

Measurement of feline lipase activity using a dry-chemistry assay with a triolein substrate and comparison with pancreas-specific lipase (Spec fPL™)

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ABSTRACT. Pancreatic lipase immunoreactivity (Spec fPL) is currently considered to be the most accurate blood test for the diagnosis of feline pancreatitis. In this study, we measured lipase activity in cats using a newer catalytic lipase assay of dry-chemistry system (FDC-v-LIP) to determine the reference range and compared the results with those for Spec fPL. Based on the results of healthy cats, the reference range of FDC-v-LIP was determined to be less than 30 U/l. FDC-v-lip did not show a strong correlation with Spec fPL in cats with various diseases, which resulted in the low sensitivity and positive predictive value. However, the relatively high (>90%) specificity and negative predictive value indicated that FDC-v-LIP could be a useful patient-side screening test for the exclusion of feline pancreatitis.

KEY WORDS: DRI-CHEM, feline, lipase, pancreatitis, Spec fPL

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Pancreatitis, especially chronic pancreatitis, is the most common exocrine pancreatic disease in cats [1, 6]. Diagnosis of feline pancreatitis remains difficult, because specific symptoms and biochemical findings have yet to be identified [3, 10]. Traditional catalytic assays for measuring serum lipase activity are believed to be unreliable in cats, because of poor sensitivity and specificity [7, 10]. Steiner *et al.* developed an assay for pancreatic lipase immunoreactivity (PLI) for dogs and cats [8, 9]. The use of immunoassays allows specific measurement of lipase produced by the exocrine pancreas. At present, feline PLI is usually measured by enzyme-linked immunosorbent assay using monoclonal antibodies (Spec fPL; Idexx Laboratories, Tokyo, Japan). The sensitivity and specificity of Spec fPL for detecting pancreatitis have been reported to be 79% and 82%, respectively [2]. Although Spec fPL is currently considered to be the most accurate blood test for the diagnosis of feline pancreatitis, its relatively high cost and its requirement for several days to obtain results are drawbacks.

Recently, a newer slide-based catalytic lipase assay using the dry-chemistry system (FUJI DRI-CHEM; FDC) was developed and introduced in Japan [4]. FDC is a system that enables measurement of various enzymatic activities in the blood easily and rapidly, and is widely used in veterinary practices in Japan. The newly developed assay slide (v-LIP-P) was designed to increase the specificity for pancreatic

lipase using triolein as the reaction substrate combined with colipase, bile salt and sodium dodecyl benzene sulphonate [5]. Measurements of lipase activity using the FDC analyzer and v-LIP-P slide (FDC-v-LIP) were validated in dogs with pancreatitis, and the results showed a strong correlation ($r=0.91$) with canine PLI (Spec cPL); FDC-v-LIP accurately distinguished healthy dogs from dogs with pancreatitis, identified based on Spec cPL values [4].

In the present study, we measured the lipase activity of feline serum samples using FDC-v-LIP to determine the reference value in cats and compared the results with Spec fPL concentrations to evaluate the effectiveness of FDC-v-LIP for diagnosing feline pancreatitis.

Serum samples were collected from healthy and client-owned cats visiting the Veterinary Medical Center of the University of Tokyo (VMC-UT). Thirty healthy cats, housed and cared for at the VMC-UT, were used as the healthy group. Routine blood chemistry and physical examinations of all 30 cats showed no clinical signs and no abnormalities. One hundred and thirty-eight client-owned cats visiting the VMC-UT between May and October 2013 with various diseases were used as the disease group. All procedures were conducted according to the animal experimentation guidelines of the University of Tokyo, and informed consent was obtained from the owners.

FDC-v-LIP was measured using a dry chemistry analyzer (FUJI DRI-CHEM 5000V, FUJIFILM Corporation, Tokyo, Japan). For Spec fPL™ analysis, serum samples were sent to a commercial laboratory (Idexx Laboratories). FDC-v-LIP and Spec fPL were measured on the day of the first visit to VMC-UT in the disease group.

The healthy group ($n=30$) consisted of 8 male (7 neutered) and 22 female (12 neutered) cats. Ages ranged from <1 to 10 years (median=4 years). Cats were of the following breeds: mongrel ($n=26$), Scottish Fold ($n=3$) and Russian

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Blue (n=1). The mean FDC-v-LIP concentration of the healthy group was 23 U/l (median 23 U/l, range 16–29 U/l). The mean Spec fPL value of the healthy group was 1.1 $\mu\text{g/l}$ (range 0.5–1.9 $\mu\text{g/l}$). As the mean + 2 SD of v-LIP in the healthy group was 29.51 U/l, we determined that the reference range of FDC-v-LIP in cats was <30 U/l, which is lower than that of dogs (<160 U/l).

The disease group (n=138) consisted of 75 male (66 neutered) and 63 female (58 neutered) cats. Age ranged from <1 to 18 years (median=10 years). The mean FDC-v-LIP concentration of the disease group was 23.27 U/l (median=22 U/l, range=1–106 U/l). The mean Spec fPL concentration was 3.97 $\mu\text{g/l}$ (median=1.7 $\mu\text{g/l}$, range=0.5–48 $\mu\text{g/l}$).

The association between FDC-v-LIP and Spec fPL (Supplementary Fig. 1) was examined using Pearson's product moment correlation coefficient. In cats with various diseases (n=138), the correlation coefficient was calculated to be $r=0.7017$, which is lower than the correlation coefficient between FDC-v-LIP and Spec cPL in dogs ($r=0.91$) [4]. In the Spec fPL test, the reference range is <3.6 $\mu\text{g/l}$, and the values ≥ 5.4 $\mu\text{g/l}$ are consistent with pancreatitis. Spec fPL values between 3.6–5.3 $\mu\text{g/l}$ are high but questionable range, and Idexx Laboratories suggests reevaluation in 2 weeks. As shown in Fig. 1A, the values of FDC-v-LIP did not correlate with those of Spec fPL when Spec fPL was less than 5.4 $\mu\text{g/l}$ (n=117, $r=-0.0132$). In contrast, a significant correlation (n=21, $r=0.894$, $P<0.0001$) was observed between the two values when Spec fPL was ≥ 5.4 $\mu\text{g/l}$ (Fig. 1B).

In the disease group, the FDC-v-LIP concentration was <30 U/l in 116/138 (84.1%) cats and ≥ 30 U/l in 22/138 (15.9%) cats. The Spec fPL concentration was ≤ 3.5 $\mu\text{g/l}$ in 107/138 (77.5%) cats, between 3.6 and 5.3 $\mu\text{g/l}$ in 10/138 (7.2%) cats and ≥ 5.4 $\mu\text{g/l}$ in 21/138 (15.2%) cats (Table 1). If Spec fPL (≥ 5.4 $\mu\text{g/l}$) is considered to be the gold standard for the diagnosis of feline pancreatitis, the sensitivity and specificity of FDC-v-LIP were 66.7% and 93.2%, respectively. Similarly, the positive predictive value (PPV) and negative predictive value (NPV) of FDC-v-LIP were 63.6% and 94.0%, respectively, in the disease group. These results indicate that care must be taken when interpreting positive FDC-v-LIP results. However, negative FDC-v-LIP results could indicate a low probability of pancreatitis (i.e., Spec fPL <5.4 $\mu\text{g/l}$). In the present study, however, we did not perform a histopathological examination of the pancreas in the healthy or disease groups. Therefore, the true sensitivity, specificity and PPV/NPV could not be calculated.

In this study, the values of FDC-v-LIP were elevated in 7 cats, without elevation of Spec fPL (<3.6 $\mu\text{g/l}$); this might cause the low PPV of FDC-v-LIP. The 7 cats were diagnosed with laryngitis (1), stomatitis (1), chronic kidney disease (1), cholangitis (1), hepatic lipidosis (1) and sclerosing encapsulated peritonitis (2). Although glycerol is known to influence the results of FDC-v-LIP [5], triglyceride, which can be degraded to produce glycerol, was not measured in the 7 cats. Therefore, blood chemistry analyses did not clarify the cause of the false positive FDC-v-LIP results in these cats. Considering that the sensitivity of Spec fPL is about 80%, these discrepancies were potentially false-negative er-

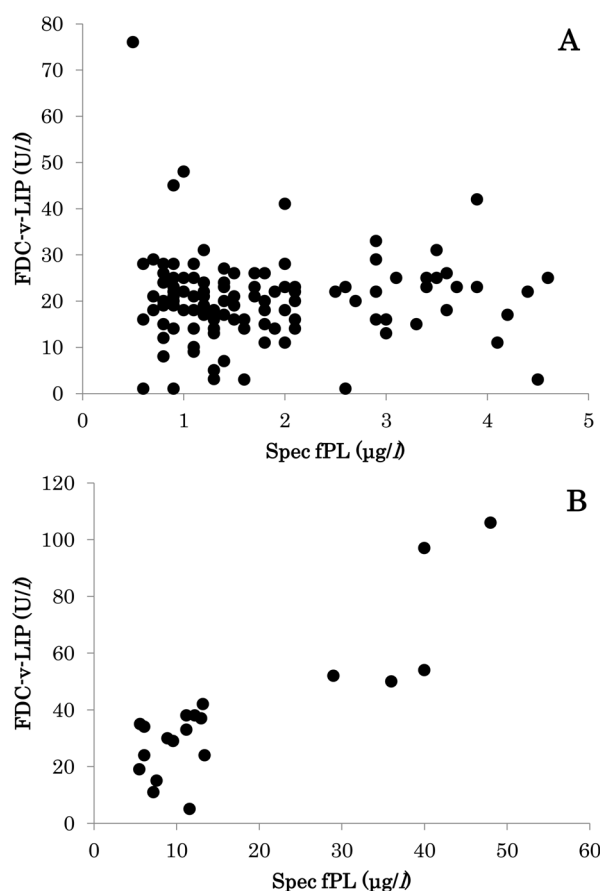


Fig. 1. Scatterplot showing distribution of serum lipase values measured by FDC-v-LIP and Spec fPL in cats with various diseases (n=138). Although values of FDC-v-LIP do not correlate with those of Spec fPL (<5.4 $\mu\text{g/l}$ (n=117, $r=-0.0132$ (A), a significant correlation (n=21, $r=0.894$, $P<0.0001$) is observed between the values when Spec fPL is ≥ 5.4 $\mu\text{g/l}$ (B).

Table 1. Contingency table of lipase values measured by FDC-v-LIP and Spec fPL in cats with various diseases (n=138)

		Spec fPL			Total
		<3.5 $\mu\text{g/l}$	3.6–5.3 $\mu\text{g/l}$	≥ 5.4 $\mu\text{g/l}$	
FDC-v-LIP	<30 U/l	100	9	7	116
	≥ 30 U/l	7	1	14	22
Total		107	10	21	138

ror of Spec fPL. A further study using cats with pancreatic biopsy is needed to know the true PPV and NPV of FDC-v-LIP using pancreatic biopsy tissue.

In conclusion, in cats, FDC-v-LIP did not show a strong correlation with Spec fPL, as Spec cPL does in dogs. The low sensitivity and PPV suggested that diagnosis of feline pancreatitis by measuring FDC-v-LIP alone would be problematic and that further diagnostic testing, including Spec fPL and ultrasound, would be required. However, the

relatively high (>90%) specificity and NPV indicated that FDC-v-LIP could be useful as a patient-side screening test for the exclusion of feline pancreatitis.

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