Notes (for explanatory notes on tests, see overleaf)

1. If there is a history of FIV vaccination (a vaccine is currently (2020) available in Asia and Australia), an uninfected cat could test antibody positive.

2. In some circumstances, for example in sick cats showing signs that can be associated with FIV infection &/or cats that are known to be at risk of FIV infection, the likelihood of a true positive test result is higher, so confirmation of a positive result with additional testing could be omitted, especially in situations of cost or time limitations. This does not apply to cats under 6 months of age (who should be retested after that age).

3. PCR is used to confirm (but not exclude) infection. Sensitivity of PCR depends on design of the assay(s) and ability to detect FIV subtype(s) that could be present in the cat being tested (geographical variation in subtypes).

4. A positive PCR result in the queen (mother) would confirm that the assay detects the subtype most likely to have infected her kittens (MDA = maternally derived antibodies, can last up to 6 months).

NOTE Confirmatory FIV antibody detection can be carried out by Western blotting and immunofluorescence (IF) testing as well as plate ELISA.

POCT = point-of-care-test
How can FIV infection be detected?

(a) **point-of-care tests (POCT)**, which are usually ELISA or immunochromatography-based, for the detection of antibodies against FIV in blood

(b) **laboratory assays** to confirm POCT results; plate ELISA, immunofluorescence (IF) assay or Western blotting, which all detect antibodies against FIV in blood

(c) **molecular methods (PCR)** for the detection of proviral DNA (viral RNA integrated as DNA) in blood

(d) **virus isolation** from heparinised whole blood for the detection of replicating virus

With a few exceptions (see footnote 2 overleaf), all positive POCT results should be confirmed for FIV infection by a diagnostic laboratory using another antibody test (b) or a test for virus (c, d), as false positive POCT results can occur. Confirmatory tests include antibody tests (immunofluorescence, plate ELISA, Western blotting), PCR and virus isolation.

**Antibody tests** are most commonly used for FIV diagnosis. All FIV tests that detect antibody (a, b) can be influenced by the presence of maternally derived antibodies (MDA). Kittens born to an FIV-infected queen become infected only rarely, but they will test positive for FIV antibodies because of the MDA that are present in their blood. Therefore, any kitten under the age of 6 months that tests positive for FIV antibodies should either be:

1. re-tested immediately using a test that detects proviral DNA (c) or replicating virus (d).
2. re-tested when older than 6 months of age, when MDA should have declined to undetectable levels.

Also, imported cats that have been vaccinated against FIV in other countries could test positive by tests that detect antibody (a, b) and such cats should be re-tested immediately using a test that detects proviral DNA (c) or replicating virus (d).

**Polymerase chain reaction (PCR)** (c) detects viral nucleic acid sequences. When FIV infects a cell, a DNA version of the viral RNA becomes incorporated into the cat host cell genome, known as provirus. PCR can detect this FIV proviral DNA even at very small amounts of viral genetic material and is therefore very sensitive. However, common variations in the FIV genome can lead to false negative PCR results. Additionally, recombination between different FIV subtypes might further confound diagnosis by PCR.

Reverse transcriptase polymerase chain reaction (RT-PCR) is only used for research purposes for the detection of FIV RNA in blood as the viral loads in infected cats are generally too low for detection.

**Virus isolation** (d) identifies replicating infectious virus in the blood by culturing peripheral blood mononuclear cells. However, it is difficult, time-consuming, requires special facilities and is not widely available. It is therefore only recommended for confirmation of positive FIV antibody test results.

Plate ELISA is reserved for diagnostic laboratories and can be used to confirm a point-of-care test (POCT).