Performing the Speed Duo FeLV/FIV® Test

Equipment needed for one test:
One test device
One single-use pipette
One bottle of reagent

1. SAMPLING
Type of sample: Serum, plasma or whole blood with anticoagulant.
Storage of samples: Serum, plasma:
   - At room temperature: 24 h
   - Between 2°C and 8°C: 48 h to 7 days
   - At -20°C: several months.
Whole blood with anticoagulant:
   - At room temperature: 24 h
   - Between 2°C and 8°C: maximum 72 h.

2. PREPARATION
- Using the pipette, place one drop of the sample into each sample well.
- Place 5 drops of reagent into each sample well.

3. READING
Time to reading: **15 minutes** of migration*
The result appears in the result window.

![Diagram of test bands]

- **Test negative for both FIV and FeLV**
- **Test positive for FIV alone**
- **Test positive for FeLV alone**

If no control band appears in the result window within the allotted time for migration, the test is invalid.

*The appearance of a test band after only 10 minutes of migration is indicative of a positive test

4. INTERPRETATION

**FeLV:**
- A positive result associated with indicative clinical signs of leucosis makes infection with FeLV highly likely.
- Vaccination does not interfere with the results of the FeLV test.

**FIV:**
- A positive result associated with indicative clinical signs makes infection with FIV highly probable.
- In kittens under 6 months of age and born to an infected queen, the persistence of colostral antibodies may give a positive result even if the kitten is free from infection.

**FeLV & FIV:**
- In areas of low prevalence and in the absence of indicative clinical signs, positive results should be confirmed with a second test. However, the predictive value of a negative result in such cases is excellent.
Guidance Notes for Speed Duo FeLV/FIV® Tests

How to use the Speed DUO test:

Here are a few practical tips to ensure the tests are run correctly and to minimise false readings:

- Tests should be at room temperature when used. If the test has been stored somewhere cold it should warm to room temperature before use.
- Do NOT allow the tests to freeze - be careful if stored in the fridge as the lower areas of a fridge can easily be below freezing.
- Do NOT store above 30°C.
- Ensure the reagent bottle matches the test kit - do NOT use reagents from other kits as each batch is carefully calibrated and matched.
- The instructions must be followed carefully - correct number of drops using reagent or the pipette supplied. Do NOT use a syringe with the needle removed to drop the blood as the volume of the drop is more and may “flood” the test.
- Hold the pipette vertical when drops are applied to the test kit.
- It is very important the test is read at 15 minutes and not any longer. If the test is left for too long then a faint positive line may appear producing a false result.
- Ensure the test is run on a flat surface otherwise migration of the liquids will not occur properly.
- The control line must be visible as a definite PINK line at 15 minutes otherwise the test is invalid.
- If a positive PINK line appears at both the control and the test line before 15 minutes it can be considered a POSITIVE result.
- The control and the test line must be pink in colouration even if it is a weak line - see images.

NEGATIVE

POSITIVE – pink colouration present on the test line

- Serum, plasma or anti-coagulated blood should be used. Whole blood sometimes produces a very weak GREY line that should NOT be considered a positive line. To be positive there must be PINK colouration present at both the test and control lines. Whole blood is, therefore, not recommended.
- Do NOT use tests beyond the expiry date.
- The clinical presentation of the cat must be considered when interpreting the results of tests.

Why do false positives and negatives occur?

By the nature of diagnostic testing false results are always possible. Tests have been designed to be both sensitive and specific for certain diseases but no test should ever be considered 100% accurate and the whole clinical presentation must be taken into account when diagnosing the patient.

Discrepancy between in-house and external laboratory tests:

- False positives and negatives are possible with any FeLV/FIV test whether it’s an in-house or an external laboratory test. Reasons are numerous and can be due to the complexity of the test - e.g. Western Blot or PCR tests involve several complicated stages.
- Different tests may look for different aspects of the disease, which may occur at various time points following infection. For example, shortly after a cat is infected with FeLV there may be detectable antigen present in the blood but within a few weeks the FeLV may have been cleared demonstrating a transient antigenaemia and not a persistent infection.
- With FIV the tests commonly detect antibodies and if blood is sampled shortly after infection or in the terminal stages of the disease the levels of antibodies may be too low to detect.
- Sample handling is important as virus may be destroyed before the sample can be used for testing, like virus isolation.
- In-house tests are essentially screening or preliminary tests that have been designed to have high sensitivity that is then confirmed by a more specific test run at external labs.
- Other factors have to be considered such as the background of the patient being tested. In terms of probability it is complicated and delves into the world of epidemiology, which is never simple to explain. But the greatest influence on whether the positive result is true is based on the local prevalence of the disease in that particular population. Essentially a positive test in a sick feral cat is more likely to be true than a positive in a healthy pet cat.
- Hence a positive test result in a healthy cat should be confirmed by an alternative test given the low prevalence of FeLV/FIV in healthy cats.