Cats Protection's LH testing protocol

Cats Protection is frequently faced with the challenge of not knowing the neuter status of female cats in the charity's care. Historically if no spay scar was identified after shaving the fur, females with unknown neuter status required exploratory laparotomy to determine whether or not they were neutered. The findings of a study[1] by Cats Protection highlights the effectiveness of the Witness LH test to determine neuter status, reducing the number of cats that require exploratory laparotomy. This not only improves cost and time efficiencies but importantly results in measurable improvements to cat welfare, avoiding surgery and reducing the time to rehoming.

[1]Cats Protection study published online first in the Journal of Feline Medicine and Surgery: Morrow LD, Gruyffydd-Jones TJ, Skillings E, Welsh Cats Protection, Murray JK (2018) Field study assessing the performance of a patient-side blood test to determine neuter status in female cats based on detection of luteinising hormone. See here.

What is LH?

LH (luteinising hormone) is a hormone, released from the pituitary gland in the brain, which plays a role in reproduction. In female cats, mating stimulates the release of LH into the bloodstream, leading to ovulation. In unneutered female cats, LH is normally maintained at low serum levels through negative feedback from the ovaries, unless coitus or spontaneous ovulation has just occurred in which case LH will rise but return to basal levels within 24 hours. Removal of the ovaries through neutering removes the negative feedback and subsequently the serum levels of LH in neutered females are higher.

What is the Witness LH test?

The Witness LH test measures the level of serum LH using a technique called Rapid Immunomigration. Levels above 1ng/ml of LH in serum is detected as a positive result and indicates that the individual has likely been neutered.

What did the Cats Protection study show?

Residual serum samples from 236 cats were blind-tested using the Witness LH test. These cats were undergoing exploratory laparotomy surgery as per usual Cats Protection protocol. Cases were recruited to the study all year round to ensure that any seasonal effects might be accounted for.

In the study, the LH test correctly detected all unneutered cats.

- **Positive results:** This study found that the LH test was 100% specific in identifying neutered females – that is all the cats that tested positive on the LH test with a single sample were correctly identified as being neutered (the positive predictive value was 1.0)

- **Negative results:** With a test sensitivity of 69%, in this study population 77% of cats that tested negative on the LH test were correctly identified as being unneutered with the remaining 23% found to be already neutered at exploratory surgery (the negative predictive value was 0.77)

The findings of this study indicate that use of this test will reduce the number of cats that require exploratory laparotomy to determine neuter status, as cats with single positive test results will not require investigative surgery.

When should I use the Witness LH test for cats of unknown neuter status in Cats Protection care?

The LH testing flowchart should be used to inform when the test should be used, as other indicators of neutered status are more practical in the first instance. For example, a missing ear tip (in the case of feral cats), the presence of behavioural signs of oestrus, pregnancy or lactation or the presence of a scar suggestive of previous neutering. Queens should have been in care and separated from entire male cats for at least 24 hours before an LH test is performed to reduce the risk of recent coitus affecting the result.

How do I perform the Witness LH test?

The LH test is performed by the veterinary practice after being stored at room temperature (between 2-25 degrees Celsius).

1. The test kit is removed from the foil packaging just ahead of use and placed on a flat level surface.

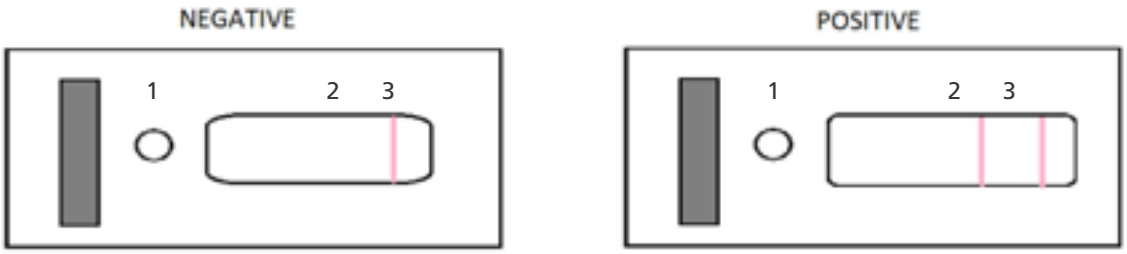
2. A serum sample is used (no buffer is required) and the serum is drawn up using the provided pipette.

3. Four drops of serum are added to the well. The pipette must be held vertically when dispensing the sample.

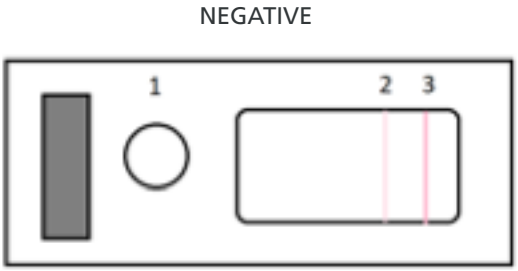
4. The test device is then left undisturbed on a flat surface and must be read 20 minutes later; a timer should be used to ensure accuracy.

A single pink line in the area marked 3 (the control line) indicates a negative result.

A pink line of similar or greater intensity to the control line in the area marked 2 indicates a positive result (see infographic below).



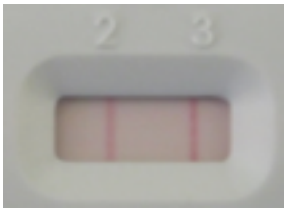
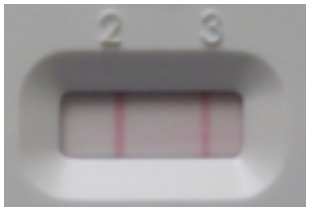
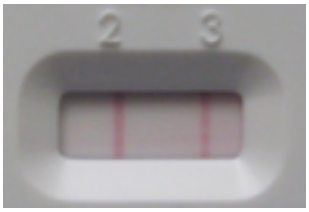
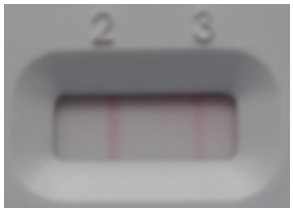
A pink line in the area marked 2 that is less intense than the control line should be interpreted as a negative result (see infographic below).



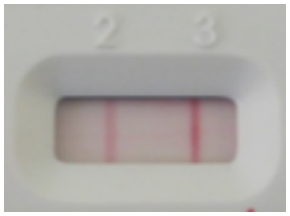
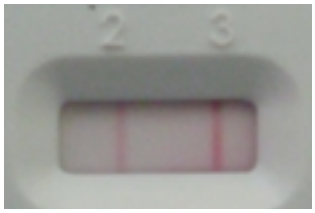
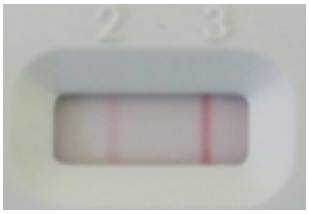
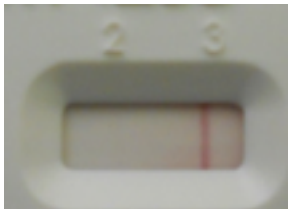
If there is no control line in the area marked 3 then the test should be repeated.

Photographic examples of test outcomes

Examples below are of a **POSITIVE TEST** indicating a neutered cat. Notice the line in area 2 can be of similar or greater intensity to the control line in area 3.

Examples below are of a **NEGATIVE TEST** indicating an unneutered cat.

Interpretation of the Witness LH test result for cats in the care of Cats Protection

• a single positive test result indicates a neutered female cat*

• a single negative test result indicates the female cat requires neutering. Please be aware that in some cases, this surgery will confirm that the cat is already neutered, as false negative results should be expected. However, use of the test will still significantly reduce the number of cats undergoing exploratory surgery

The test should be used in accordance with Cats Protection's LH testing flowchart.

*The test package insert suggests that positive test results should be repeated to avoid detecting the short sporadic LH surge that occurs around ovulation. However, the Cats Protection study showed that when used in field conditions, a single positive test result was always reliable. Therefore, for pragmatic reasons, in the Cats Protection procedure there is no need to repeat the test. Adopters will be informed of the test result with guidance to monitor for signs of oestrus behaviour.

How do I order Witness LH test kits?

Witness LH test kits are available from veterinary wholesalers in packs of six.

Please order the Witness LH test kit from Covetrus using the product code 'PFWIT01', description 'Witness LH Test 6 Test'. Shelf life is approx. 12 months (please check individual boxes).

Informing the adopter

Adopters should be informed of the procedure that has been carried out on their cat in order to ascertain neuter status. The results should be clearly stated on the Cats Protection Medical Summary Form.

A handout is available for adopters.

Guidelines for fractious cats

If a cat is too fractious to tolerate conscious shaving to look for a scar, or becomes very stressed during this process, Cats Protection would recommend the following approach:

• pre-medicate the cat with a suitable pre-med to enable blood sampling and shaving (as appropriate for the cat, assess on an individual basis)

• take a blood sample and spin blood down

• in the meantime, shave left flank, midline and then right flank to look for a scar

• if scar present, assume neutered

• if no scar present or uncertain, perform single LH test

• single test positive – assume neutered, no need to repeat test

• single test negative – proceed to full general anaesthetic for neutering/exploration

In order to make this process as time efficient as possible, you may wish to start prepping the cat for surgery while the LH test is running. This is at the attending vet's discretion.

If the cat is unstable under pre-med and you don't feel it is in the cat's best interest to wait for the LH test to run, you may wish to proceed straight to general anaesthesia for neutering/exploration. This is at the attending vet's discretion.

If you feel that a cat is too fractious to shave/blood sample under pre-med, consider the use of an appropriate injectable general anaesthesia combination in the first instance. This should be assessed on a case by case basis and is at the attending vet's discretion.