

THE FIP-SPECIFIC TITRE TEST

A Second-Generation Test for the Detection of the Causative Agent of Feline Infectious Peritonitis (FIP)

The Need For a New Test

The coronavirus titre test first appeared 2 decades ago, and has remained a topic of controversy ever since. The basic test, of which there are numerous variants on the market, involves growing large quantities of coronavirus, usually porcine coronavirus (PCV), or canine coronavirus (CCV), in culture and coating proteins purified from these preparations on ELISA plates for detection of serum anti-coronavirus antibodies in the cat to be tested. By their very nature, tests of this sort are incapable of differentiating between antibodies which result from the infection of the cat by any of the large, extended family of coronaviridae, including exposure to mouse, canine, or even human coronaviruses. These problems exist in the test even before one addresses the question of whether the test can differentiate between cats infected with the various members of the cat coronavirus family, including FECV, TGEV, FIPV, or the Primucell FIP vaccine (it can't).

Not surprisingly, the clinical interpretation of the coronavirus titer is difficult for the practitioner. For example, it has been reported that a high, rising titre indicates active infection. But with what? If the virus involved is a non-FIP coronavirus (i.e. FECV), then the cat will never develop FIP, and probably does not need to be culled from a cattery. Decreasing titre could be expected to be a good result, except that many cats show depressed titres precisely during the interval in which they enter the fulminant terminal stage of FIP. It has been suggested that at best, a cattery with persistently high levels of anti-coronavirus antibody has poor husbandry standards, and is thus more likely to be a source of FIP cases than a negative cattery; while there is unquestionably truth to this, it is also a poor justification for performing the test as a "FIP" screening assay. In view of all this, it has also been suggested that the best scenario that could be hoped for regarding the use of coronavirus titre testing in catteries is that the test will be run, but no action taken based on the result (Pederson, 1994). Clearly, there is a need for a serology screening test which is capable of detecting antibodies to FIPV to the exclusion of all other coronaviruses.

The Origin of the FIP-Specific Titre Test

Since 1995, Antech, under license from Hoffman-La Roche, has offered the first FIP-specific test commercially available: the FIPV-PCR assay. This test was developed by running a computer-generated comparison between the known gene sequences of a variety of coronavirus types and strains in order to identify gene regions unique to FIPV, and which were not found in related viruses (especially not in TGEV and FECV). A region of the 7E gene, expressed in FIPV strains 79-1146 and field strains FIP/SBIO-1, and SBIO-2 but deleted in FECV-1683, TGEV and the Primucell FIP

vaccine, was found to have these characteristics, and was chosen as the target for the PCR assay.

The clinical value of the PCR assay directed to the 7B gene has been conclusively demonstrated on effusive fluids from suspect FIP cases, showing a positive predictive value of 95% and a negative predictive value of 78% in a recent 40 cat study published in the May 1997 Antech newsletter (2).

To create the FIP specific test, the protein coded for by the 7B gene has been produced as a protein for use in an ELISA-based serological assay. To accomplish this, the 7B gene was cut out from the FIPV viral genome by recombinant DNA technology, inserted into an *E. coli* bacterium, and the 7B protein then produced by the gene purified. The pure 7B protein is coated on ELISA plates and sera from cats to be tested allowed to bind. Antibodies specific for the 7B protein are detected by the use of anti-cat antibody reagents. The serum is tested using the KELA kinetics dependant ELISA assay, with background irrelevant binding of sera removed by the use of various control proteins. This is the same method employed at Cornell Vet Labs for their coronavirus testing system. Unlike the coronavirus titre, however, antibodies to the 7B protein indicates infection with pathogenic FIPV strains such as 79-1146, SBIO-1, or SBIO-2 to the exclusion of non-pathogenic coronaviruses, as well as the Primucell FIP vaccine. Overall titre values range from 0 to 1:640, lower than the values the practitioner is used to for coronavirus assays, but reflecting the proportion of antibodies truly specific for FIPV.

Recently, reports have emerged which indicate that expression of the 7B gene protein product by a FIP virus is the key factor which determines whether the virus is pathogenic or non-pathogenic. 7B has been reported to function as a virus growth factor, allowing the fatal multiplication of virus in peritoneal macrophages. Evidence in favor of this includes: loss of virulence has been shown to correlate with loss of 7B expression; and attenuated FIP strains created for vaccine purposes also lose 7B expression when they are selected for non-virulence (3,4).

An extensive study (Vennema, et al, 1998) of various mutations in regulatory genes which lead to the pathogenic state shows that the mutations invariably occur in either the 7B gene, or in the 3C gene which may regulate 7B expression. For conversion from a "benign" to a "malignant" form of virus, both possessing, and expressing an intact 7B gene product seems to be required. Thus, while many coronaviruses have been shown to possess at least partial 7B sequences, expression of functional 7B protein is the marker that shows that conversion to pathogenic FIPV has occurred. This expression is detected by both the PCR and ELISA assays to 7B.

Clinical Validation of the FIP-specific Titre Assay

The clinical validation of the 7B assay falls into three major groups: tests of sera obtained from laboratory cats, sera from PCR+, histopathology confirmed cats, and sera obtained from various catteries to track the progression of FIP cases. Each is discussed separately below:

A. Data from Laboratory Cats

Results of 7B ELISA testing on the sera of 10 cats exposed to various experimental protocols are shown in Table 1.

<u>CAT #</u>	<u>Status</u>	<u>Coronavirus Titre</u>	<u>7B FIP Titre</u>
1.	FECV strain 1683 infected	1:960	0
2.	FECV strain 1683 infected	1:1048	0
3.	FIPV strain 1146 infected	1:1920	1:160+
4.	FIPV strain 1146 infected	1:4096	1:640++
5.	Primucell vaccine infected	1:1000	0
6.	Primucell vaccine infected	1:2000	0
7.	FIP UCD strain exposed	0	0
8.	FIP UCD strain infected	0	1:160+
9.	SPF (pathogen free)	0	0
10.	SPF (pathogen free)	0	0

Results clearly indicate that the designed specificity of the genetically engineered 7B protein to contain FIPV-only serological markers has been observed. The closely related strain FECV 1683, which has deletions in the 7B region, does not induce antibodies in the infected cat which bind to the recombinant 7B protein. Similarly, sera from Primucell infected cats also do not react to the 7B protein; however, cats infected with FIPV strains 1146 and UCD are positive to 7B. The complete lack of utility of the standard coronavirus test is also illustrated here, with 4 out of 6 false positives (cats 1, 2, 5 and 6) and even a false negative (cat 8).

B. Association of 7B Titre with PCR+, Histopath+, Cats

There is a very clear association of positive 7B titre with cats presenting with classical effusive FIP confirmed with PCR and/or histopathology examination postmortem. Results are summarized in the Table below:

<u>Cat#</u>	<u>7B Status</u>	<u>Clinical Findings</u>	<u>Outcome</u>
1. 428	1:160	Clin+ FIP, fluid, clin/path +	dead
2. 430	1:320	Clinical FIP, clin/path+	dead
3. 201	1:640	Clin+ PCR+	dead
4. 406	1:320	fluid, Clinical+, PCR+	dead
5. 362	1:160	fluid, Clinical+, PCR+	dead
6. 414	1:160	fluid, Clinical+, PCR+	dead
7. 512	1:320	fluid, Clinical+, PCR+	dead
8. 711	1:640	Clinical+, histology +	dead
9. 589	1:320	histology+, necropsy +	dead
10. 511	1:160	cyto+, histology+	dead
11. 710	1:320	histology+	dead
12. 8	1:320	Ocular FIP, cytology+	dead

C. Predictive Value of 7B Titre on Outcome-Cattery Studies

The value of the 7B ELISA in predicting which cats should be removed from a cattery to prevent spread of disease is the subject of an on-going, longterm collaborative study between Synbiotics and numerous catteries country-wide. The results from two of these catteries, who have been followed long enough to obtain first results, are shown below.

Cattery #1

<u>Cat#</u>	<u>Status When Tested</u>	<u>7B Result</u>	<u>Status 3 months later</u>
1.	normal	0	normal
2.	normal	0	normal
3.	normal	0	normal
4.	normal	1:80	normal
5.	normal	0	normal
6.	normal	0	normal
7.	normal	1:160	dead of FIP (clin/path+)
8.	normal	0	normal
9.	normal	0	normal
10.	normal	1:320	dead of FIP (clin/path+)

Cattery #2

<u>Cat#</u>	<u>Status When Tested</u>	<u>7B Result</u>	<u>Status 2 months later</u>
1.	normal	0	normal
2.	normal	0	normal
3.	normal	1:40	normal
4.	normal	1:320	dead of FIP (PCR+)
5.	normal	0	normal
6.	normal	0	normal
7.	normal	1:40	normal
8.	normal	1:40	normal
9.	normal	0	normal
10.	normal	1:160	dead of FIP (clinical)

The results clearly show an association between detectable 7B titres (>1:80) and an eventual outcome of clinical FIP; no cats with a negative titre have yet developed disease in these two catteries while 4 out of 8 7B positive cats have died of clinical FIP within 3 months after testing.

Independent Studies and Incidence Data

Tests of the ability of the 7B system to differentiate between cats infected with a variety of laboratory strains of coronavirus were undertaken at a second laboratory independent of Antech. In the first experiment, 10 cats were first vaccinated with the coronavirus vaccine

All cats tested by the old coronavirus titer rapidly became positive to the vaccine, while none were 7B antigen positive. The cats were then subsequently challenged with the FIP strain 79-1146. Tests with the old corona titer assay gave no further useful information, as all cats were already positive to the vaccine; however, the 7B test revealed that 9/10 cats gradually became positive after virus exposure. This demonstrates two clear advantages of the 7B FIP-specific test: ability to exclude vaccine reactions, and the ability to selectively detect the spread of pathogenic virus in an infected animal.

The second independent experiment included a study of sera from cats experimentally infected with FECV-RM strain. All cats were also FIV+ at the time of infection, adding an extra complication; however, the results obtained were clear-cut. All 22 cats rapidly became positive to the old coronavirus assay while none became 7B antigen positive. Again, this clearly demonstrates the utility of testing by this method as FECV, the most likely coronavirus which the clinician would like to exclude from consideration, does not induce 7B antibodies.

Incidence data so far obtained from Antech samples indicates that, out of a group of 124 cats obtained from an adoption shelter, 120 were coronavirus positive to various degrees, again underlining the poor utility of this test. For 7B detection, 32 were found positive to varying degrees (10 high titer, 22 low titre, rest negative); all 32 cases are being followed for eventual outcome.

Conclusions

This new form of testing for the presence of FIPV, utilizing recombinant DNA technology, is the first commercially available test that can detect antibodies to FIPV in the blood of a test cat to the exclusion of related forms of non-pathogenic feline coronaviruses. By eliminating these sources of confusing and conflicting results, the 7B titre assay promises to make serology testing for FIPV a more useful reality.