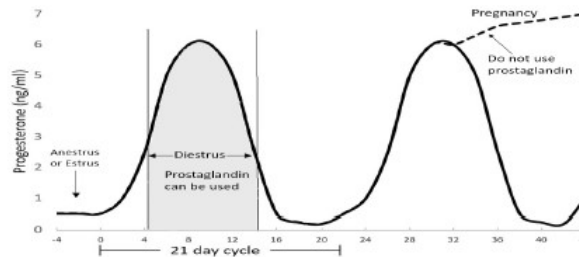
≥10 ng/ml: No line appears in the test line region.

Maintenance of pregnancy



The Equine Progesterone test can be used to monitor Progesterone concentrations during pregnancy. Progesterone concentrations of 5 ng/ml and above are needed to maintain pregnancy. The average gestation period of a mare is 342 days. From day 120-150, Progesterone stops being produced by the mare and the fetoplacental unit becomes responsible for producing progesterone that maintain pregnancy. From day 180 onward, Progesterone becomes undetectable in the mare. The rapid test can be used after ovulation until about 120 days post insemination to monitor if adequate Progesterone levels are being maintained. The expected result in a healthy mare would be a faint or no test line appearing. If a strong test line is observed, the mare may need to be supplemented with Regu-Mate® or similar supplements to avoid late-term abortion. A veterinarian should be consulted for the best course of action in such cases. From day 110-300 post breeding, urine oestrone sulphate can be used to monitor pregnancy.

Prostaglandin treatment



Prostaglandin can be used when the mare has a persistent corpus luteum (High Progesterone levels) in order to induce estrus (Progesterone drops to <1 ng/ml). In an un-bred mare, an extremely faint or no test line indicates that the mare is in diestrus or has a persistent corpus luteum. Prostaglandin can be used when progesterone levels are high in order to bring the mare into estrus. About 48-72 hours after prostaglandin treatment, the Equine Progesterone test should have a test line of similar intensity to the control line (0-1 ng/ml result). In a mare that has been bred, if no test line is observed after 17-20 days, then prostaglandin should not be used as it will lower progesterone and cause an abortion of pregnancy.

Related items available from ADI

Catalog#	Description
BP4-RDT-5 Ovulation test (milk)	TruStrip™ Bovine Progesterone
CP4-RDT-5 Ovulation Test (serum)	TruStrip™ Canine Progesterone
FP4-RDT-5	TruStrip™ Feline Progesterone Test
1955 Quantitative	Progesterone ELISA Kit, 96 tests,



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