Novel Lipase Assay for a Rapid, Inexpensive, and Reliable Diagnosis of Pancreatitis in Dogs and Cats

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Introduction

- Pancreatitis in dogs and cats occurs commonly, but it is often underdiagnosed due to its nonspecific clinical signs, physical examination findings, and CBC and serum biochemistry results
- Biochemical assays for lipase have been traditionally used in the diagnosis of pancreatitis. However it is produced in multiple other tissues, including intestinal and adipose tissue and the biochemical assays have been unable to distinguish the source of the lipase.
- Immunoassays for the pancreatic specific lipase have been developed and validated for dogs (cPLI) and cats (fPLI) and have high sensitivity and specificity (1) for pancreatitis. However, they are expensive compared to biochemistry tests and need to be oursourced, which may take days before results are available. Frequently, values are reported as being greater than a moderate level of lipase. Alternatively, point-of-care Snap® tests may be used for rapid but qualitative or semiquantitative results.
- Recently, a biochemical lipase assay was described that has high sensitivity and specificity in dogs and cats (1-3). It uses a novel substrate (DGGR) and activators (colipase, calcium, bile acids) with high specificity for pancreatic lipase.

REACTION MECHANISM

CHROMOGENIC SUBSTRATE
1,2-o-Dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin)

↓ LIPASE

UNSTABLE INTERMEDIATE
Glutaric acid-(6-methylresorufin)

↓ Spontaneous breakdown

ABSORBANCE MEASURED AT 570nm
(methyl resorufin)

Reference Range for Healthy Dogs and Cats

	Cat	Dog	Cat	Dog
	DGGR-Lipase		Amylase	
Mean	15	28	960	735
SD	4	17	212	225
n	77	68	70	68
Range	9 – 25	8 – 81	403 – 1330	225 - 1190

- no significant anaemia, inflammation, liver or kidney disease, nor any clinical signs of pancreatitis such as vomiting or colic
- There is a 3 fold difference in cats versus dogs, but amylase values are about the same

Methods

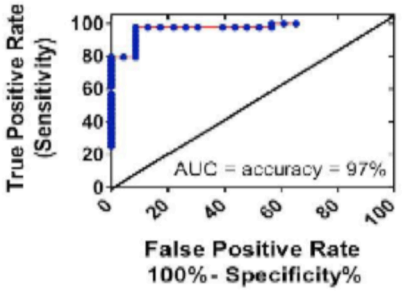
- A retrospective study of hospital records was made.
- All canine or feline records were retrieved for which there were determinations of lipase by both a) quantitative pancreatic immunoassay, and b) by DGGR-lipase: 67 dogs and 31 cats. Additionally, records for dogs for which point-of-care immunoassay snap-test (Snap® cPL™) and DGGR-lipase determinations had been made were retrieved: 42 dogs. Amylase data was also recovered for all these dogs and cats.
- Records from animals with clinical signs indicating a differential diagnosis of pancreatitis, including one or more of: vomiting, abdominal pain, diarrhea, weight loss, anorexia, and fever.
- DGGR and amylase assays (LI 3837 and AY 3805, Randox, UK) were performed on a Randox Imola® biochemistry analyzer according to manufacturer's recommendations.
- Snap-test results are qualitative, and considered to be either positive or negative. Equivocal tests were considered negative.

Data Analysis

- Linear regression analysis was performed. For correlations, the Spearman's r was calculated (GraphPad Prism 5.01, La Jolla, USA). Correlation was considered excellent (r>0.93), good (r=0.80-0.92), fair (r=0.59-0.79) or poor (r<0.59).
- ROC curves for enzymatic lipase and amylase constructed
- Normality checked with the D'Agostino & Pearson's omnibus test

Results

ROC Curve for Lipase >80



Optimal, Diagnostic Cut-offs & cPL Concordance for DGGR-Lipase and Amylase in Cats and Dogs

cPL		DGGR-Lipase		Amylase	
Diagnostic Cut off Values (IU/L)					
Cat	Dog	Cat	Dog	Cat	Dog
5.3	200	25	80	1400	1200
Concordance with PL (%)					
-	-	97	96*	71	75

*DGGR-lipase was only 90% concordant with snap test
67 dogs were tested with 67% cPL positive
31 cats were tested with 36% fPL positive

Comparisons of Sensitivity & Specificity of DGGR-Lipase, Snap Test & Amylase at Predicting cPL Test Result

	DGGR Lipase	Snap	Amylase
Sensitivity	93	86	58
Specificity	95	83	85

DGGR-Lipase Correlation with cPL Test

	cPL>200	cPL >300	cPL>400	Snap Test
Correlation (r)	1.0	0.84	0.74	0.67

- In order to compare, categorical values of 0 or 1 were assigned to each test result and check for correlation
- Best cut-off value to maximize correlations was 80 U/L for lipase
- Best cut-off value was a cut-off of 200 ug/L for cPL the upper limit of normal
- Snap test was much less effective than either cPL or lipase

Conclusions

•Snap® cPL and fPL are only semi quantitative, not giving a value for a clear positive and having lower accuracy then either the quantitative test or DGGR-lipase assay

•DGGR Lipase is of equivalent diagnostic value as quantitative cPL and fPL

•DGGR Lipase can be run on biochemistry analyzers found in most clinical pathology labs as opposed to outsourcing quantitative fPL or cPL giving more rapid turnover of results

• DGGR-lipase assay is most cost effective: costing us only an average of 50¢ per assay for reagents, whereas the quantitative PL test cost us €41 and the Snap® test cost €25

References

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