Utility of feline coronavirus antibody tests

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Abstract

Eight different tests for antibodies to feline coronavirus (FCoV) were evaluated for attributes that are important in situations in veterinary practice. We compared four indirect immunofluorescent antibody tests (IFAT), one enzyme-linked immunosorbent assay (ELISA) (FCoV Immunocomb; Biogal) and three rapid immunochromatographic (RIM) tests against a panel of samples designated by consensus as positive or negative. Specificity was 100% for all but the two IFATs based on transmissible gastroenteritis virus (TGEV), at 83.3% and 97.5%. The IFAT and ELISA tests were best for obtaining an antibody titre and for working in the presence of virus. The RIM tests were the best for obtaining a result quickly (10–15 mins); of these, the Speed F-Corona was the most sensitive, at 92.4%, followed by FASTest feline infectious peritonitis (FIP; 84.6%) and Anigen Rapid FCoV antibody test (64.1%). Sensitivity was 100% for the ELISA, one FCoV IFAT and one TGEV IFAT; and 98.2% for a second TGEV IFA and 96.1% for a second FCoV IFAT. All tests worked with effusions, even when only blood products were stipulated in the instruction manual. The ELISA and Anigen RIM tests were best for small quantities of sample. The most appropriate FCoV antibody test to use depends on the reason for testing: in excluding a diagnosis of FIP, sensitivity, specificity, small sample quantity, rapidity and ability to work in the presence of virus all matter. For FCoV screening, speed and sensitivity are important, and for FCoV elimination antibody titre is essential.

Introduction

There are several reasons for testing cats for antibodies to feline coronavirus (FCoV), and a number of different tests may be used for this purpose. The choice of which to use depends on the reason for the examination. In this study, we compared several FCoV antibody tests for their precision (ie, specificity and sensitivity) and certain other important attributes. Based on the results, we suggest how each might be used most appropriately in a variety of situations in clinical practice.

FCoV is a common infection of cats with a tropism for the epithelial cells of the gut and for monocytes/macrophages.[1,2] Most FCoV-infected cats remain asymptomatic, but up to 10% develop a perivascular pyogranulomatosis known as feline infectious peritonitis (FIP),[1,2] which is almost always fatal. Infected cats, whether asymptomatic or manifesting clinical disease, usually mount an immune response, and antibodies to the virus are found in their blood. If a cat recovers and ceases to excrete virus, the antibodies decline and may disappear altogether.[2,3]

Tests for FCoV antibodies have been available for almost 40 years.[4] Today, commercially available tests fall into one of three categories: indirect immunofluorescent antibody tests (IFAT) using cells infected with FCoV[4,5] or the related porcine transmissible gastroenteritis virus (TGEV)[6–8] as the antigen; enzyme-linked immunosorbent assays (ELISA)[9–11]; or rapid immunochromatographic (RIM) tests. The ELISA and RIM formats are available for use in-house. A fourth method,

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immunoblotting, is only available commercially in specialised laboratories.

Several factors should be considered when choosing a test. The appropriate choice in any situation depends on the reason for determining the FCoV antibody status of the cat. For example, where a fast result is required, in-house tests are usually preferred (eg, for eliminating FIP as a diagnosis in a sick cat, or for screening a breeding queen immediately before mating). Where sequential tests are required (eg, to ascertain when a cat is no longer infected), a test that provides an antibody titre is desirable.

Unfortunately, in the minds of many clinicians, FCoV antibody (or, indeed, FCoV reverse transcriptase polymerase chain reaction [RT-PCR]) testing is misunderstood to be a test for FIP, a mistake encouraged by the mislabelling of some tests as 'FIP' tests instead of 'FCoV' tests. FCoV antibody testing is used more often in the elimination of FIP as a diagnosis than for any other use. FIP is usually classified as effusive ('wet') or non-effusive ('dry'). These definitions are useful but not rigid; for example, a non-effusive case may become effusive as the disease progresses.[1,2] In consequence, FIP is a clinical challenge, with a similar presentation to many other diseases. In cats suspected of suffering from dry FIP, the list of differential diagnoses is especially long, and a great many clinical pathology tests may have to be performed upon a sample before a diagnosis can be achieved. Consequently, in this case an antibody test that requires only a small volume of sample can be advantageous. It is useful to be able to utilise the effusion in wet FIP, as it is often available in large quantities. Another factor to be considered is that, as we showed previously,[12,13] the presence of a large amount of virus in a sample can reduce, or even block, antibody detection.

Taking these issues into consideration, we identified five desirable qualities in a FCoV antibody test: high sensitivity; high specificity; a requirement for a small quantity of sample; the ability to use effusion, as well as whole blood, plasma or serum; and the sensitivity of the test in the presence of virus. In addition, two other qualities in tests may be required for the purpose of screening cats: the speed of the result and the determination of an antibody titre.

The study then evaluated the utility in clinical situations of some of the most commonly used FCoV antibody tests.

Materials and methods

FCoV antibody tests

A number of commercial veterinary laboratories and FCoV antibody test manufacturers were approached and offered the opportunity to take part in this study: some refused or sent insufficient test devices to give statistically significant results. Assessment was blinded and was strictly confidential: manufacturers and laboratories were given the option not to be included in this publication once they had seen the results.

Four laboratories offering an IFAT participated. Two used feline cells infected with FCoV: Biobest Laboratories (Penicuik, UK) and Veterinary Diagnostic Services (University of Glasgow, Glasgow, UK). Two used cells infected with TGEV: Clinical Laboratory, Vetsuisse Faculty (University of Zurich, Switzerland) and UMR 1161-Virologie-INRA-ENVA-ANSES (Maisons-Alfort, France). Each of these tests provided an antibody titre.

One ELISA was studied: FCoV Immunocomb (from Biogal Galed Labs).[9,10] This test produces grey spots that can be read in an ordinary photograph scanner, with software provided by the manufacturer.[9] Results are provided on a scale of 1–6, depending on the intensity of the colour of the spots, which, in a previous study, correlated well with FCoV IFA titres.[10] The absence of a spot gave a result of zero, correlating with FCoV IFAT titre of <1:10, which was deemed to be negative. In a previous study,[10] the spots were read by eye, but in this study they were read using a scanner (Epson 4000), which increased the precision of the tests, that is the repeatability of the results, as the analysis of scanned images is probably more reproducible and objective than a visual analysis that is operator dependent and may also suffer from variables (eg, sources of light).

Three RIM tests were compared: Speed F-Corona (BVT); FASTest FIP (MegaCor Diagnostik); and Anigen Rapid FCoV Ab Test Kit (Bionote). The manufacturers of the latter two do not indicate on their instruction sheets that the tests were suitable for use in effusions. The RIM tests were given subjective assessments of the intensity of the signal, ranging from 0 for a complete absence of a band in the test zone; 1 for a distinct, but not strong, positive result; 2 for a strong signal; 3 for an intense signal; and 4 for a band greater than the control band. Very faint, or 'ghost', lines were subjectively allocated values of <1 but >0. All three RIM tests were tested in batches, in parallel, under the same laboratory conditions as each other, according to the manufacturers' instructions.

FCoV RT-PCR

FCoV quantitative RT-PCR tests were performed as previously described at the veterinary faculties of the Universities of Bari, Glasgow and Zürich, and the École Nationale Vétérinaire d'Alfort (ENVA).[12,14]

Sample panel

The samples originated from naturally infected cats, some of which were healthy, while others were sick with FIP or another condition in which FIP was suspected. Samples were stored at −80°C or −20°C. The panel contained 101 positive samples and 126 negative samples. Not all samples were tested by each test.

Test systems are often evaluated by comparison with a reference 'gold standard'.[15] However, as Enoe et al[16] have pointed out, a reference test is often less than perfect. The gold standard in FCoV antibody tests is generally regarded as the IFAT. However, as we found in this and a previous study,[17] IFATs from different laboratories do not always give the same result on any one sample. Therefore, to assess the sensitivity and specificity of a test, a panel of samples on which a consensus of results had been obtained was chosen in preference to a gold standard. Each sample was carefully characterised individually as FCoV antibody-positive or negative.

The negativity and positivity of samples in the panel had previously been determined.[12,17] Assessing the sensitivity of a FCoV antibody test requires the ability to determine accurately when a false-negative result has occurred. The sample panel included some challenging positive samples that gave false-negative or inappropriately weak-positive results on one or more FCoV antibody tests owing to the presence of large amounts of coronavirus in the sample. Seventeen of these samples were described in detail in a previous study in which we showed that the presence of increasing amounts of coronavirus in the sample correlated with an increased possibility of unexpectedly low, or false-negative, results in FCoV antibody tests.[12] Therefore, samples that gave conflicting results in our antibody test comparison were tested further by FCoV quantitative RT-PCR.

The panel also enabled us to obtain an accurate picture of specificity. As described previously,[17] we found that some tests, especially IFATs using TGEV-infected cells, could produce false-positive results for some samples owing to the presence of antinuclear antibodies.

Most samples were easily characterised, with all tests giving either a positive or negative result. However, the panel also contained some complex samples in which different tests gave different results. The challenge was then to determine the true result for the sample, discovering which test results were falsely positive and which falsely negative. Interpretation of the result of a diagnostic test depends not only on the actual test result(s), but also on information external to this result; this external information must be combined with the data to yield the so-called updated, posterior estimates of the true test characteristics.[18] An example of external information pertinent to this analysis would be knowledge of whether or not the sample contained virus (see below).

Great lengths were taken to give each laboratory or test the benefit of the doubt. For example, if a laboratory gave a positive result on a sample that other tests found to be negative, another aliquot of the sample was submitted to the laboratory; and if the second aliquot was negative, the first result was considered to be a false-positive. However, if the second result was also positive, it was considered that the test could possibly be more sensitive than the other tests. This approach was especially important in tests of some high virus samples in which the FCoV Immunocomb ELISA initially appeared to give false-positive results,[12] when, in fact, it was detecting antibodies that some other tests failed to detect.

To solve the problem of classification of samples that gave conflicting results across a variety of tests, a Bayesian approach was used to calculate the probability that a sample really contained anti-FCoV antibodies when tested on one, two or more independent antibody tests. The probability that a sample was truly positive was calculated using the following equation:

$$P\,(Pos)\,=\,\frac{(Sensitivity\ of\ test)\,\times\,(TP)}{(Sensitivity\,\times\,TP)\,+\,(FP\,\times\,TN)}$$

where P (Pos) is the probability that a positive signal really indicates presence of antibodies; TP is a true positive; FP is a false positive; and TN is a true negative

To calculate the probability of a test giving a false-positive result on two or more independent antibody tests, the figure(s) for TP in the previous test(s) were used. Owing to 100% specificity in most of the FCoV antibody tests (ie, FP was zero) the probability that a positive result was correct was 100% for most tests. Thus, if a sample tested positive by four kinds of IFAT, one ELISA and two RIM tests, despite being negative on one RIM test, the chance that it was giving a false-positive result on all seven tests was zero. For the two tests with <100% specificity, the probability of a sample really being positive when it gave a positive signal on both tests was 98%; however, no sample was categorised based only on results from those two tests.

Because different tests utilise different dilutions of sample, and therefore generate differing antibody titres, for the purposes of clarity, the samples were further categorised relative to consensus IFAT as negative; borderline positive; low positive; moderate positive; high positive; or very high positive, as shown in Table 1, and, accordingly, were given a score of 0–5, as previously described.[12] All samples which titrated beyond a dilution of 1:1280 were considered very high.

Sensitivity and specificity determination

Sensitivity was determined using the following equation, where 'true positive' means correctly identified as positive:

$$Sensitivity\,(\%)\,=\frac{True\ positive\ (TP)\times100}{TP+False\ negative}$$

Specificity was determined using the following equation, where true negative means correctly identified as negative:

$$Specificity\,(\%)\,=\frac{True\ negative\ (TN)\times100}{TN+False\ positive}$$

Table 1 How FCoV immunofluorescent antibody titre correlates with FCoV shedding

	Classification of panel of samples	Corresponding IFAT titre	Likelihood of shedding virus*
0	Negative	<1:10	~10%
1	Borderline positive	10 to 25	~15%
2	Low positive	26 to 99	33%
3	Moderate positive	100 to 399	60%
4	High positive	400 to 1280	75%
5	Very high positive	>1280	75%

Some samples titrated out to dilutions >1:10,240. Consequently, samples that titrated beyond 1:1280 were considered to be very high and allocated number '5'. This table shows that the higher the FCoV antibody titre, the greater the chance of the cat shedding FCoV and that, importantly for the purposes of using FCoV antibody tests in screening and quarantine of uninfected cats, cats with low and moderate antibody titres have a significant chance of shedding virus

IFAT = immunofluorescent antibody test

*Based on Addie and Jarrett[3] and Pedersen et al[21]

Results

FCoV antibody test results
Table 2 shows a summary of the tests that were included in the study.

Sensitivity and specificity
The sensitivity and specificity of each test is shown in Table 3.

FCoV Immunocomb ELISA
As shown in Table 3, all of 121 negative and 78 antibody positive samples were correctly identified.

FCoV IFAT (Biobest)
Biobest laboratory is able to detect when samples give non-specific fluorescence, and reports on the fact if requested in advance, otherwise reporting the result as negative.

Sensitivity was 96.2%: two samples with very low titres gave negative results.

FCoV IFAT (University of Glasgow)
The University of Glasgow reports when samples are non-specific and has the ability to offer Western blot confirmation.

TGEV IFAT (ENVA)
This laboratory reports when samples fluoresce non-specifically and recorded non-specific fluorescence in 11 samples. One false-positive was reported; one sample with a moderate titre was falsely negative and one sample of low titre was reported as non-specific.

TGEV IFAT (Vetsuisse Faculty, University of Zurich)
Two negative samples were reported with antibody titres of 25, giving a specificity of 83.3%. This laboratory reports when samples fluoresce non-specifically.

Speed F-Corona antibody RIM test
The test correctly identified 46 samples as negative. For one sample, the control band did not show, so although

there was a band in the test area, it could not be counted. (The same sample behaved the same in the FASTest FIP device, which is why those two test numbers total only 99, not 100.) Faint lines in the test window were given scores of <1 (the instruction manual states that these should be counted as positive results). This test was the most sensitive of the RIM tests, at 92.4%, although 10 of the test devices gave weak signals scored <1. A breakdown of the results for 53 positive samples is shown in Table 3.

FASTest FIP antibody RIM test
Although the manufacturer's instructions state that only blood, plasma or serum samples should be used, this test was found also to work well on effusions. As for the Speed F-Corona device, for one sample the control band did not show, so although it showed a band in the test area, it could not be counted. A breakdown of the results for 52 positive samples is shown in Table 3: sensitivity was 84.6%, although five of the test devices gave signals <1.

Anigen Rapid FCoV (antibody RIM test)
A breakdown of the results for 53 positive samples is shown in Table 3: sensitivity was 64.1%, although eight of the test devices gave signals <1. In the absence of manufacturer's instructions to the contrary, very faint results were counted as positive rather than negative.

FCoV RT-PCR testing of the sample panel
Financial constraints precluded testing the entire sample panel for FCoV by quantitative RT-PCR, but this was performed on 59 samples, of which 27 samples were positive (see Table 4) and 32 were negative. There were two reasons for RT-PCR testing. First, to try to reveal if any false-negative results occurred across all of the antibody tests owing to the presence of virus, which is known to occur even in IFAs;[12,13] and, second, to determine how the presence of virus affected test sensitivity, especially in samples giving false-negative results on some tests. Of 47 antibody-positive samples screened by RT-PCR, 27 were positive for

Table 2 Volume of sample required for each test and whether effusion can be used

Test	Type of test	Antigen	Amount of sample (µl)	Performing the test	Time to result*	Antibody titre?	Can effusion be used?
FCoV Immunocomb	ELISA	FCoV	5	In-house	40 mins	Yes	Yes
Anigen Rapid FCoV	RIM	Unknown	10	In-house	10 mins	No	Not advised in instruction booklet, but does work to some extent with effusion
Biobest	IFAT type II FCoV	Whole virus (FCoV type II) in CRFK cells	25	External laboratory	>1 day	Yes	Yes
VDS Glasgow	IFAT type II FCoV	Whole virus (FCoV type II) in feline embryo A cells	25 (ask for 1 ml sample but can do the test with much less)	External laboratory	>1 day	Yes	Yes
Speed F-Corona	RIM	Recombinant TGEV N	40	In-house	15 mins	No	Yes
FASTest FIP	RIM	Recombinant FCoV antigens	40	In-house	10 mins	No	Not advised in instruction booklet, but works well with effusions
Zurich	IFAT TGEV	Whole virus (TGEV Purdue) in porcine kidney cell line PD5	50 of plasma/ serum requested; 10 used (the rest is in case the test has to be repeated)	External laboratory	>1 day	Yes	Yes
ENVA	IFAT TGEV	Whole virus (TGEV) in porcine kidney cells	100	External laboratory	>1 day	Yes	Yes

The amount of sample each test requires is shown: the tests are shown in ascending order of quantity of sample required
VDS = Veterinary Diagnostic Services; ENVA = École Nationale Vétérinaire d'Alfort; ELISA = enzyme-linked immunosorbent assay; N = nucleocapsid antigens; RIM = rapid immunochromatographic; IFAT = immunofluorescent antibody test; TGEV = transmissible gastroenteritis virus
*Including shipment

viral RNA. The results of sensitivity in the presence of virus for each test examined are shown in Table 4.

It is usual for blood samples to be negative for virus, but, interestingly, 10/23 (43%) antibody-positive effusions from FIP cases were negative. Four of these samples were from cats confirmed by histopathology as having FIP, while the other cases were diagnosed as having FIP by being positive on most parameters of an FIP diagnostic algorithm.[19]

Amount of sample required and whether an effusion can be used

The amount of sample each test required and whether the test worked on effusions, as well as whole blood or serum or plasma, are shown in Table 2. The ELISA and Anigen Rapid FCoV RIM test required the least amounts of sample (5 µl and 10 µl, respectively). The FASTest FIP

and Anigen Rapid FCoV RIM tests were quickest, with results available within 10 mins, with the Speed F-Corona a close second with results available in 15 mins. The ELISA took upwards of 40 mins to perform. IFATs require to be mailed, so it took >1 day to obtain results. However, the IFATs and ELISA had the added benefit of giving an antibody titre.

Discussion

This study differed from the usual assessment of diagnostic tests in that we rigorously defined each individual sample on our test panel as being positive or negative, rather than arbitrarily deciding that one FCoV antibody test was the gold standard and then assessing the other tests relative to that. The sensitivity and specificity of each FCoV antibody test were measured relative to the sample panel results. The gold standard in FCoV antibody

Table 3 Sensitivity and specificity of eight FCoV antibody tests

	IFAT FCoV		IFAT TGEV		ELISA	RIM					
	Biobest	VDS Glasgow	ENVA	Zurich	FCoV Immunocomb	Speed F-Corona	<1	FASTest FIP	<1	Anigen Rapid FCoV	<1
Very high (5.0)	100.0 (11/11)	100.0 (15/15)	100.0 (25/25)	100.0 (18/18)	100.0 (18/18)	93.3 (14/15)	2	93.3 (14/15)	1	93.3 (14/15)	2
High (4.0–4.5)	100.0 (15/15)	100.0 (14/14)	100.0 (16/16)	100.0 (13/13)	100.0 (26/26)	94.7 (18/19)	2	94.7 (18/19)	2	73.7 (14/19)	3
Moderate (3.0–3.5)	100.0 (18/18)	100.0 (16/16)	90.0 (9/10*)	100.0 (2/2)	100.0 (24/24)	93.3 (14/15)	3	73.3 (11/15)	1	33.3 (5/15)	2
Low positive (1.0–2.5)	77.8 (7/9)	100.0 (7/7)	100.0 (5/5)	100.0 (3/3)	100.0 (10/10)	75.0 (3/4)	3	33.3 (1/3)	1	25.0 (1/4)	1
Sensitivity (positive/total)	96.1 (50/52)	100.0 (52/52)	98.2 (55/56)	100.0 (36/36)	100.0 (78/78)	92.4 (49/53)	–	84.6 (44/52)	–	64.1 (34/53)	–
Specificity (Negative/total)	100.0 (53/53)	100.0 (69/69)	97.5 (39/40)	83.3 (10/12)	100.0 (121/121)	100.0 (46/46)	–	100.0 (46/46)	–	100.0 (47/47)	–

Results are given as percentages, with the total number of samples analysed in brackets. To give an idea of the disparity of the intensity of signals relative to the true result, the number of positive RIM tests in which the signals were extremely faint is given in the columns headed <1. Low and borderline positive samples were combined to achieve adequate numbers

IFAT = immunofluorescent antibody test; TGEV = transmissible gastroenteritis virus; ELISA = enzyme-linked immunosorbent assay; RIM = rapid immunochromatographic; VDS = Veterinary Diagnostic Services; ENVA = École Nationale Vétérinaire d'Alfort; FIP = feline infectious peritonitis
*When the one false negative sample was resubmitted, a positive result was obtained

Table 4 FCoV antibody test sensitivity of samples containing virus

Test	IFAT FCoV	IFAT FCoV	IFAT TGEV	IFAT TGEV	ELISA	RIM	RIM	RIM
	VDS, Glasgow	Biobest	ENVA	Zurich	FCoV Immunocomb	Speed F-Corona	FASTest FIP	Anigen Rapid FCoV
Sensitivity %	100.0	100.0	100.0	100.0	100.0	84.6	84.6	53.8
Number of samples	10/10	7/7	24/24*	19/19	14/14	11/13	11/13	7/13

The number of virus-positive samples subjected to each test is shown. Results are given as percentages, with the total number of samples analysed given in the second row
IFAT = immunofluorescent antibody test; TGEV = transmissible gastroenteritis virus; ELISA = enzyme-linked immunosorbent assay; RIM = rapid immunochromatographic; VDS = Veterinary Diagnostic Services; ENVA = École Nationale Vétérinaire d'Alfort
*The re-sampling of this sample has been counted, discounting the false-negative in the initial test

Table 5 Uses of FCoV antibody testing

	Reason for testing	Positive result	Negative result	Desirable criteria in FCoV antibody test and other comments
1	To rule out a diagnosis of effusive FIP	Does not necessarily indicate FIP, as many sick cats may be seropositive for FCoV owing to a prior transient infection	Rules out FIP unless test is insensitive or inhibited by large amounts of virus	Good sensitivity: because a negative result is more useful than a positive. Specificity is also important to avoid erroneous FIP diagnoses. Useful to have test which works on effusion. Essential that the test works in presence of large amounts of virus
2	To rule out a diagnosis of non-effusive FIP	Does not necessarily indicate FIP, as many sick cats may be seropositive for FCoV owing to a prior transient infection	Rules out FIP unless test is insensitive	Sensitivity matters, although most dry FIP cases have a very high antibody titre. A negative test is more useful than a positive result. Specificity is very important to avoid mistaken FIP diagnoses. Using a small amount of sample is an advantage
3	To monitor FIP treatment	Re-test in 2–3 months; continue treatment, gradually reducing corticosteroid dose, if applicable	Provided clinical signs, haematology and biochemistry parameters have returned to normal, it is now safe to discontinue treatment	Less useful than monitoring AGP, globulin, lymphocyte count and haematocrit in early stages, but good for knowing when to stop treating a recovered cat. FCoV antibody titre essential: hoping for decreasing titre if treatment effective. Not useful to test more frequently than every 8–12 weeks. Bear in mind that high doses of steroids can artificially reduce the FCoV antibody titre, so interpret results with caution
4	Diagnosis of FCoV associated with gastrointestinal signs	The clinical signs may be related to FCoV infection, but as many cats with diseases other than coronaviral enteritis will also be seropositive, other parameters must be examined and differential diagnoses carefully eliminated	Provided the test is sensitive enough and that the kitten or cat had time to seroconvert (ie, at least 3 weeks) FCoV or FIP are unlikely to be the causes of the gastrointestinal signs	Negative result is more useful than positive; therefore, good sensitivity essential. A positive test can be followed up with RT-PCR of the faeces to establish if virus is being shed
5	To check cats in contact with FIP case or known FCoV excretor	Positive result expected, especially where litter trays are communal. Re-test every 2–3 months until negative. Minimise virus dose exposure and stress, and optimise nutrition to try to avert development of FIP	No risk of FIP (unless test has poor sensitivity) and safe to introduce a new cat	Guardians of an FIP cat often have two questions about the survivor(s): 1 Will the survivor(s) develop FIP? As the chances are that the cat will be seropositive and will be monitored, a test that gives a FCoV antibody titre is best first choice 2 Is it safe to get another cat or is the survivor going to infect him or her (see row 6)?
6	Screen household of cats before bringing in a new kitten or cat	If the cats in the household are seropositive, it is not advisable to introduce another cat, especially if it is FCoV seronegative	If the cats in the household are negative, it is safe to bring in a seronegative cat but not a seropositive one	Any kind of test can be used, provided sensitivity and specificity are good. If positive – see row 8 for how to proceed

(Continued)

Table 5 (Continued)

	Reason for testing	Positive result	Negative result	Desirable criteria in FCoV antibody test and other comments
7	Screen cat before entry into multi-cat household or FCoV-free country	If the cats in the household are negative, it is not advisable to admit a seropositive cat	If the cats in the household are seropositive, it is not advisable to admit a seronegative cat	In-house positive/negative tests suitable for this purpose, provided adequately sensitive
8	In a household in which FCoV is being eliminated FCoV antibody testing is used to separate infected and uninfected cats	Re-test antibody titre in 2–3 months (in kittens <6 months old, re-test in 1 month). Consider testing faeces by RT-PCR	This cat has eliminated FCoV, provided the test was sensitive enough. Separate the cat from cats that are still seropositive	Antibody titre essential for this purpose: the higher the antibody titre, the greater the chance that the cat is shedding virus. The seropositive cat has an approximately 1 in 3 chance of being actively infected (ie, 66% of seropositive cats are actually not shedding virus at any one time)
9	Screen stud cat prior to mating	May be shedding FCoV; therefore, use controlled matings to prevent virus transmission.* Faeces can be tested for virus	FCoV-free (provided test is sensitive enough); therefore, preferably use FCoV-free queen	Speed may be required. In-house positive/negative tests suitable for this purpose, provided adequately sensitive
10	Screen queen cat prior to mating	May be shedding FCoV; therefore, delay mating until cat becomes FCoV-free or use controlled matings to prevent virus transmission. Take steps to prevent infection of kittens. Test faeces for virus	FCoV-free (provided test is sensitive enough); therefore, preferably use FCoV-free stud. No risk of virus transmission to kittens	A rapid result is often required. In-house positive/negative tests suitable for this purpose, provided adequately sensitive. Specificity is also important
11	Screening kittens of a FCoV seropositive queen to check whether they have become infected	Have become infected with FCoV, so should not be rehomed in household with uninfected cats – rehome where only cat. Re-test monthly. The kitten has around a 1 in 10 chance of developing FIP	FCoV-free (provided test is sensitive enough); therefore, no risk of FIP developing	Test kittens after 10 weeks of age.[22] Sensitivity of the test is important, as is the ability to detect low levels of FCoV antibody
12	Screen cat prior to stressful procedure; eg, rehoming, neutering or other elective surgery	If possible, delay the stress until the cat has become seronegative. Re-test in 2–3 months	FCoV-free (provided test is sensitive enough); therefore, no risk of FIP developing	In-house positive/negative tests suitable for this purpose, provided they are adequately sensitive, but positive cats will need FCoV antibody titre to monitor becoming seronegative. See comments in row 8
13	Screen cat prior to immunosuppressive treatment; eg, high-dose steroids, ciclosporin A, chemotherapy	If feasible, RT-PCR test faeces to determine if actively infected. If so, delay the treatment until the cat has become negative. Re-test FCoV antibody titre in 2–3 months	FCoV-free (provided test is sensitive enough); therefore, no risk of FIP developing	In-house positive/negative tests suitable for this purpose, provided they are adequately sensitive, but positive cats will need a follow-up test to establish FCoV antibody titre. RT-PCR testing can be useful, but five consecutive negative tests required to establish that the cat is no longer infected[3]

(Continued)

Table 5 (Continued)

	Reason for testing	Positive result	Negative result	Desirable criteria in FCoV antibody test and other comments
14	Screen a prospective blood donor cat prior to transfusion	Use of FCoV seropositive blood donor is not recommended	Safe to use a FCoV seronegative blood donor, provided test is sensitive enough	Speed is usually essential, so in-house tests are likely to be chosen. There is some evidence that passively transferred FCoV antibody could endanger the recipient in the event of becoming infected with FCoV[23,24]
15	Screen cat prior to FIP vaccination	No point vaccinating: the cat is either incubating FIP already or has natural immunity[3,25–27]	Vaccinate	Rapid result usually desired, so in-house tests usually chosen. There is no point in vaccinating a seropositive cat with the FIP vaccine, as the cat has already been exposed to infection
16	Screen prior to FeLV vaccination	Vaccination not recommended; either screen faeces for FCoV virus shedding or wait 2–3 months and re-test for FCoV antibodies	Safe to go ahead and vaccinate, provided test is sensitive enough	In-house tests usually chosen. There is concern among cat breeders that FeLV vaccination of FCoV-infected kittens can trigger FIP; therefore, a pre-vaccination screen of rescued cats or pedigree kittens for FCoV infection is advisable

FIP = feline infectious peritonitis; FeLV = feline leukaemia virus; RT-PCR = reverse transcriptase polymerase chain reaction; AGP = alpha-1-acid glycoprotein
*Controlled matings are where the stud and queen are put together for only the time needed to mate. The cats are not left together and do not have contact with a litter tray, so are not exposed to each other's faeces

tests is generally regarded as the IFAT; however, IFATs can be based on FCoV or TGEV, and – as we found in this and a previous study – tests from different laboratories do not always all give the same results on any one sample.[17] One limitation of IFATs is that they rely on a subjective reading by a human, who may mistake non-specific fluorescence for a positive result.

Most of the sample panel results agreed across all antibody tests assessed; when tests performed on a sample gave conflicting results, it was essential to be able to differentiate false-positive from false-negative results. The sample panel included some challenging positive samples that gave discordant – that is, false-negative or inexplicably low – results on one or more FCoV antibody tests, as described previously,[12] and this could have had the effect of lowering the apparent sensitivity of some tests.

Many commercial laboratories were invited to take part in this study. Some refused, others requested that their results be kept confidential and still others revealed that they use a test or reference laboratory already under assessment. Thus, the tests presented herein represent those that produced the most accurate results, and the manufacturers of which agreed to be assessed by an independent body. Specificity was very high for all of the tests presented here, although some laboratories gave false-positive results by mistaking non-specific fluorescence. Thus, one laboratory (not recorded in this study) reported 6/16 negative samples as falsely positive. We showed previously that this problem can occur owing to the presence of anti-nuclear antibodies in the sample, but can also be inexplicable.[17]

Sensitivity was an issue with some tests and, as we have previously shown, the presence of large amounts of virus in the sample can affect all types of FCoV antibody test, resulting in false-negative results or reduced FCoV antibody titre.[12] Therefore, where possible, samples negative for FCoV antibodies were screened for viral RNA. Although in this instance all of the consensus negative samples tested were negative by RT-PCR, we have previously encountered rare samples in which even IFAs were falsely negative.[13] To be fair to the tests being assessed in this study, the panel was probably more challenging than is generally encountered in day-to-day veterinary practice and contained a disproportionate number of effusions, relative to plasma or serum samples. This was done because large quantities of sample were required to assess many antibody tests and it is probably not possible to obtain very large blood samples from pet cats.

It was curious that 43% of effusions from cats with FIP were negative by RT-PCR. This is a well-known phenomenon, but the reason for it is unknown: it may be that the virus is cell-associated and confined to the cells of the perivascular granuloma. The pathogenic process that leads to the development of an effusion destroys the

connection between endothelial cells, allowing pure plasma to leak out: sera and plasma are often negative for virus, even in cats with FIP.[20] Other reasons include inhibition of the RT-PCR by an interfering substance in the effusion, or that the viral RNA was destroyed in the sample during mailing or storage. It is useful to be able to use both FCoV RT-PCR and serology in the diagnosis of FIP either together or sequentially: a negative antibody test (provided it is adequately sensitive) allows FIP to be ruled out of the differential diagnosis list of an effusion, whereas a negative RT-PCR test would not rule out FIP. Positive serology on an effusion is not diagnostic of FIP. However, large amounts of virus, detected by RT-PCR, indicates that FIP is extremely likely.[1]

Table 5 presents a number of situations in which tests for the detection of anti-FCoV antibodies are employed, and, based on the comparison described above, which test is most appropriate for each purpose. The choice of FCoV antibody test depends, in part, on the test being of good quality, and partly on the purpose of running the test. Where speed is of the essence (eg, for a breeding queen on the way to a stud cat), an in-house test (RIM or ELISA) will be chosen, so it is important that these tests are adequately sensitive. In-house tests take between 10 and 40 mins to perform compared with at least overnight for tests that require the mailing of a sample to a reference laboratory.

For cats undergoing diagnostic testing, the choice of test may be influenced by whether or not it can be performed on an effusion. We found that although some test instructions do not state that an effusion can be used, the tests worked on both effusions and blood components. In a cat suspected of dry FIP, where there is no effusion, sample quantity may be limited and in-house tests can have an advantage over tests performed at a reference laboratory, requiring as little as 5–40 µl of sample. However, most laboratories that state on their sample submission forms that they require one full millilitre of blood, will often admit to being able to perform their FCoV antibody test on as little as 50 µl of serum or plasma.

Overall, the most sensitive in-house test was the FCoV Immunocomb; the best RIM was the Speed F-Corona, with the Megacor FASTest FIP coming a close second. In the presence of virus, the RIM tests seemed more prone than ELISA or IFAs to give false-negative results or extremely faint bands. Even in the absence of virus in the sample there were some false-negative, or very faint, results, which might have been due to tiny blood or fibrin clots in the sample clogging the membrane.

For sequential antibody testing of cats (eg, where FCoV is being eliminated from a household), an FCoV antibody titre is important, so the sensitivity of tests was examined using samples of medium and low antibody titre. It was in the samples of medium and low titre,

rather than those of high titre, that the greatest differences in test sensitivity were revealed, especially between the RIM tests.

The FCoV Immunocomb had improved in both sensitivity and specificity since it was previously assessed,[10] probably owing to the mechanisation of reading the ELISA spots, which eliminates the element of subjectivity in interpreting the result spots and so reduces the chances of human error. It was the best test overall, requiring small sample size, being able to be performed in-house, and having excellent sensitivity and specificity.

We hope that this study will contribute to reducing the prevalence of misdiagnosis of FIP based on misunderstanding the nature of FCoV antibody tests, attributable, in part, to manufacturers erroneously labelling FCoV tests as FIP tests. Two companies deserve special mention as having responded to an appeal from one of the authors (DDA) to re-name their tests: Biogal and BVT re-named their tests as FCoV, not FIP, tests (although, unfortunately, the Food and Drugs Administration forced Biogal to change the name of the FCoV Immunocomb back to FIP Immunocomb for sale in the USA).

Conclusions

FCoV antibody testing is useful for a variety of reasons in the veterinary surgery and veterinary diagnostic laboratory. A flexible approach is useful in selecting FCoV antibody tests, choosing the test most appropriate to the reason for testing, rather than adopting one test and sticking to it rigidly. The FCoV Immunocomb required the least amount of sample. All tests worked on effusions, as well as plasma or serum samples, even when not stipulated to do so in their instruction sheets. However, a large amount of virus in the sample correlated with decreased antibody signal in all tests, but was most marked in the RIM tests. Specificity was 100% for most tests. Sensitivity was 100% for two IFA tests and the ELISA test. The FCoV Immunocomb was the most sensitive of the in-house tests, and the Speed F-Corona was the most sensitive of the RIMs.

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