Version 03/2018

*FAST*est® LH

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***In vitro* diagnosticum**

Test-kit for the qualitative detection of luteinising hormone (LH) in serum of the dog and the cat

INSTRUCTIONS FOR USE

Supplied By
Vetlab Supplies Ltd
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Vetlab Supplies
Specialists in Veterinary Laboratory Supplies

1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit ***FAST*est® LH** contains:
- 2, 5 or 25 test cassettes coated with monoclonal antibodies
- 2, 5 or 25 disposable plastic pipettes
- 1 instructions for use

STABILITY AND STORAGE

Store at 15–25 °C

Expiry date – see label

APPLICATION AND ABBREVIATIONS

For veterinary use only

LOT Lot number

In vitro diagnosticum

! Do not use test-kit components from different kits, lot numbers or beyond stated expiry date.

Follow instructions for use precisely

T – TEST line, **C** – CONTROL line, **LF** – Lateral flow

LIABILITY

The entire risk due to the performance of this product is assumed by the purchaser. The manufacturer shall not be liable for indirect, special or consequential damages of any kind resulting from the use of this product.

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2. INTRODUCTION

The luteinising hormone (LH) is a peptide hormone belonging to the sex hormones. It has an influence in female (facilitation and initiation of ovulation) as well as in male animals (facilitation of sperm maturation).

LH indications:

Fertility status: ovariectomised – intact (table 1)
***FAST*est® LH** can differ between fertile/intact (LH concentrations below 1 ng/ml, ***FAST*est® LH** negative) and infertile/ovariectomised (LH concentration above 1 ng/ml, ***FAST*est® LH** positive) female cats and dogs and therefore is completely suitable for evaluation of the sex status of unknown found female animals or for the control of a conducted ovariectomy.

Ovulation time point in bitches (table 2)
As an optimal starting time point for ***FAST*est® LH** testing, day 4–5 of the prooestrus (vaginal cytology: 50 % cornification, progesterone ≥ 1.5 ng/ml) is recommended. The first positive ***FAST*est® LH** (LH > 1 ng/ml) after daily measurements in the interval of 12, maximum 24 hours, marks the LH peak and thereby day zero of the cycle. Because ovulation usually occurs 2 days after the LH peak, the ovulation time (duration Ø 12–24 hours, progesterone amount ca. 4–10 ng/ml) can be exactly timed. Most important for breeders, the best date for mating (fertile period) normally follows 2–3 days later (progesterone > 10 ng/ml).

Planning whelping date in bitches
Determining LH peak, the date of birth can be predicted precisely on +/– one day by addition of 65 days of average duration of pregnancy. This enables the veterinarian and the breeder to prepare all necessary precautions for an easy and uncomplicated birth.

Therefore, ***FAST*est® LH** is qualified a reliable on-site screening test in cats and dogs for differentiation between fertile (intact ovaries) and infertile (ovariectomy or chemically castrated animals, respectively) as well as in the bitch for optimal determination of ovulation time/mating time/artificial inseminating time and for determination of expected date of whelping.

3. INFORMATION ON THE SPECIMEN MATERIAL

For determination of LH, **only serum** must be used!

For the test, approximately **150–200 µl (4 drops of attached plastic pipette) 15–25 °C warm serum (S)** are needed. Mix the sample material well before use!

Non-cooled (**15–25 °C**), S should be tested immediately or up to maximum 2 hours after sampling! At **2–8 °C**, S can be stored up to 24 hours. Serum samples can be permanently stored **at minimum −20 °C**.

Keep in mind that the sample material, as well as all used test-kit components, should have reached **room temperature** at the time of application.

Endogeneous and exogeneous interfering substances of the sample (e.g. hemoglobin, albumin, fibrinogen, lipids, CRP, heterophilic antibodies, especially type IgA, as well as viscosity, pH-value and excess EDTA) **can cause interferences** (matrix effects) **that can influence the target measurement (impaired LF and/or unspecific reactions on T and C).**

Due to red hemoglobin background of the test membrane, caused by hemolytic blood samples, the visibility of T could be from worse to not visible. Therefore, hemolysed samples should not be used.

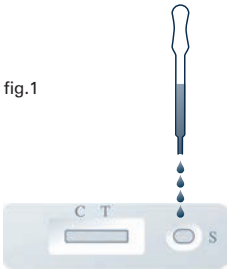
4. SPECIMEN PREPARATION

- No specimen preparation necessary.

5. TEST PROCEDURE

1. Remove the test cassette from its foil pouch shortly before use. Place it on a flat surface.
2. Take the disposable plastic pipette and express **4 drops (approx. 150–200 µl) of serum** into the sample window S of the test cassette. Hold the pipette vertically (fig.1).
3. **No need for buffer diluent!**

fig.1



6. READING OF THE TEST RESULT

20 min

Read the test result **20 minutes** after the four drops have been added into the sample window S.

The colour intensity of the TEST line in proportion to the CONTROL line is important!

POSITIVE TEST RESULT

fig.2A fig.3A fig.4B fig.5B

NEGATIVE TEST RESULT

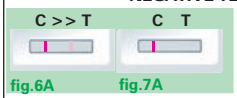


fig.6A fig.7A fig.8B fig.9B

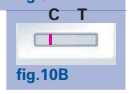


fig.10B

INVALID TEST RESULT
No CONTROL line visible. The test should be repeated using a new test cassette.

7. PRECAUTIONS FOR USERS

- Label sample material and associated test cassette to ensure a precise assignment.
- Use a new pipette for each sample.
- The sample material must be seen as potentially infectious and disposed of accordingly, together with the used test-kit components.

8. TEST PRINCIPLE

The ***FAST*est® LH** is based on an immunochromatographic "sandwich principle".

LH molecules present in the serum are binding to specific monoclonal anti-LH antibodies in the conjugate pad. These antigen-antibody complexes are migrating along the nitrocellulose membrane ("lateral flow", **LF**) and bind to fixed monoclonal anti-LH antibodies conjugated with gold particles forming a pink-purple TEST line (**T**).

A correct test procedure will be indicated by a second pink-purple CONTROL line (**C**).

9. IMPORTANT TIPS FOR TEST INTERPRETATION

- The interpretation of the test result should always be based on anamnestic and clinical data as well as the therapy and prophylaxis possibilities.
- Any non-described colour or contour variation of T and C (e.g. greyish, shadow-like lines) has to be considered as unspecific reaction and therefore as negative test result.

10. INFORMATION FOR THE INTERPRETATION

A) DOG (BITCH)/CAT (QUEEN): OVARIECTOMISED or INTACT/FERTILE?

table 1	1st LH test	test repetition with a new sample	2nd LH test	interpretation
♂ ♀	positive	after 2 h	positive	**ovariectomised**
	positive	after 2 h	negative	**intact**
	negative	optional	negative	**intact**

POSITIVE ≥ 1 ng/ml = ovariectomised (fig.2A and 3A)
Pink-purple TEST line **of greater** (2A) **or similar (a bit weaker or equal)** (3A) **intensity** than CONTROL line. To exclude pulsatile LH fluctuations in intact animals, a positive test result should be confirmed 2 hours later with a new serum sample.

***FAST*est® LH** "false positive" (LH > 1 ng/ml, but intact ovaries). Reason: Animal still in oestrus ("heat")/in silent oestrus ("silent heat"; acoustic stimulus, spontaneous GnRH release → LH ↑) → repetition of test after 2 h with a new sample

NEGATIVE < 1 ng/ml (fig.6A and 7A)
No pink-purple TEST line (7A) appears or the pink-purple TEST line is **really weaker** (6A) than the CONTROL line.

***FAST*est® LH** "false negative" (LH < 1 ng/ml, but no ovaries). Reasons: residual ovaries (incomplete removal), physiological discontinuous LH release. Cat: LH ↓ due to stress, anesthesia, sedativa. Dog: test time after ovariectomy (< 1 year significant lower LH concentration than after > 1 year) → test repetition with a new sample + vaginal cytology

B) DETERMINATION OF LH PEAK/OVULATION IN THE BITCH

table 2	before LH peak (prooestrus)		LH peak (oestrus)	after LH peak (metoestrus)	
LH test	variably negative – positive if positive, test repetition after 2 h → negative		positive test repetition after 24 h → positive	negative test repetition after 2 h → negative	
LH value ng/ml	pulsatile LH emission fluctuates between < 1 and > 1		**praeovulatory LH ↑ > 1**	LH basal values < 1	
cycle state	early prooestrus	late prooestrus	oestrus	early metoestrus	late metoestrus
progesterone value ng/ml	basal value: < 0,5	↑ to ca. 1	↑ increase > 2–4	ovulation: 4–10	after ovulation: > 10

POSITIVE ≥ 1 ng/ml (fig.4B and 5B)
Pink-purple TEST line usually **with higher** (4B) **or same** (5B) **intensity** than CONTROL line.

Note! If the requested daily ***FAST*est® LH** tests for LH peak determination (see 2. Introduction) always show a distinct but a little less intense TEST line compared to CONTROL line, this should be taken as a hint for the LH peak! In case of doubt, a progesterone test should be done in parallel to LH test and 2–4 days after the last LH test.

NEGATIVE < 1 ng/ml (fig.8B, 9B and 10B)
No pink-purple TEST line (10B) or a pink-purple TEST line **with lower intensity** (8B, 9B) than the pink-purple CONTROL line appears.