



OVUCHECK®
Premate 5

OVUCHECK®
Premate 10

Monographie

*Trousse de détermination du taux de progestérone sérique
et plasmatique chez les chiens et les chats.*

Insert

*Kit for Determination of the Level of Serum or Plasma
Progesterone in Dogs and Cats.*

Monografía

*Kit de determinación de la concentración de la progesterona
en el suero y en el plasma de perras y gatas.*

INTRODUCTION

OVUCHECK® PREMATE is a semi-quantitative test for the measurement of progesterone in a drop of plasma or serum. The quantity of progesterone present is indicated by a change in colour, which is compared with high and low progesterone standards.

This kit is quick and simple to use. It gives reliable information which allows you to:

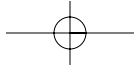
- plan mating of the bitch at the optimum time;
- determine the time of whelping of the bitch;
- investigate the causes of infertility of the bitch: anovular cycles, silent heats with normal ovulation;
- determine if the queen has ovulated.

PHYSIOLOGICAL BASIS

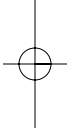
Bitch:

Hormonal changes occurring in the bitch in the course of pro-oestrus, oestrus, beginning of dioestrus, and gestation are described below.

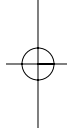
At the end of pro-oestrus, the drop in oestrogen levels, secreted by the maturing ovarian follicle, causes the luteinising hormone (LH) surge. Ovulation normally happens 24 to 48 hours after the LH surge.



The follicles gradually produce progesterone above basal levels (<0,5 ng/mL) a few days before the LH surge. When the LH surge occurs, progesterone levels may reach 2 to 4 ng/mL. At the time of ovulation, the progesterone level is generally between 4 and 10 ng/mL. This normally happens 11 to 13 days after the start of pro-oestrus (characterised by the presence of blood and swelling of the vulva). After ovulation, progesterone levels continue to rise to reach maximal levels 2 to 3 weeks after the start of dioestrus. However, because of the large variations between breeds and individuals, one can only be certain that ovulation has taken place when the level of progesterone has exceeded the value of the high standard of the PREMATE test (10 ng/mL).



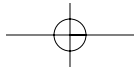
At the end of gestation, 12 to 24 hours before giving birth, the level of progesterone falls again to reach values of less than 2 ng/mL. Thus, a level of progesterone higher than the low standard of the PREMATE test shows that parturition will not occur in the next 12 to 24 hours.



Queen:

The oestrus cycle of the queen is seasonal and polycyclic. There are 5 phases in the queen's oestrus cycle: pro-oestrus, oestrus, inter-oestrus, dioestrus, and anoestrus.

In queens, ovulation is induced by vaginal penetration, leading to development of the corpus luteum (luteal phase) which synthesises and secretes progesterone, whether or not the mating was fertile.

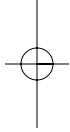


Basal progesterone levels (<math><0,5\text{ ng/mL}</math>) rapidly increase with the development of the corpus luteum. Progesterone levels reach 5 ng/mL 3 days after the beginning of the luteal phase, and over 20 ng/mL after 16 to 25 days. This rise is similar whether the queen is in gestation or in pseudogestation. If the queen is in gestation, the progesterone levels remain elevated while they drop in the case of pseudogestation.

SAMPLING

Timing of sampling:

Bitch:



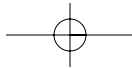
In order to determine the time of ovulation, the first samples are taken and tested after the appearance of signs which mark the start of the oestrus (slight losses of blood, acceptance of the male, or characteristic vaginal smear), which corresponds to 6 to 9 days after the start of pro-oestrus.

In the case of a bitch which has a known history of failed conception, or if you suspect that ovulation is early, it is preferable to start the tests from the pro-oestrus in order to establish basal progesterone levels for further comparison.

Queen:

Samples should be taken a minimum of 7 days following mating.





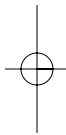
Collection of the samples:

Serum:

Collect a sample of blood in the usual manner in a dry tube (1 mL of blood is enough). Allow to coagulate (for about half an hour at ambient temperature) and use the serum on the top for the measurement.

Plasma:

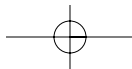
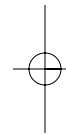
Collect a heparinised blood sample in a heparin-coated tube and separate the plasma from the red blood cells by centrifugation, then use the plasma for the measurement.



OPERATING PROCEDURE

Bring all the constituents to room temperature before use (about 30 minutes).

Make sure every solution is properly mixed before use.



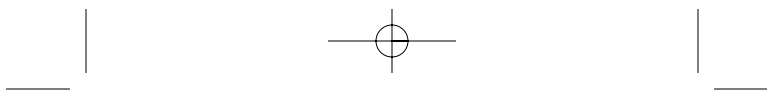
Preparation of the substrate:



- Carefully remove the screw cap from bottle D. Then, take out and discard the polyethylene stopper.
- Without touching it, eject the substrate tablet into bottle D by pressing the back of the metal foil.
- Place the nozzle supplied into bottle D. Put on the cap and screw firmly to ensure the nozzle fits securely into the neck of the bottle.
- Mix at regular intervals, until the tablet has completely dissolved (15 to 30 minutes). The solution must now be yellow.

Storage of the substrate:

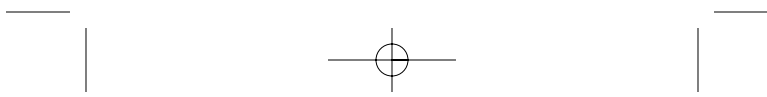
- Write the date on the label. The mixture prepared in bottle D is now usable for a period of up to three months if kept refrigerated (2 - 6°C).
- This solution can be frozen for a longer period of conservation. The use-by-date is then shown on the outside label on the kit box. You can fractionate solution D, avoiding freeze/thaw cycles. For example, you can use insulin syringes (0,6 mL per syringe). This way, you will only have to take one syringe from the freezer.
- The colour of the solution of bottle D (when activated) can change with time but this does not affect the interpretation of the results.

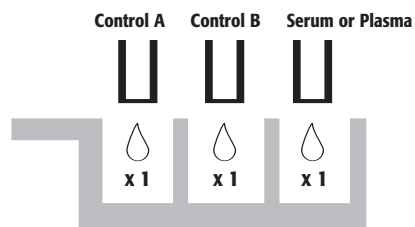


Carrying out the test:

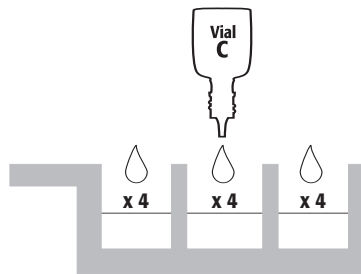
Two standards are provided (A and B). They need to be used each time an assay is performed.

- Take out a strip of wells from the plastic.
- Select the necessary number of wells ($2 + n$, n being the number of samples to test) by breaking the plastic between the wells. Put the unused wells back in the plastic bag. Mark for identification purposes the top of the first well you will use.

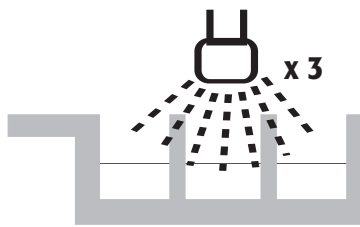




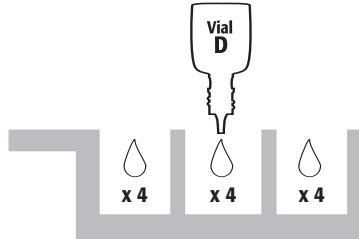
- Keeping the pipette vertical, add 1 drop low standard (A) to one well.
- Keeping the pipette vertical, add 1 drop of high standard (B) to another well.
- Keeping the pipette vertical, add 1 drop of the sample to be tested to a further well. Each sample to be tested shall be added to a different well. Always use a new pipette for each sample or standard. Wells must either contain a standard or a sample, not both.



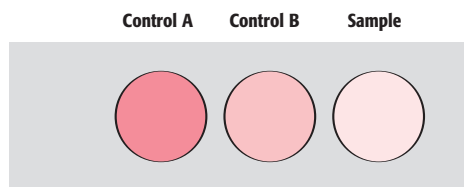
- Keeping bottle C vertical, add 4 drops of reagent C to each well.
- Cover the wells and let them stand for 15 minutes at room temperature.



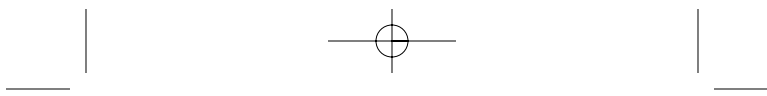
- Empty the contents of the wells into the sink and rinse gently the wells three times, using tap water (warm). Dry by tapping onto absorbent paper.



- Keeping bottle D vertical, add 4 drops of the prepared substrate from bottle D to each well.
- Cover the wells and leave for 15 minutes at room temperature.

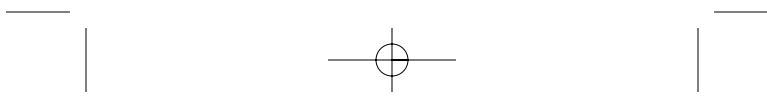


- Agitate the wells gently to mix the contents and compare the colours in the test wells with those of the high and low standards.



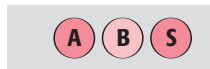
INTERPRETATION OF THE RESULTS

- To help visualise the results, place the wells on top a white background and look at the wells from the top.
- It is important to first check that standard A (low progesterone) is of a darker pink than standard B (high progesterone).

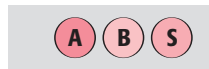


BITCHES :

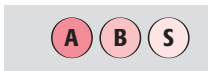
Heath detection:



- If the sample is as pink or more pink than A (low standard), the progesterone concentration is lower or equal to that of the low standard.
- The bitch is still in the pro-oestrus phase.
- Test again in two days.



- If the sample is of a lighter pink than A, but darker than B (intermediate between A and B), progesterone concentration is between 3 and 10 ng/mL.
- Ovulation is imminent.
- Test again the next day.



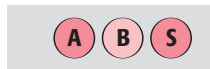
- If the sample is of a lighter pink than B (high standard), progesterone concentration is higher than 10 ng/mL.
- Ovulation has probably taken place.
- Proceed to mating without any further delay.



- If the sample has the same colour than B (high standard), it is probable that ovulation has taken place.
- A period of 2 to 3 days is then necessary for the ovum to mature.
- Because of the relatively long survival period of spermatozoa in the genital tract of the bitch, it is necessary to mate the bitch in the 24 to 48 hours following this result.

BITCHES :

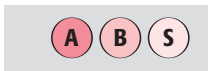
Term detection:



- If the sample is of a darker pink than A (low standard), progesterone concentration is less than 3 ng/mL.
- The bitch is probably due to whelp. Whelping should take place within 12 to 24 hours.



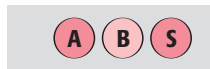
- If the sample is of a lighter pink than A, but darker than B (intermediate between A and B), progesterone concentration is between 3 and 10 ng/mL.
- The bitch is not due to whelp.
- Test again the next day.



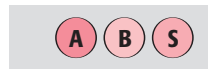
- If the sample has the same colour or is lighter than B (high standard), progesterone concentration is higher than 10 ng/mL.
- The bitch is not due to whelp. Whelping will probably not take place before 48 hours.
- Test again in two days.

BITCHES :

Prolonged anæstrus

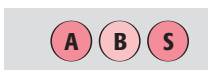


- If the sample is of a darker pink than A (low standard), progesterone concentration is less than 3 ng/mL.
- The bitch does not secrete progesterone. This can be that ovulation has not taken place yet or that the bitch has not had heat for the last 2 months.

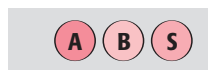


- If the sample is of a lighter pink than A, but darker than B (intermediate between A and B), the bitch secretes progesterone.
- Heat has probably occurred unnoticed or the bitch suffers from ovarian pathology.
- Test again in one month in order to document the duration of high progesterone levels.

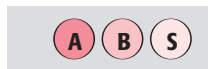
QUEENS :

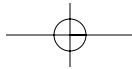


- If the sample is of a darker pink than A (low standard), progesterone concentration is less than 3 ng/mL.
- If mating has occurred more than a week ago, the queen has not ovulated.



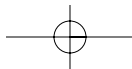
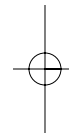
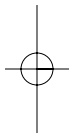
- If the sample is of a lighter pink than A, but darker than B (intermediate between A and B), ovulation has occurred.
- The queen has been mated and is likely in gestation.





PRECAUTIONS

- Keep the kit refrigerated (2-6°C). DO NOT FREEZE.
- Do not use wells more than once.
- The stabilising solution in bottles A, B, and C contain preservative.
- When one empties the contents of the wells into the sink, rinse away with a large amount of tap water.
- For *in vitro* veterinary diagnostic use only.
- Keep out of reach of children.
- Do not pipette solutions by mouth.
- If the product splashes into the eyes or onto the skin, wash thoroughly with tap water.
- For more information, contact technical services at Biovet Inc.



KIT CONTENTS

PREMATE 5	PREMATE 10	
A 1 X 1,0 mL	A 1 X 1,0 mL	Ready-to-use Low Standard (3 ng/mL progesterone)
B 1 X 1,0 mL	B 1 X 1,0 mL	Ready-to-use High Standard (10 ng/mL progesterone)
C 1 X 5,0 mL	C 1 X 8,5 mL	Ready-to-use Conjugate
D 1 X 8,5 mL	D 1 X 8,5 mL	Substrate Buffer
1 Tablet	1 Tablet	Substrate
2 X 8	4 X 8	Coated Microwells
18	35	Plastic Pipettes
1	1	Rubber Bulb for Plastic Pipettes