

## ELISA for the serology of FIP virus

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### SUMMARY

*An enzyme linked immunosorbent assay (ELISA) for feline infectious peritonitis (FIP) virus serology is described. The assay is analogous to a previously developed indirect heterologous immunofluorescence test (IFT) in which transmissible gastroenteritis (TGE) viral antigen was used. Comparative testing of selected feline sera in both assays resulted in corresponding titers, which justifies the conclusion that the ELISA is a reliable test for the serology of FIP virus.*

Feline infectious peritonitis (FIP) is a disease of felines caused by a coronavirus which was shown to be widely distributed among cats all over the world (2). The disease almost invariably leads to the death of the animal and is only incidentally observed in most cat populations. However, serological studies indicated that high percentages of cats may be infected subclinically (4, 5, 7). Antibodies against FIP virus in cats were first demonstrated in an indirect immunofluorescence test (IFT) using liver sections from a FIP virus infected kitten as antigen (7). The successful propagation of the virus in the brains of suckling mice permitted us to perform a similar test on cryostat sections of infected mouse brain (6), and also resulted in the first demonstration of neutralizing antibodies in sera from cats with FIP (3). Based on the confirmed antigenic relationship between FIP virus and transmissible gastroenteritis (TGE) virus of pigs

(5, 9, 10, 11, 12) we developed a heterologous indirect IFT for the detection of anti-FIP viral antibodies (5): A mixture of TGE virus infected and uninfected porcine thyroid cells served as antigen preparation. In this paper we describe a heterologous enzyme linked immunosorbent assay (ELISA) for this purpose, using an analogous indirect system.

### MATERIALS AND METHODS

#### Sera

Serum samples were collected from cats of different populations, with or without a history of FIP:

- 20 sera from a colony of specified pathogen free (SPF) cats (Centraal Proefdieren Bedrijf - Zeist),
- 39 sera from cats of the 'open population' (OP) in the Netherlands. Eight showed symptoms of FIP, confirmed by post mortem examination; the others were healthy,
- 14 sera from SPF cats, 14 to 30 days after experimental infection (EI) with about  $10^4$  MID<sub>50</sub>

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suckling mouse brain adapted FIP virus (13th passage). Two samples were taken from cats showing symptoms of FIP, confirmed by post mortem examination.

## ELISA

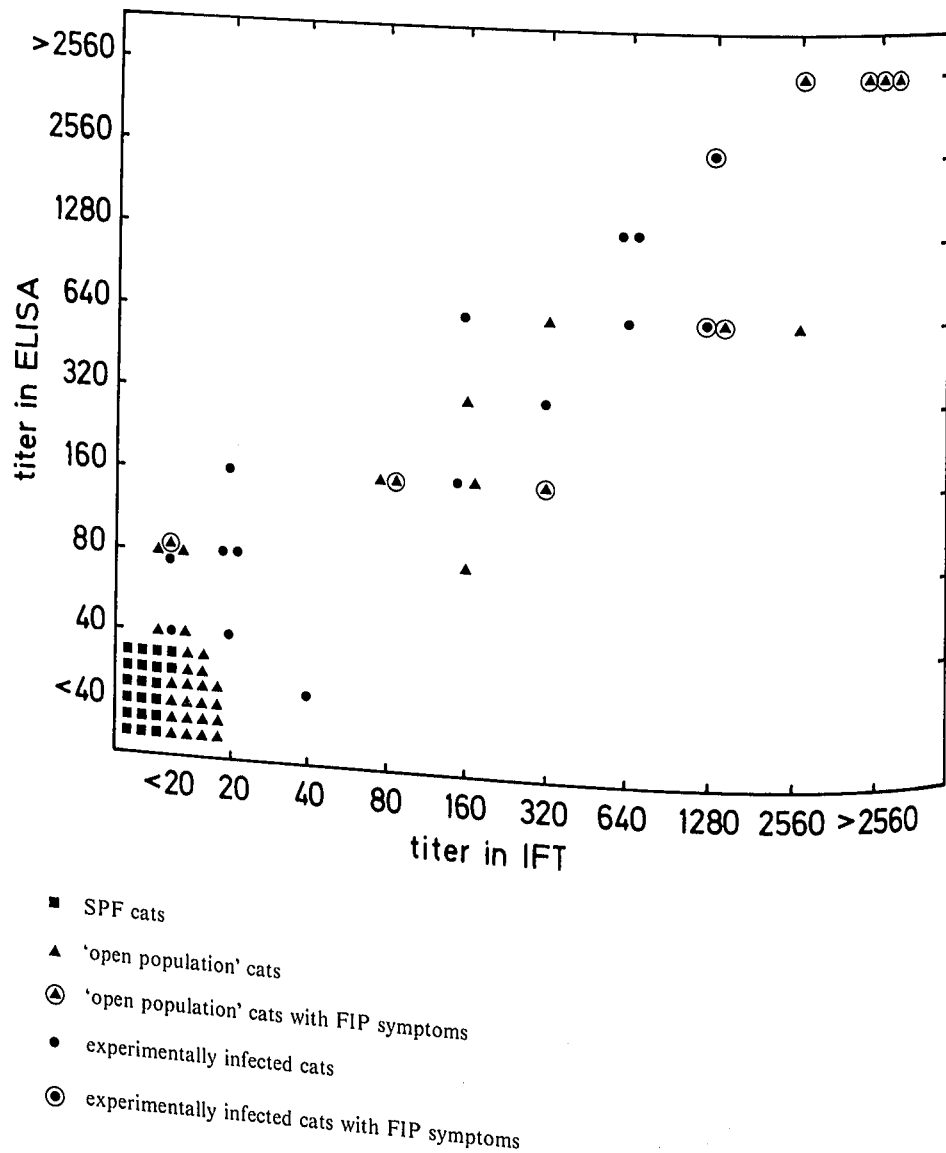
### Antigen/coating

TGE virus (strain Purdue) was propagated in monolayers of PD<sub>3</sub> cells. When about 75% of the cells showed CPE, approximately  $5 \times 10^7$  cells were harvested in 5 ml 0.5 carbonate buffer, pH 9.6. After three cycles of freezing and thawing the cellular debris were removed by low speed centrifugation and the supernatant was used as antigen in a 1:4 dilution. Control antigen was prepared in the same way from uninfected PD<sub>3</sub> cells. U shape wells of polystyrene microtiter plates (Cooke) were coated with TGE viral or control antigen by filling the wells with 100  $\mu$ l antigen and drying in a 20° C

### Indirect IFT

The procedure followed was essentially the same as described previously (5). However, a mixture of TGE virus infected and uninfected cells of the porcine line PD<sub>3</sub> (kindly provided by Philips Duphar, Weesp) dried onto epoxycoated microprint slides (Cooke Eng. Comp. Alexandria Va.) served as antigen preparation. Titers  $\geq 20$  were considered positive.

Fig. 1.



air stream. The coated plates were stored at 4° C. Before use the wells were filled with deionized water containing 0.05% Tween 80 and 0.5% bovine serum albumin, incubated for one hour at 37° C and rinsed three times with ELISA buffer (PBS, containing 0.05% Tween 80 and 0.1 mM EDTA, pH 7.1).

#### Conjugate

The rabbit anti-cat IgG serum which was used for the preparation of the FITC conjugate for the IFT was also employed to prepare a peroxidase conjugate. Conjugation was carried out essentially according to a method described by Ellens *et al.* for the conjugation of a calf anti-rotavirus serum (1). The conjugate was stored in 20 µl quantities at -20° C and diluted in ELISA buffer supplemented with 1% calf serum before use. The optimal dilution of the conjugate (1:200) was determined by checkerboard titration.

#### Substrate

The enzyme substrate used was hydrogen peroxide with 5-aminosalicylic acid (5-AS, Merck). 5-AS was purified as described by Ellens *et al.* (1). Before use 10 mg of the purified 5-AS were dissolved in 10 ml 0.01 M phosphate buffer (final pH 6.0) and 1 ml 0.05% hydrogen peroxide was added.

#### Assay

Volumes of 100 µl of twofold dilutions of the sera in ELISA buffer, using a starting dilution of 1:40, were added to the wells.

After an incubation period of one hour at 37° C the plates were washed three times and 100 µl/cup of the diluted conjugate was added. Again the plates were incubated for one hour at 37° C, rinsed three times and 100 µl of the substrate was added. After an incubation period of two hours at 4° C the plates were read by visual inspection.

## RESULTS AND DISCUSSION

The results of a comparative testing of the feline sera in both IFT and ELISA are shown in Fig. 1. Sera from 40 clinically

healthy cats (20 SPF sera and 20 OP sera), which were negative in the IFT were also negative in the ELISA. Eight sera gave discordant results: seven IFT negative (< 20) sera (five OP sera and two EI sera) showed low titers in the ELISA (titers 40 and 80); one serum with a low titer in the IFT (= 40) was negative in the ELISA (< 40). This difference in sensitivity is not significant ( $X^2$  test: P-value 8%). All remaining sera were positive in both tests. The geometric mean of the ratios between IFT and ELISA titers (in the range of 20-2560 and 40-2560 respectively), is not significantly different from 1, using the Student test for paired values (IFT titer/ELISA titer = 0.7; 95% confidence interval between 0.5 and 1.1). Consequently there is no reason to suppose that differences in these analogous assays — e.g. in indicator molecule (FITC and peroxidase respectively) — cause a difference in titer. It may be concluded from these results that the ELISA is a reliable test which, depending on technical facilities, may replace the IFT for FIP virus routine serology. The sera from the OP were not randomly selected, but sera from clinically healthy cats and cats with FIP symptoms with low, middle and high titers in the IFT were chosen. Hence, the distribution of antibody titers among clinically healthy and diseased cats is not representative. In most cases, cats with clinical FIP have high antibody titers whereas in cats without clinical symptoms low titers are found (8).

## ACKNOWLEDGEMENTS

The authors wish to thank Prof. Dr. M. C. Horzinek for helpful suggestions.

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