

INTERPRETATION

Results

For Visual Method:

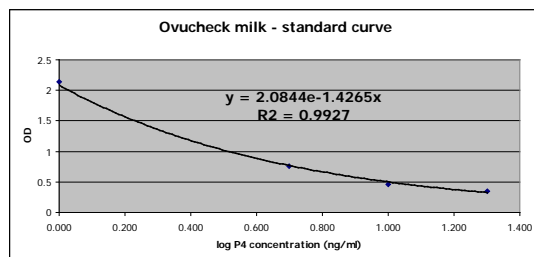
- Compare colour intensity of each sample with that of standards 5 and 10 ng/mL.
- A colour the same as, or more intense than that of standard 5 ng/mL, indicates a lower progesterone level, i.e., oestrus or non-pregnant.
- A colour equal to or paler than that of standard 10 ng/mL, indicates a higher progesterone level, i.e., pregnant or mid-luteal.
- Samples in between should be re-sampled and tested the next day.

For Quantitative Method:

- Set plate reader to read absorbance at 405 nm. Zero instrument on air. Draw a standard curve by plotting standard absorbance values on the graph paper provided or by using appropriate software (e.g. Excel).
- The progesterone concentration of the samples can then be calculated from the curve.

Example of Standard Curve

P4 standard (ng/ml)	OD (405 nm)
1	2.141
5	0.758
10	0.459
20	0.351



Example of calculation from the curve: $\log \text{concentration P4} = - [\ln (\text{DO}/2.0844) / 1.4265]$

Specificity

The interference (cross-reactivity) from steroids other than progesterone is insignificant (less than 1%) except for:

11 α -Hydroxy-progesterone	66.0%
5-Pregnan-3 β -ol-20-one	16.0%
Deoxy-corticosterone acetate	3.0%
5 β -Pregnan-3, 20-dione	4.5%
5 α -Pregnan-3, 20-dione	3.3%

Interpretation

Background:

Progesterone concentrations in bovine milk reflect those in blood. There is little or no detectable progesterone at oestrus and for approximately the next three days. The level then rises progressively until mid-cycle with the growing *corpus luteum*.

If, at 17-18 days, the cow is not pregnant, the *corpus luteum* regresses and the progesterone level falls.

If pregnant, the cow's progesterone level remains at the mid-cycle level and continues throughout pregnancy.

Confirmation of Pregnancy:

Test milk samples 24 days after artificial insemination. If no low values (5 ng/mL or less) are observed by the 24th day after insemination, pregnancy can be assumed and should be confirmed using an appropriate method (e.g. rectal palpation, ultrasonography, Bovine Preg-Test 29[®]) after suitable interval. If low values are observed on three consecutive days, between days 16-24, oestrus may be assumed.

Measurement of Progesterone in Bovine Milk Ovucheck[®] Milk Insert

2017-01-06

The OVUCHECK[®] MILK ELISA is an immunoenzymatic test which provides a simple, reliable and precise measurement of progesterone in whole milk from dairy cows. The concentration range covered by the reagents is 1 to 20 ng/mL. Each kit contains sufficient reagents for up to 92 tests plus four standards.

OVUCHECK[®] MILK is used for oestrus detection and assessment of pregnancy status/luteal function in cows.

PRINCIPLE OF THE TEST

The OVUCHECK[®] MILK test is based on the competitive binding of unlabelled progesterone present in the standard or whole milk sample, and a fixed quantity of progesterone labelled with the enzyme alkaline phosphatase (AP) (conjugate), to binding sites on a limited amount of specific progesterone antibodies.

The wells are pre-coated with specific progesterone antibodies, providing a solid phase for the capture of the progesterone present in the samples, standards or the conjugate. After incubation, all components other than those bound to the plate wells are washed away.

The amount of bound AP-labelled progesterone remaining in the wells is inversely proportional to the concentration of the unlabelled progesterone present in the sample. The bound labelled progesterone is then measured by making the AP react with its substrate during a second incubation.

The colour produced is measured spectrophotometrically and the concentration of progesterone in the milk is determined from a standard curve. Alternatively, the colour can be interpreted visually.



MATERIAL

Components

Components	Quantity
• 12 strips of 8 wells coated with progesterone antibodies	1
• Ready-to-use progesterone standards ** (Each of: 1; 5; 10; and 20 ng/mL)	1.0 mL
• Ready-to-use conjugate-(Prog.-AP)	26 mL
• Ready to use substrate buffer***	25 mL
• Ready-to-use stop solution	20 mL
• Graph paper	1

****It is important to store the standard tubes in UPRIGHT position in order to prevent contact of the standards with the inner of the cap**

*****Substrate containing black insoluble stabilizing pellets.**

Materials Required but not Provided:

- Purified water
- Adjustable single- and multi-channel micropipettes
- Single-use micropipette tips
- Milk collection bottles
- ELISA 96-well microplate reader equipped with 405 nm filter

PRECAUTIONS

- Store the kit at 2-7°C. DO NOT FREEZE.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents from different serial numbers.
- The standards and the conjugate contain a preservative. When emptying the wells' contents into a sink, thoroughly flush away with an excess volume of tap water.
- For *in vitro* veterinary diagnostic use only. The components or their residues must not be allowed to come into contact with livestock.
- Do not pipet by mouth.
- If eyes or skin are splashed, wash thoroughly with tap water.
- The material used in this kit must be considered as infectious. Therefore, all waste must be decontaminated before being discarded.
- Dispose of the substrate and the stop solution according to local regulations for chemicals.

EXECUTION

A. Sample Collection

Milk samples for testing should be taken from whole milk (morning milking). Samples should be put into clean milk collection bottles properly identified.

If sampling by hand from the udder, avoid foremilk by discarding the first five squirts from each quarter. Then collect an equal amount from each quarter into the container.

Samples can be kept 24 hours at 2-7°C before being assayed. If samples need to be kept for more than 24 hours, they need to be frozen at - 20 ± 4°C.

B. Test Procedures

For the detection of oestrus or indication of pregnancy, the OVUCHECK® MILK assay may be used in one of two ways.

- For a visual interpretation of the colour development, use standards 5 and 10 ng/mL.
 - For a quantitative measurement of the milk progesterone level, use the four standards provided. The colour development of the samples and standards should be determined using an ELISA plate reader. For increased precision of quantitative measurements duplicates are recommended.
1. Bring all components and samples to room temperature (22 ± 3°C) before use.
 2. Homogenize milk samples, standards and reagents just before use except substrate which is important to not homogenized (cf #10)
 3. Free the microtitration plate from the protected packaging. Take the quantity of microwells needed for the test (4 wells for the standards and 1 well per milk sample to be tested). Wells can be separated from one another. Return unused microwells strips to storage at 2-7°C.
 4. To prevent any mistake, it is recommended to identify the wells used by making a schematic representation of the plate.
 5. Add 10 µL of each standard to be used to the appropriate wells.
 6. Add 10 µL of each milk sample to be tested to the appropriate wells.
 7. Immediately add 200 µL of conjugate to every used well. Take care not to contaminate the micropipette tips with the samples. Gently shake the wells to homogenize reagents.
 8. Incubate for 30 minutes at room temperature away from light.
 9. Empty wells and gently wash them with a pipette or a wash bottle using purified water at room temperature. Repeat twice more. Tap dry on absorbent paper. Do not let the wells dry completely.
 10. Add 200 µL of substrate to all wells. Take care not pipetting black pellets, pipet supernatant only
 11. Incubate for 30 minutes at room temperature away from light.
 12. Add 100 µL stop solution to all wells.
 13. Read the results at 405 nm. The reading should be done no later than 15 minutes after the addition of the stop solution. See the "RESULTS" section.
 14. At the end of testing, return components to storage at 2-7°C.

