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# OVUCHECK®
# Premate 5

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# 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-œstrus, œstrus, beginning of diœstrus, and gestation are described below.

At the end of pro-œstrus, the drop in œstrogen 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 follicle gradually produces 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 (characterized 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 œstrus cycle of the queen is seasonal and polycyclic. There are 5 phases in the queen's œstrus cycle: pro-œstrus, œstrus, inter-œstrus, dicœstrus, and anœstrus.

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 (<0.5 ng/mL) 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 œstrus (slight losses of blood, acceptance of the male, or characteristic vaginal smear), approximately 6 to 9 days after the start of pro-œstrus.

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 pro-œstrus in order to establish basal progesterone levels for further comparison.

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

***Collection of samples:***

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

*Plasma:*
Collect a blood sample in a heparin-coated tube and separate the plasma from the red blood cells by centrifugation, then use the plasma to perform the test.

## *OPERATING PROCEDURE*

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

Make sure every solution is properly mixed before use.

## *Substrate preparation:*


- Carefully remove the screw cap from bottle D. Then, take out and discard the stopper.
- Without touching it, eject the substrate tablet into bottle D by pressing the back of the metal foil.
- Place the supplied nozzle 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.

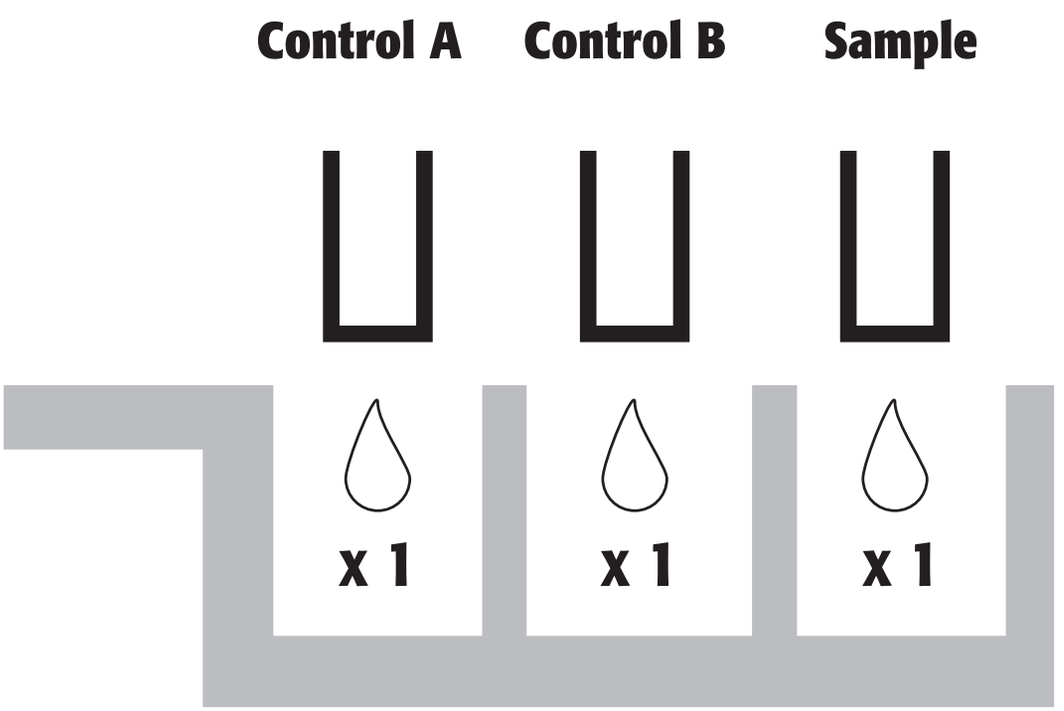
## *Storage of the substrate:*

- Write the date on the label. The mixture prepared in bottle D is now stable for up to three months if kept refrigerated (2 - 6°C).
- This solution can be frozen for a longer period of conservation. The expiration date is then shown on the outside label on the kit box. You can aliquot solution D, to avoid repeated 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 out of the freezer each time you perform the test.
- The colour of the solution of bottle D (when activated) can change with time but this does not affect results interpretation.

## *Carrying out the test:*

Two standards are provided (A and B). They must both be used each time an assay is performed to validate the test.

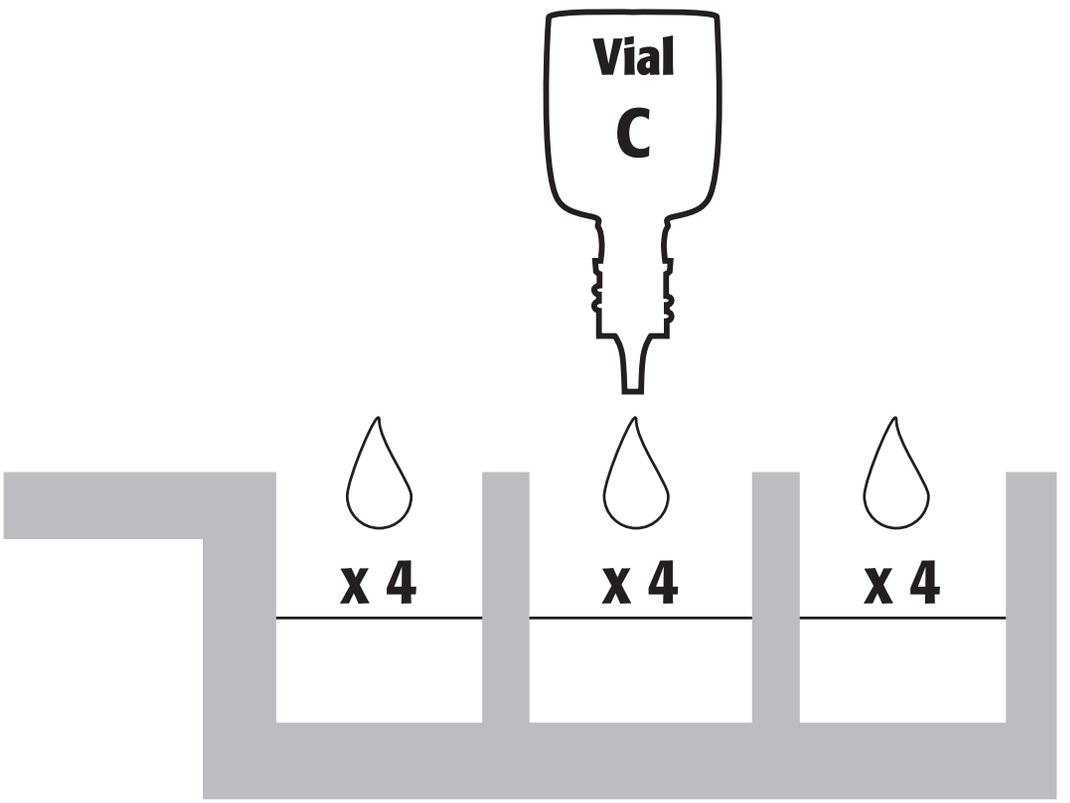
- Take out a strip of wells from the plastic bag.
- Select the necessary number of wells (2 + n, n being the number of samples to test) by breaking the plastic between the wells. Put the unused wells back in the plastic bag. Mark for identification purposes the top of the first well you will use.



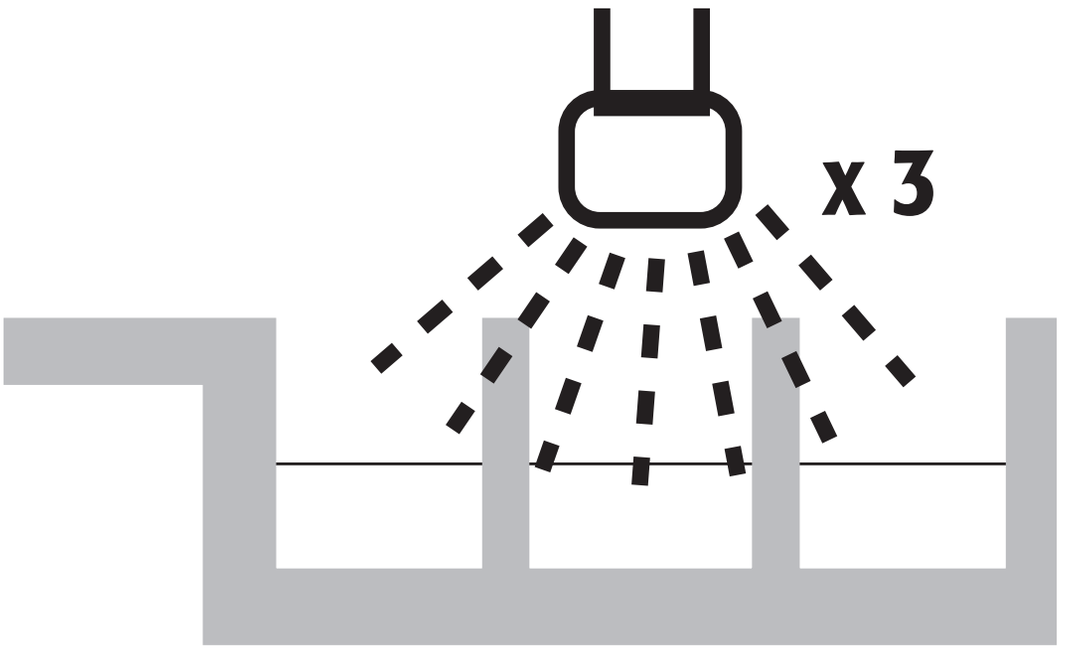

Always use a new pipette for each sample or standard. Keeping the pipette vertical, add:

- 1 drop of low standard (A) to the first well.
- 1 drop of high standard (B) to the second well.
- 1 drop of the sample to be tested to the third well. Each sample to be tested shall be added to a different well. Wells must either contain a standard or a sample, not both.

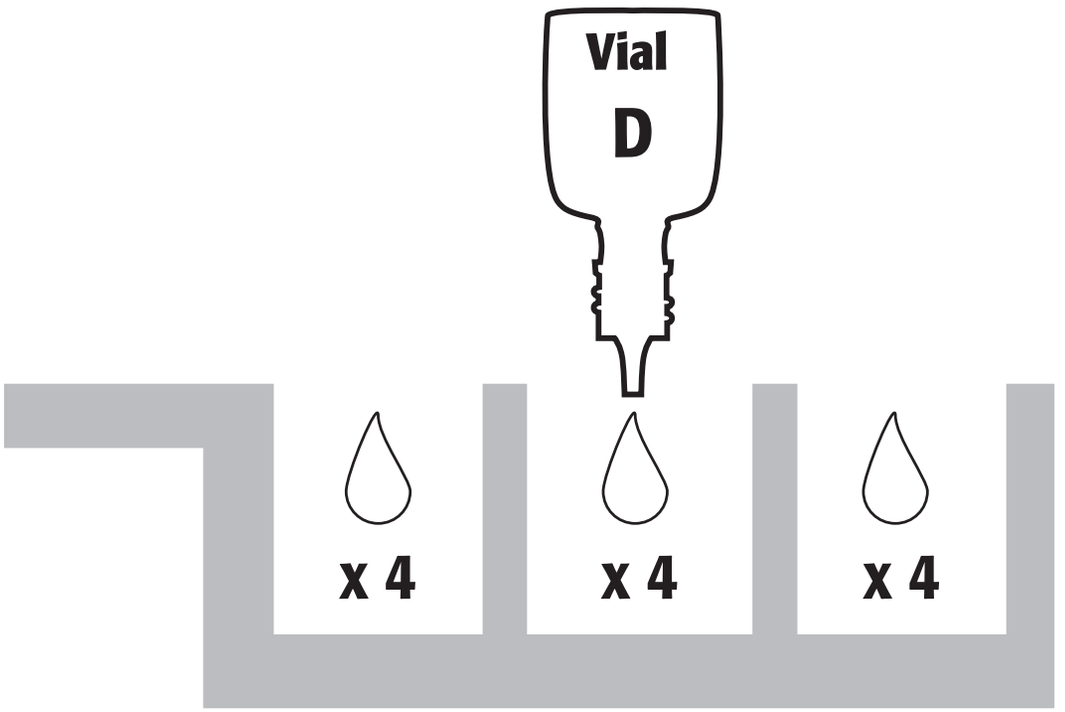
*Important note: If the pipette is not held in a vertical position, the drop volume could be smaller and affect the accuracy of the test.*



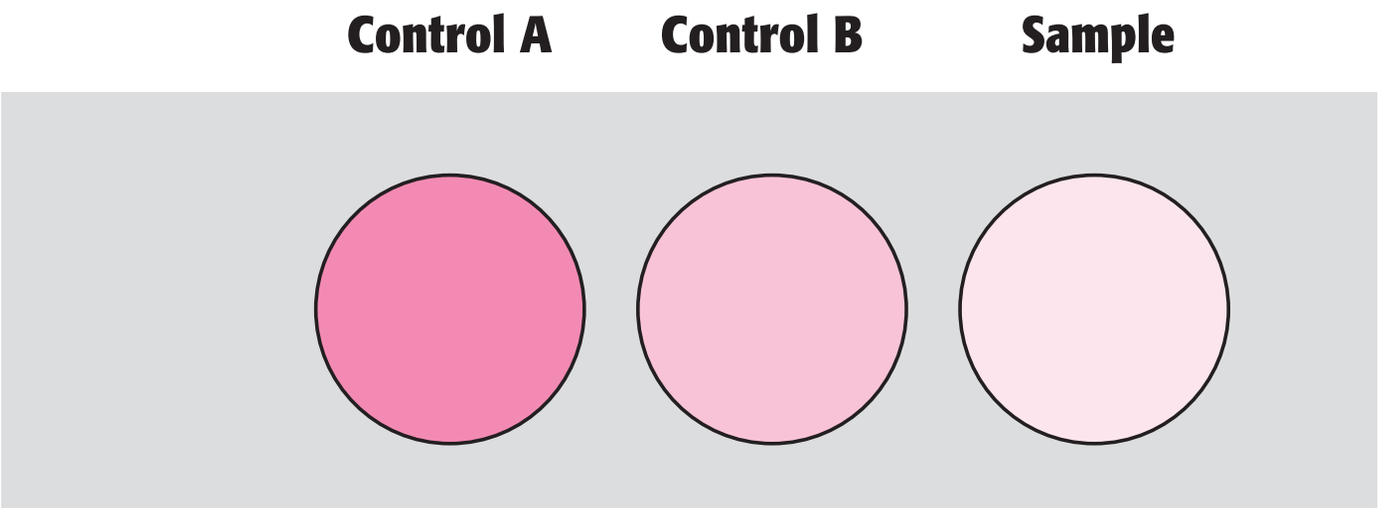

- Keeping bottle C vertical, add 4 drops of reagent C to each well.
- Cover the wells and incubate for 15 minutes at room temperature.




- Empty the contents of the wells into the sink and gently rinse the wells three times, using lukewarm tap water. Dry by tapping onto absorbent paper. To allow better colour differentiation, avoid overdrying.




- Keeping bottle D (or insulin syringe) vertical, add 4 drops of the prepared substrate to each well.
- Cover the wells to protect them from light and incubate for 15 minutes at room temperature.



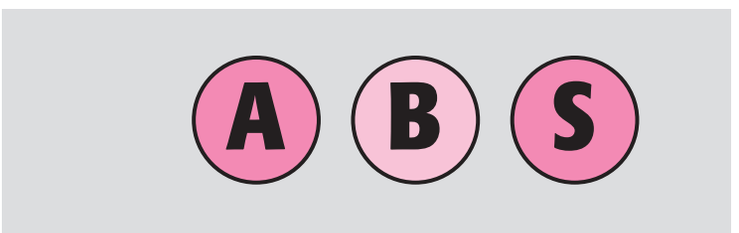

- Agitate the wells gently to mix the contents and compare the colour of the sample to the colours of the high and low standards.

## *RESULTS INTERPRETATION*

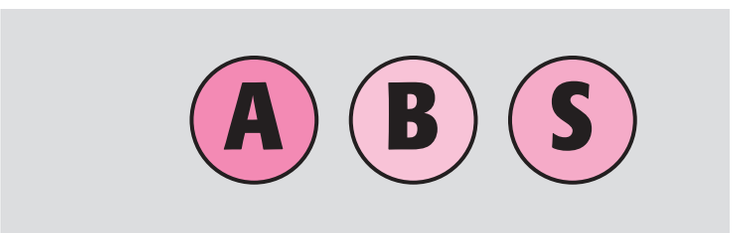
- To help visualise the results, place the wells on top of a white background and look at the wells from the top.
- It is important to first check that standard A (low progesterone) is of a darker pink than standard B (high progesterone).

## *BITCHES:*

### *Heat detection:*




- If the sample is the same shade of pink or darker than A (low standard), the progesterone concentration is lower or equal to the low standard (3 ng/mL).
- The bitch is still in the pro-œstrus phase.
- Test again in two days.



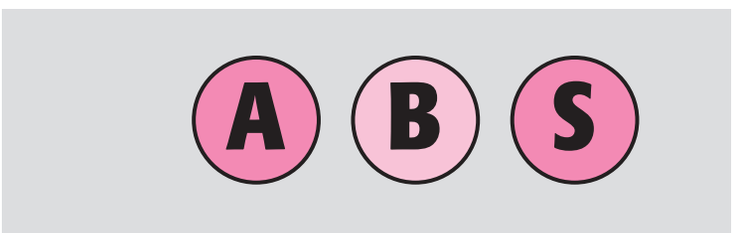

- If the sample is lighter than A, but darker than B (intermediate between A and B), progesterone concentrationis between 3 and 10 ng/mL.
- Ovulation is imminent.
- Test again the next day.


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

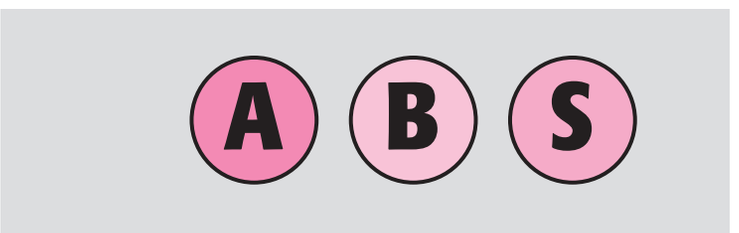

- If the sample is the same colour as B (high standard), ovulation has probably taken place.
- A period of 2 to 3 days is then necessary for the ovum to mature.
- Because of the relatively long survival of spermatozoa in the genital tract of the bitch, you can mate the bitch in the next 24 to 48 hours.

## *BITCHES:*

### *Term detection:*




- If the sample is darker 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.

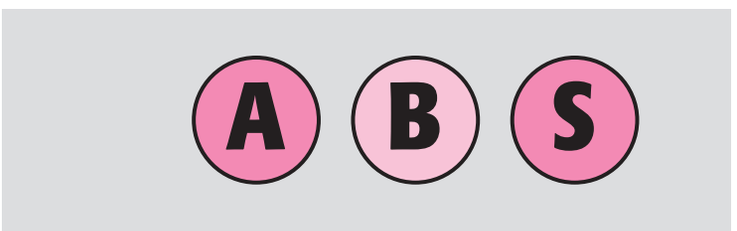



- If the sample is lighter than A but darker than B (intermediate between A and B), progesterone concentration is between 3 and 10 ng/mL.
- The bitch is not due to whelp.
- Test again the next day.

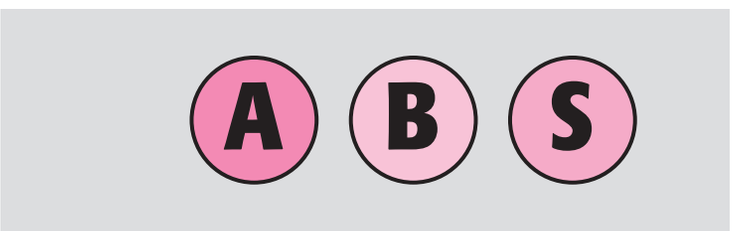

- If the sample is the same colour or lighter than B (high standard), progesterone concentration is higher than 10 ng/mL.
- The bitch is not due to whelp. Whelping will probably not take place before 48 hours.
- Test again in two days.

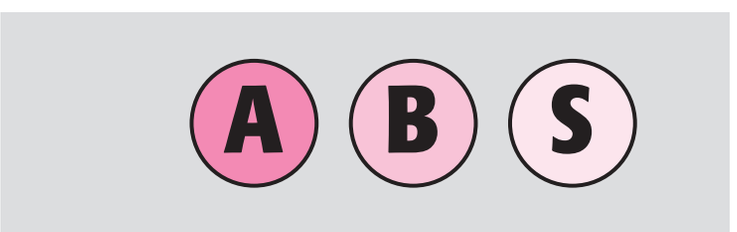
## *BITCHES:*

### *Prolonged anœstrus:*



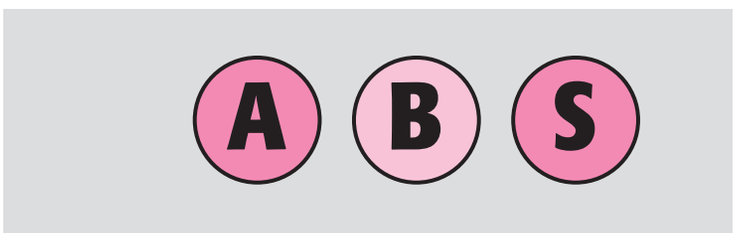

- If the sample is darker than A (low standard), progesterone concentration is less than 3 ng/mL.
- The bitch is not secreting progesterone. There are two possibilities: she has not ovulated or she has not been in heat for the past 2 months.



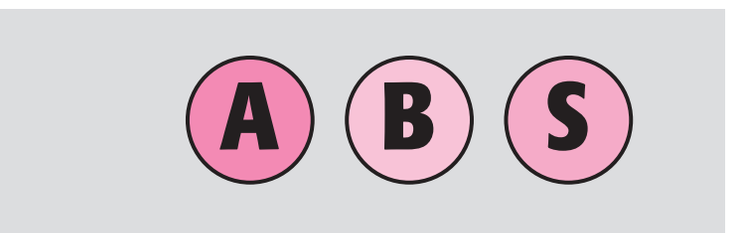



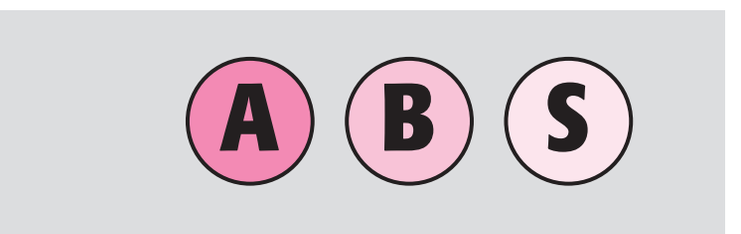

- If the sample is lighter than A, but darker than B (intermediate between A and B), or lighter than B, the bitch is secreting progesterone.
- Heat has probably occurred unnoticed or the bitch suffers from an ovarian pathology.
- Test again in one month in order to document the duration of the rise in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

## *QUEENS:*




- If the sample is darker 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 lighter than A, but darker than B (intermediate between A and B), or lighter than B, ovulation has occurred.
- Mating has occurred and the queen is likely pregnant.

## *PRECAUTIONS*

- Keep the kit refrigerated (2-6°C). DO NOT FREEZE.
- Do not use wells more than once.
- The solutions in bottles A, B, and C contain a preservative.
- When emptying 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. (support@biovet-inc.com).

## KIT CONTENTS

| PREMATE 5 | PREMATE 10 | |
|---|---|---|
| ***A*** 1 X 1,0 mL | ***A*** 1 X 1,0 mL | Ready-to-use<br>Low Standard<br>(3 ng/mL progesterone) |
| ***B*** 1 X 1,0 mL | ***B*** 1 X 1,0 mL | Ready-to-use<br>High Standard<br>(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<br>Plastic Pipettes |

## NOTES

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**Fabriqué par / Manufactured by / Fabricado por :**

Biovet®

www.biovet.ca

Biovet – Canada

4375, Beaudry
St-Hyacinthe (Québec)
J2S 8W2
CANADA
Tel.: 1-888-824-6838
1-450-771-7291
Fax: 1-450-771-2996

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