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# Use of recombinant nucleocapsid proteins for serological diagnosis of feline coronavirus infection by three immunochromatographic tests



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## ABSTRACT

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Three types of immunochromatographic assays (ICAs) were designed to detect anti-feline coronavirus (FCoV) antibodies. Recombinant FCoV nucleocapsid protein (rNP) was used as a conjugate or test line in all 3 ICA kits ( $C_{1}IgG/rNP$ ,  $C_{1}NP/rNP$ , and  $C_{1}NP/rPA$ ). All three ICA kits were capable of detecting anti-FCoV antibodies; however, non-specific positive reactions of anti-FCoV antibody-negative plasma samples with the test line were observed in 2 ICA kits ( $C_{1}IgG/rNP$  and  $C_{1}NP/rNP$ ), in which rNP was used as the test line. On the other hand, the specific detection of anti-FCoV antibodies was possible in all plasma, serum, whole blood, and ascitic fluid samples using the ICA kit with protein A blotted as the test line ( $C_{1}NP/rPA$ ). In addition, the specificity and sensitivity of ICA ( $C_{1}NP/rPA$ ) were equivalent to those of the reference ELISA. The development of simple antibody test methods using the principle of ICA ( $C_{1}NP/rPA$ ) for other coronavirus and feline viral infections is expected in the future.

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## 1. Introduction

Feline infectious peritonitis virus (FIPV), a feline coronavirus (FCoV) of the family *Coronaviridae*, causes a fatal disease called FIP in wild and domestic cat species. FCoV is mainly composed of nucleocapsid (N) proteins, membrane proteins (M), and peplomer spike (S) proteins, and has been classified into serotypes I and II according to the amino acid sequence of its S protein (Hohdatsu et al., 1991a; Motokawa et al., 1995). Both serotypes consist of two biotypes: FIPV and feline enteric coronavirus (FECV). FECV infection is asymptomatic in cats, whereas FIPV infection has been shown to cause FIP (Pedersen, 2009). FIPV has been proposed to arise from FECV due to a mutation (Brown, 2011; Chang et al., 2010, 2012); however, the exact mutation and inducing factors have not yet been clarified. Therefore, no reliable method has been established to distinguish FIPV and FECV.

The antemortem diagnosis of FIP is extremely difficult (Addie et al., 2009). Although exudate can be used to diagnose FIP in cats, accompanied by ascites and pleural effusion, not all cats with FIP retain exudate. Therefore, a biopsy is necessary for the antemortem diagnosis of FIP without exudate retention. Tru-cut and fine needle biopsies are minimally invasive, highly specific diagnostic methods; however, their sensitivity is low (Giordano et al., 2005). FIP has typically been diagnosed comprehensively

based on the clinical condition, hematological profile, and results of FCoV genomic RNA and FCoV-antibody measurements in cats suspected of FIP (Addie et al., 2009). An indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and immunochromatographic assay (ICA) are currently used to measure FCoV-antibodies. IFA and ELISA are highly sensitive and specific, but are also cumbersome, expensive, and time-consuming. ICA is a simple antibody detection method, the results of which can be rapidly obtained at a low cost. However, the sensitivity of commercially available ICA test kits to detect the FCoV antibody was shown to be low (Meli et al., 2013).

The human coronavirus (HCoV) N protein is attracting attention as an important target of antibody test methods because of its high antibody reactivity (Severance et al., 2008). Several antibody-binding linear epitopes were shown to be present in the FCoV N protein, and were common to type I and II FCoV (Satoh et al., 2011). Based on these findings, it is assumed that FCoV antibodies can be detected with high sensitivity using the FCoV N protein regardless of serotypes.

Three types of ICA test kits were prepared using the recombinant N protein of FCoV, and differences in their sensitivity and specificity for FCoV antibodies were investigated.

## 2. Materials and methods

### 2.1. Recombinant N protein (rNP)

A cDNA segment representing the N protein of the type I FIPV KU-2 strain was ligated into pGEX4T-1 (GE Healthcare, NJ,

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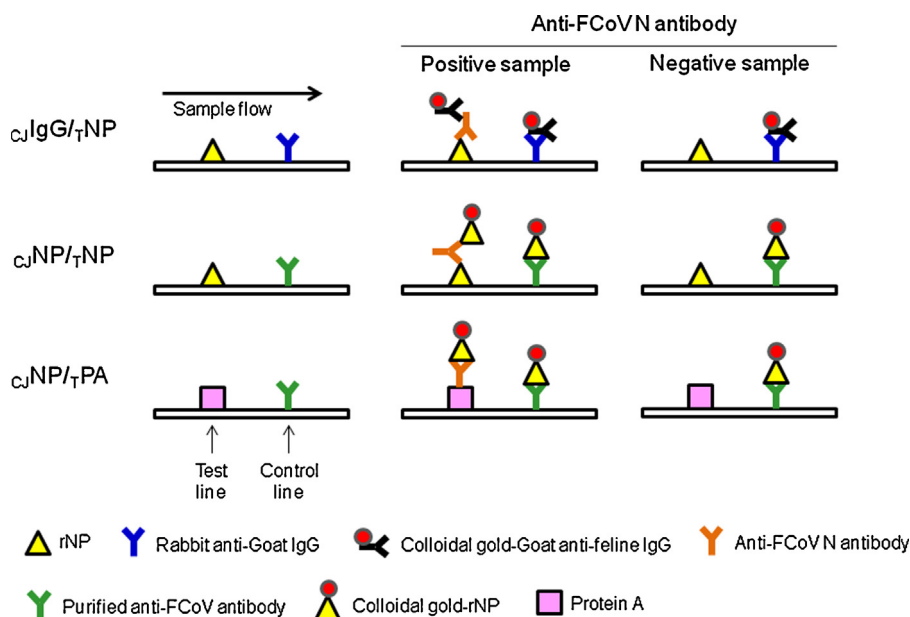


Fig. 1. 3 schematic diagrams of the 3 types of immunochromatographic tests.

USA), and transfected into *Escherichia coli* strain BL-21. Expression of the glutathione-S-transferase (GST) fusion protein was examined using the GST gene fusion system following the manufacturer's instructions. rNP was purified by affinity chromatography on glutathione-Sepharose 4B (GE Healthcare, NJ, USA).

## 2.2. Monoclonal antibodies (MAbs)

MAbs E22-2 (IgG1) and F19-1 (IgG1) were used as hybridoma cell culture supernatants. These hybridoma cells were previously established (Hohdatsu et al., 1991b). MAb E22-2 was shown to react with the N protein of FCoV, while MAb F19-1 reacts with the M protein of FCoV (Hohdatsu et al., 1991b).

## 2.3. Western blotting

rNP was separated employing 12% SDS-PAGE and transferred to a nitrocellulose membrane. A standard protein marker (Precision Plus Protein Standards) was purchased from Bio-Rad (Hercules, CA, USA). The membrane was blocked with 5% non-fat dry milk powder in TBST (20 mM Tris-HCl, 150 mM sodium chloride, and 0.05% Tween-20, pH 8.0) for 1 h at 37 °C, incubated for 1 h at 37 °C with MAb F19-1 or E22-2, and then incubated with peroxidase-conjugated goat anti-mouse IgG (MP Biomedicals, LLC-Cappel products, CA, USA) for 1 h at 37 °C. It was then visualized in the substrate for 10 min.

## 2.4. Sample specimens

Plasma, serum, and whole blood samples were collected from control uninfected specific pathogen-free (SPF) cats inoculated orally with FIPV. Twenty-three cats were inoculated with the type I FIPV KU-2 strain. Twenty-four cats were inoculated with the type II FIPV 79-1146 strain. Samples from 37 control uninfected SPF cats were used as negative controls.

## 2.5. Preparation of the ICA test strip

The ICA test strip consisted of three main components: a sample pad (C083 Cellulose Fiber Sample Pad Strips, Millipore Corporation, MA, USA), nitrocellulose membrane (Hi-Flow Plus 240 Membrane

Cards, Millipore Corporation, MA, USA), and absorbent pad (the same one as the sample pad). Three types of ICA test strips were prepared in this study (Fig. 1). Preparation methods for the ICA strips were as follows:

ICA (conjugate: IgG/test line: NP) [ICA ( $C_J$ )IgG/ $TNP$ ]: purified rNP was dispensed on the test line of the nitrocellulose membrane using a brush. Rabbit anti-goat IgG (Bethyl Laboratories, Inc., TX, USA) was dispensed on the control line of the nitrocellulose membrane using a brush.

ICA (conjugate: NP/test line: NP) [ICA ( $C_J$ )NP/ $TNP$ ]: Purified rNP was dispensed on the test line of the nitrocellulose membrane using a brush. Affinity-purified cat anti-FCoV IgG (prepared by the laboratory of the present study) was dispensed on the control line of the nitrocellulose membrane using a brush.

ICA (conjugate: NP/test line: PA) [ICA ( $C_J$ )NP/ $TPA$ ]: Protein A (Sigma-Aldrich, MO, USA) was dispensed on the test line of the nitrocellulose membrane using a brush. Affinity-purified cat anti-FCoV IgG was dispensed on the control line of the nitrocellulose membrane using a brush.

## 2.6. Preparation of colloidal gold-labeled goat anti-cat-IgG

Goat anti-cat-IgG (MP Biomedicals, Tokyo, Japan) was diluted with 20 mM Borax (20 mM sodium tetraborate containing 1% bovine serum albumin (BSA) and 0.1% sodium azide) to 0.5 mg/ml, and 40  $\mu$ l of this dilution was added to 1 ml of colloidal gold solution (40 nm; BBI Solutions, Cardiff, UK). After stirring well, the mixture was left standing for 30 min at room temperature, followed by the addition of 100  $\mu$ l of 20 mM Borax containing 10% BSA, following which the mixture was once again left standing for 30 min at room temperature. After centrifugation at 22,000  $\times$  g for 10 min, the precipitate was suspended in 0.5 ml of preserving solution (1% (w/v) BSA, 0.1% (w/v) sodium azide, and 150 mM sodium chloride in 20 mM Tris-HCl buffer, pH 8.0). This suspension (colloidal gold-labeled goat anti-cat-IgG) was used as a conjugate in ICA ( $C_J$ )IgG/ $TNP$ ).

## 2.7. Preparation of colloidal gold-labeled rNP

rNP (0.5 mg/ml) was diluted with PBS to 0.5 mg/ml, and 30  $\mu$ l of this dilution was added to 1 ml of colloidal gold solution. After

stirring well, this mixture was left standing for 30 min at room temperature, followed by the addition of 100  $\mu$ l of 20 mM Borax containing 10% BSA, following which the mixture was once again left standing for 30 min at room temperature. After centrifugation at 22,000  $\times$  g for 10 min, the precipitate was suspended with 0.75 ml of 20 mM Borax. This suspension (colloidal gold-labeled rNP) was used as a conjugate in ICA ( $C_{JNP/TNP}$ ) and ICA ( $C_{JNP/TPA}$ ).

### 2.8. Procedure for ICA

Plasma was diluted 80 times with eluent solution (PBS containing 3% Tween-20), 40  $\mu$ l of this dilution was mixed with 20  $\mu$ l of the colloidal gold-labeled agent, and it was then absorbed in the sample pad of the ICA strip. The appearance of the test line or/and control line was confirmed after 10 min.

### 2.9. Reference ELISA

The reference ELISA for anti-FCoV antibodies was performed as described by Takano et al. (2008). Briefly, detergent-disrupted, purified type II FIPV 79-1146 virions were diluted appropriately with carbonate buffer (0.05 M, pH 9.6). A total of 100  $\mu$ l of the dilution was pipetted into each well of a 96-well flat-bottomed plate. The plates were allowed to stand overnight at 4  $^{\circ}$ C, washed with PBS containing 0.02% Tween-20, and 100  $\mu$ l of the test serum sample was then added to each well. Horseradish peroxidase-conjugated goat anti-cat IgG (ICN Pharmaceuticals, Inc., USA) was diluted to the optimal concentration with PBS containing 10% FCS and 0.05% Tween-20, and 100  $\mu$ l of the dilution was added to each well of the plates. After incubation at 37  $^{\circ}$ C for 30 min, 100  $\mu$ l of the substrate solution was added to each well and plates were incubated at 25  $^{\circ}$ C for 20 min in a dark room. The substrate solution was prepared by dissolving *o*-phenylenediamine dihydrochloride at a concentration of 0.4 mg/ml in 0.1 M citric acid and 0.2 M  $Na_2HPO_4$  buffer (pH 4.8), and 0.2  $\mu$ l/ml of 30%  $H_2O_2$  was then added. The reaction was stopped with 3 N  $H_2SO_4$  solution and the optical density (O.D.) at 492 nm was determined. The positive limit value of reference ELISA was ELISA O.D. = 0.10.

## 3. Results

### 3.1. Analysis of rNP using western blotting

The specificity of rNP was investigated by western immunoblotting using MAbs. As shown in Fig. 2, rNP did not react with F19-1, and only reacted with E22-2, which recognized the FCoV N protein.

### 3.2. Determination of the optimum concentrations of samples

Plasma was diluted 8, 80, and 800 times with PBS, and these samples were mixed with colloidal gold-labeled goat anti-feline

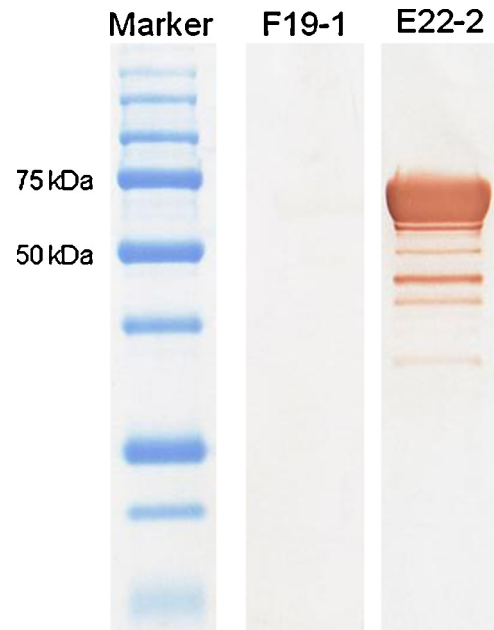


Fig. 2. Setting the dilution rate of plasma for addition to the 3 types of immunochromatographic tests.

IgG. These mixtures were then applied to nitrocellulose membranes with rNP blotted as the test line (Fig. 3:  $C_{JNP/TNP}$ ). Line formation was confirmed with the 800 times dilution of plasma from control uninfected SPF cats, whereas that with the 8 times dilution of plasma from FCoV-infected cats was unclear. Similar findings were noted in samples mixed with colloidal gold-labeled rNP (Fig. 3:  $C_{JNP/TNP}$ ). Plasma was diluted 8, 80, and 800 times with PBS, and these samples were then mixed with colloidal gold-labeled rNP. These mixtures were then applied to nitrocellulose membranes with Protein A (0.1 mg/ml) blotted as the test line (Fig. 3:  $C_{JNP/TPA}$ ). When the plasma of control uninfected SPF cats was applied to the sample pads, no line formed at any concentration. In contrast, the formation of lines with the 8 and 800 times dilutions of plasma from FCoV-infected cats was unclear. Based on these findings, the dilution rate of plasma for mixing with colloidal gold-labeled agents was set at 80 times.

### 3.3. Determination of the optimum concentrations of capturing reagents for application as the control line

Rabbit anti-goat IgG was diluted to 0.02, 0.1, 0.5, and 1.0 mg/ml with PBS, and these IgG dilutions were applied to nitrocellulose membranes (Fig. 4A,  $C_{JNP/TNP}$ ). The presence or absence of line formation was judged 10 min after applying only colloidal gold-labeled goat anti-feline IgG to the sample pads. Since clear line

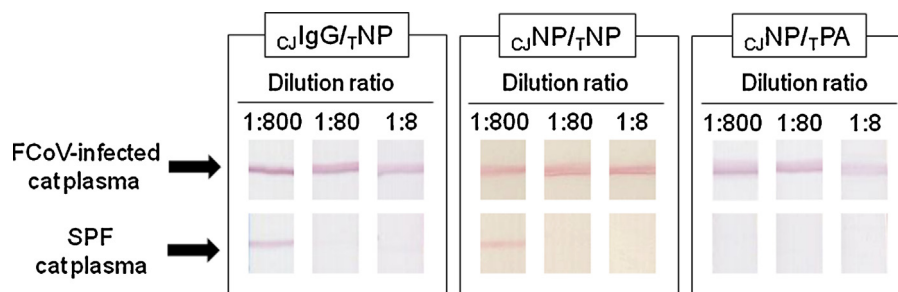
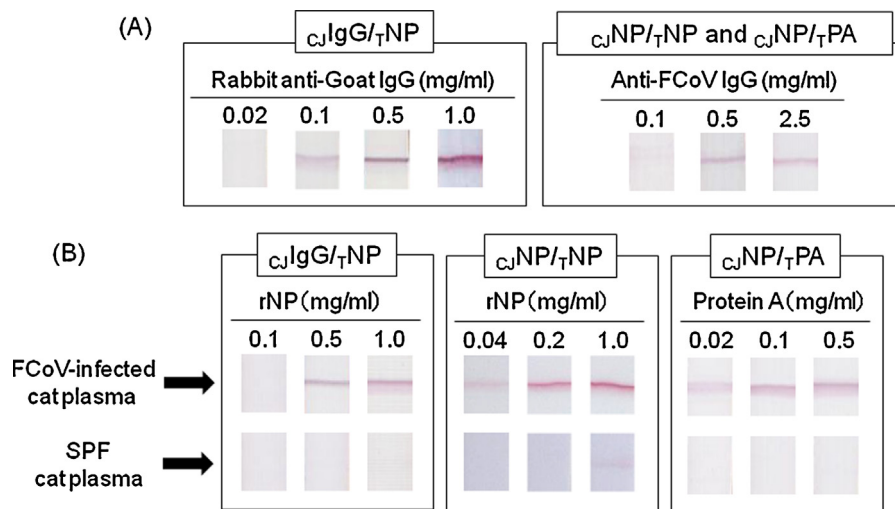


Fig. 3. Establishment of the control and test line concentrations in the 3 immunochromatographic tests. (A) Establishing the antibody concentration for the control line. (B) Establishing the protein concentration for the test line.



**Fig. 4.** Analysis of the expressed recombinant GST-tagged FIPV KU-2 nucleocapsid protein using western immunoblotting. F19-1, anti-FCoV M MAb (IgG1); E22-2, anti-FCoV N MAb (IgG1).

formation was observed at 0.5 mg/ml, the rabbit anti-goat IgG concentration for application as the test line of ICA (c<sub>J</sub>IgG/τNP) was set at 0.5 mg/ml.

Affinity-purified cat anti-FCoV IgG was diluted to 0.1, 0.5, and 2.5 mg/ml with PBS (Fig. 4A, c<sub>J</sub>NP/τNP, and c<sub>J</sub>NP/τPA), and these IgG dilutions were applied to nitrocellulose membranes. The presence or absence of line formation was judged 10 min after applying only colloidal gold-labeled rNP to the sample pads. Since clear line formation was observed at 0.5 mg/ml, the affinity-purified cat anti-FCoV IgG concentration for each of the test lines of ICA (c<sub>J</sub>NP/τNP) and ICA (c<sub>J</sub>NP/τPA) was set at 0.5 mg/ml.

#### 3.4. Determination of the optimum concentrations of capturing reagents for application as the test line

rNP was diluted to 0.04, 0.1, 0.2, 0.5, and 1.0 mg/ml with PBS (Fig. 4B, c<sub>J</sub>IgG/τNP and c<sub>J</sub>NP/τNP). These IgG dilutions were applied to nitrocellulose membranes. When colloidal gold-labeled goat anti-feline IgG and the plasma of FCoV-infected cats (ELISA O.D.=0.82) were applied to the sample pads, clear line formation was observed at 0.5 mg/ml. When colloidal gold-labeled goat anti-feline IgG and the plasma of control uninfected SPF cats (ELISA O.D.=0.06) were applied to the sample pads, no line formed at any concentration. Therefore, the rNP concentration for the test line of ICA (c<sub>J</sub>IgG/τNP) was set at 0.5 mg/ml. Similarly, when colloidal gold-labeled rNP and the plasma of FCoV-infected cats were applied to the sample pads, clear line formation was observed at 0.2 mg/ml (Fig. 4B, c<sub>J</sub>NP/τNP). When colloidal gold-labeled rNP and the plasma of control uninfected SPF cats were applied, faint line formation was noted at 1.0 mg/ml, while no line formed at 0.2 mg/ml or lower. Therefore, the rNP concentration for the test line of ICA (c<sub>J</sub>NP/τNP) was set at 0.2 mg/ml.

Protein A was diluted to 0.02, 0.1, and 0.5 mg/ml with PBS (Fig. 4B, c<sub>J</sub>NP/τPA). These protein A dilutions were applied to nitrocellulose membranes. When colloidal gold-labeled rNP and the plasma of FCoV-infected cats were applied to the sample pads, clear line formation was observed at 0.1 mg/ml. When colloidal gold-labeled goat anti-feline IgG and the plasma of control uninfected SPF cats were applied, no line formed at any concentration. Therefore, the protein A concentration for the test line of ICA (c<sub>J</sub>NP/τPA) was set at 0.1 mg/ml.

#### 3.5. Specificity of the 3 types of ICAs

The specificity and sensitivity of the 3 types of ICAs were demonstrated using plasma samples from cats infected experimentally with FCoV and SPF cats. The specificities of the 3 types of ICAs are shown in Table 1. Positive reactions were observed in plasma samples from type I FCoV infected cats (ELISA O.D. > 0.80) in all ICA tests. Regarding plasma samples from type II FCoV infected cats (ELISA O.D. > 0.80), one of the 24 cats was negative in ICA (c<sub>J</sub>NP/τNP), whereas all others were positive in all ICA tests. Regarding plasma samples from control uninfected SPF cats (ELISA O.D. < 0.06), 9 and 11 of the 37 cats were positive in ICA (c<sub>J</sub>IgG/τNP) and ICA (c<sub>J</sub>NP/τNP), respectively, whereas a positive reaction was not observed in any of the 37 cats in ICA (c<sub>J</sub>NP/τPA). These findings strongly suggested that ICA (c<sub>J</sub>NP/τPA) is the optimum ICA test to detect anti-FCoV antibodies. Accordingly, only ICA (c<sub>J</sub>NP/τPA) was used in the experiments below.

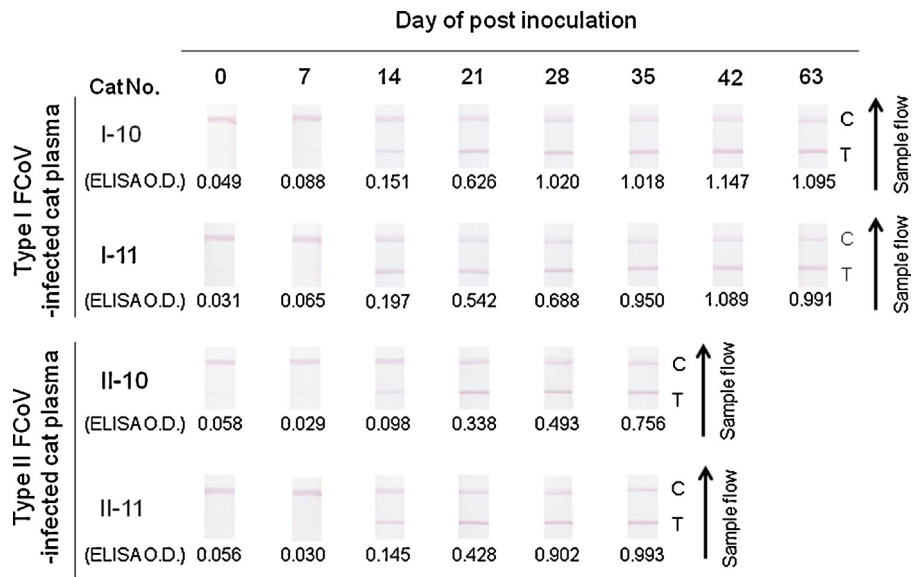
#### 3.6. Sensitivity of ICA (c<sub>J</sub>NP/τPA)

The sensitivity of ICA (c<sub>J</sub>NP/τPA) for anti-FCoV antibody detection was compared to that of ELISA (Fig. 5). SPF cats were inoculated orally with type I (FIPV KU-2 strain) or type II (FIPV 79-1146 strain) FCoV, and plasma was collected weekly. Anti-FCoV antibodies were detected in these plasma samples using ICA (c<sub>J</sub>NP/τPA) and ELISA. ELISA O.D. values increased slightly in all plasma samples on day 14 after the FCoV inoculation, and appeared to rise on day 21. A positive reaction was noted in ICA (c<sub>J</sub>NP/τPA) on day 14 after the FCoV inoculation, and all samples showed positive reactions on day 21 and thereafter. The ELISA O.D. value of the plasma sample of Cat No. II-10 on day 14 was close to the positive limit value (0.10), but was clearly positive in ICA (c<sub>J</sub>NP/τPA).

The sensitivity of ICA (c<sub>J</sub>NP/τPA) was confirmed in detail. Based on the positive limit value (0.10) of ELISA O.D., plasma samples from cats infected experimentally with FCoV were divided into 2 groups,

**Table 1**  
Comparison of anti-FCoV antibody detection sensitivities among the 3 types of immunochromatographic tests.

Method	Specificity (%)
c <sub>J</sub> IgG/τNP	75.7
c <sub>J</sub> NP/τNP	70.3
c <sub>J</sub> NP/τPA	100



**Fig. 5.** The detection of anti-FCoV antibodies using the immunochromatographic test ( $C_{JNP/T}PA$ ) and ELISA. Control uninfected SPF cats were inoculated with the type I FIPV KU-2 or type II FIPV 79-1146 strain, and plasma was collected weekly. Anti-FCoV antibodies in plasma samples were detected using ICA and ELISA. C: control line. T: test line.

**Table 2**  
Sensitivity of the immunochromatographic test ( $C_{JNP/T}PA$ ) for detecting the anti-FCoV antibody relative to ELISA.

ELISA (O.D.)	ICA		
	Positive	Negative	Positive %
<0.10	3 <sup>a</sup>	36 <sup>b</sup>	8.6
≥0.10	76	0	100.0

<sup>a</sup> ELISA O.D. values in 3 cats were 0.098, 0.096, and 0.095, respectively.

<sup>b</sup> ELISA O.D. values were lower than 0.08 in all 36 cats.

and their reactivity in ICA ( $C_{JNP/T}PA$ ) was investigated. All plasma samples with an ELISA O.D.  $\geq 0.10$  were positive (Table 2). Three of 39 plasma samples with an ELISA O.D. < 0.10 were positive, and the ELISA O.D. values of these 3 samples were close to the positive limit value (0.10).

**3.7. Sensitivity of ICA ( $C_{JNP/T}PA$ ) for serum, whole blood, and pleural effusion**

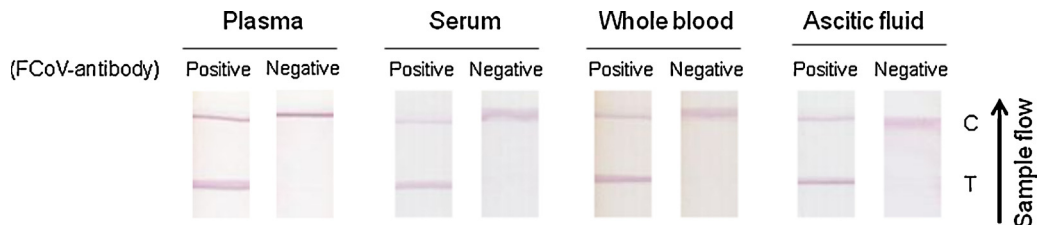
The reactivity of ICA ( $C_{JNP/T}PA$ ) with serum and whole blood samples from cats infected experimentally with FCoV and control uninfected SPF cats was investigated. Its reactivity with all specimens was similar to that with plasma samples (Fig. 6).

The reactivity of ICA ( $C_{JNP/T}PA$ ) with exudate samples judged as anti-FCoV antibody-positive ( $n=9$ ) and -negative ( $n=6$ ) on ELISA was investigated, and the results of ICA ( $C_{JNP/T}PA$ ) were the same as those of ELISA (Fig. 6).

**4. Discussion**

FCoV infection is reported frequently in domestic and wild cats, and outbreaks often occur in multi-cat environments, such as catteries and animal shelters (Pedersen, 2009). When an outbreak of virulent FCoV (FIPV) infection occurs in these environments, most cats in the environment are likely to develop FIP (Wang et al., 2013). An accurate and rapid test method is necessary to prevent an epidemic of FCoV infection. A low-cost FCoV test method is also needed to measure feline FCoV antibodies in animals maintained in multi-cat environments. The most appropriate diagnostic method meeting these conditions may be ICA. The detection of anti-FCoV antibodies using ICA requires no special device or reagent, and the results can be simply and rapidly obtained. Several ICA test kits to detect anti-FCoV antibodies are currently available commercially; however, their reactivity is lower than those of IFA and ELISA (Meli et al., 2013).

Three types of ICAs were prepared in the present study with the aim of developing an ICA test kit with high specificity and sensitivity. The optimum conditions of the ICA test kit were investigated using a simple trial kit comprised of a sample pad, nitrocellulose membrane, and absorbent pad. All the ICA test kits examined were capable of detecting anti-FCoV antibodies, while non-specific positive reactions of anti-FCoV antibody-negative plasma samples with the test line were noted in 2 ( $C_{JNP/T}NP$  and  $C_{JNP/T}NP$ ) of the 3 kits. rNP was used as the test line in these 2 kits, and a non-specific reaction with serum was also observed (data not shown). The cause of this non-specific reaction remains unclear.



**Fig. 6.** The reactivity of ICA ( $C_{JNP/T}PA$ ) with plasma, serum, whole blood, and ascitic fluid samples. Anti-FCoV antibody positive samples:  $n=9$ . Anti-FCoV antibody negative samples:  $n=6$ .

The N protein was used as a conjugate or test line in all 3 kits. In SARS coronavirus-infected humans, anti-N protein antibodies were shown to be present longer and were more abundant than antibodies against other structural proteins (Woo et al., 2004). The major antibody-binding domain was also shown to be present in the SARS-CoV N protein (He et al., 2004). Based on these findings, the N protein may be appropriate to detect anti-coronavirus antibodies. The trial ICA examined in this study can be applied to the detection of antibodies during infection not only with FCoV, but also with other coronaviruses.

Both the sensitivity and sensitivity of ICA ( $C_{jNP/T}PA$ ) were high in the 3 kits examined. Several studies have reported the usefulness of protein A as the test line in an ICA test kit, similar to that in ICA ( $C_{jNP/T}PA$ ), and the specificity and sensitivity were high in all reports (Cui et al., 2008; Jin et al., 2012; Li et al., 2012). Protein A specifically binds to the Fc fragments of mammalian IgG. Theoretically, ICA ( $C_{jNP/T}PA$ ) is also applicable to animals other than domestic cats, i.e., ICA ( $C_{jNP/T}PA$ ) developed in this study can be applied to the serological surveillance of FCoV in wild animals.

Affinity-purified cat anti-FCoV IgG was used as the control line in ICA ( $C_{jNP/T}PA$ ). As large amounts of serum and plasma from FCoV-infected cats had been stored prior to initiating the present study, a sufficient amount of affinity-purified cat anti-FCoV IgG could be prepared for the experiments conducted. However, on the assumption of commercializing ICA ( $C_{jNP/T}PA$ ), it is practically difficult to secure large amounts of serum and plasma from FCoV-infected cats. Whether several FCoV N protein-recognizing monoclonal antibodies can be used for the control line is currently being investigated to overcome this problem.

Although the presence or absence of the anti-FCoV antibody can be assessed using the ICA developed in the present study, measuring antibody levels is not possible, i.e., ICA is a qualitative test. However, quantitative testing may be possible using serially diluted samples. Therefore, to investigate the application of ICA to quantitative testing, the ELISA O.D. values of serially diluted samples should be compared with the reactivity of ICA.

Three types of ICAs were prepared in this study to detect FCoV antibodies, their reactivities were investigated with FCoV antibodies, and both the specificity and sensitivity of ICA ( $C_{jNP/T}PA$ ) were shown to be high. Modifying the test line and adding a conjugate pad and plastic cassettes is required to prepare a commercial kit for ICA ( $C_{jNP/T}PA$ ). The development of simple antibody test methods using the principle of ICA ( $C_{jNP/T}PA$ ) is expected for other coronavirus and feline viral infections.

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