LymphoPro Feline Dx

Executive Summary

Benefits

LymphoPro Feline Dx™ is an ancillary diagnostic to conventional histopathology, providing veterinarians with additional molecular data for a more accurate differential diagnosis of small cell lymphoma (SCL) and inflammatory bowel disease (IBD) in cats. LymphoPro Feline Dx employs histology guided mass spectrometry (HGMS) profiling and improves feline care by offering the following advantages as compared to existing secondary tests:

- **Improved Accuracy:** Method validation was performed using 54 feline duodenal specimens (30 SCL and 24 IBD cases) and resulted in a sensitivity of 86.7% and a specificity of 91.7%. An indeterminant rate of 3.7% was observed among specimens analyzed and was included in the sensitivity and specificity calculations.
- **Objective Classifications:** LymphoPro Feline Dx leverages histology in combination with mass spectrometry imaging and machine learning algorithms to classify lymphocyte populations as either SCL or IBD.
- **Faster Results:** Test results are generally available to the ordering veterinarian within five (5) business days.
- **Less Tissue:** HGMS only requires three (3), 5-micron thick sections of feline duodenal tissue.
- **Enhanced Insight:** LymphoPro Feline Dx profiles the proteomic content of lymphocyte populations and provides a molecular snapshot of the active state of the cells, not just the genetic potential for malignancy.

LymphoPro

Feline Dx

The most accurate test to differentiate between feline small cell lymphoma and inflammatory bowel disease

89%	87%	92%
Accuracy	Sensitivity	Specificity

Accuracy, sensitivity, and specificity were evaluated relative to the consensus diagnoses rendered by a review panel composed of expert veterinary specialists.

Clinical Overview

Differential diagnosis of feline SCL from IBD is a challenge for veterinary specialists.[1,2] Accurate diagnosis is essential for the proper treatment and improved prognosis of cats with chronic enteropathy.[3,4] Both ailments result in similar clinical presentations often requiring veterinarians to obtain biopsy specimens for histopathological assessments and additional molecular testing. While histopathological evaluation of hematoxylin and eosin (H&E) staining is considered a standard procedure, in many cases, pathologists cannot confidently yield a differential diagnosis.[1,5] Due to the subjective nature of histopathology, there is also a high level of diagnostic variability when assessing intestinal tissues.[6]

Many pathologists rely on data from additional molecular tests such as immuno-histochemistry (IHC) and PCR for Antigen Receptor Rearrangements (PARR) to provide diagnostic clarity on challenging lesions.[1,5] IHC is used for immunophenotyping of lymphocytes, and while it provides a deeper molecular insight than histopathology alone, assessments are also subjective in nature. PARR, also called clonality testing, is used to determine if lymphocyte populations are derived from one or many precursor cells. Feline patients, where gastrointestinal (GI) specimens show lymphocyte clonality, are interpreted as having SCL, suggesting that lymphocytes derived from a parent neoplastic cell.

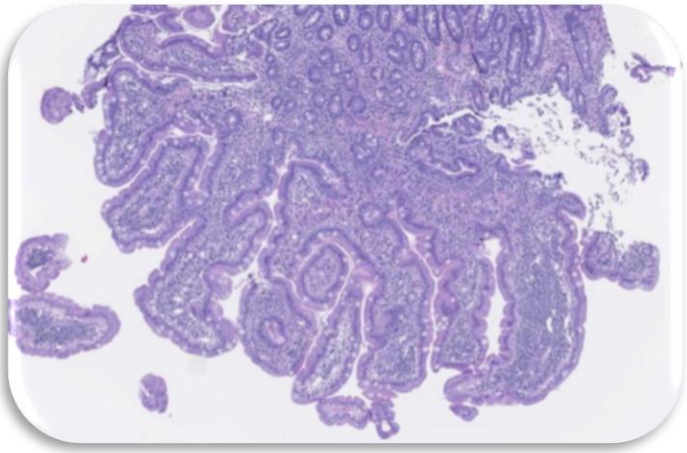

Figure 1: Standard H&E image of a small intestine biopsy.

While PARR is less subjective, there are significant limitations to its utility in differentiating between feline SCL and IBD. Studies have documented a limited sensitivity of PARR to detect feline SCL between 60-78%.[7,8] While specificity of PARR has been reported as high as 90% in detecting feline SCL, this study only compared feline SCL cases to non-reactive lesions, not reactive IBD lesions.[9] A recent study comparing feline SCL to IBD lesions showed the specificity of PARR to be as low as 30% relative to the consensus diagnoses of a panel of expert veterinary specialists, including pathologists, oncologists, and internists.[10] In a separate study of 20 clinically healthy, client owned cats with similar demographic characteristics to cats with chronic enteropathy, there was a significant discrepancy between the number of cats that were diagnosed with SCL based on histopathology alone (2 cats) as compared to the integrated results from H&E, IHC, and PARR (12 cats). Seven (7) of these cats had PARR analysis performed at two independent laboratories, and only three (3) of the seven (7) cats had PARR results that were in agreement between the two testing facilities.[11] Due to the lower and wide range of accuracies exhibited by PARR, this technique has limited value in the differentiation of SCL and IBD in cats.[7,8,10]

Intended Use

LymphoPro Feline Dx is intended for use in patients whose duodenal lesions can not be definitively assigned a diagnosis of SCL or IBD based solely on clinical and histopathological evaluations. The test provides additional molecular data to help the veterinarian more accurately diagnose these ambiguous cases in felines.

Test Process

LymphoPro Feline Dx requires the ordering veterinarian to request the collection of a total of three (3), 5-micron thick formalin fixed

paraffin embedded (FFPE) serial sections of biopsy tissue or, alternatively, one (1) FFPE tissue block. If the ordering veterinarian submits serial tissue sections, one tissue section will be mounted on a standard microscope slide and the remaining two tissue sections will be mounted on mass spectrometry compatible slides. One of the mass spectrometry slides is reserved as a backup in case the analysis needs to be repeated. The tissue section on the standard microscope slide must be sandwiched by the two mass spectrometry tissue sections. The tissue sections are then shipped to New River VDL where a veterinary pathologist identifies and annotates the areas of lymphocytic component to be targeted for proteomic analysis using HGMS. A minimum of five (5), 50-micron annotations of lymphocyte rich locations are required for analysis.

HGMS analysis requires tissue processing steps to increase access of proteolytic enzymes to cleavage sites of the proteins in the tissue sections. Following specimen preparation, the slide is loaded into the mass spectrometer and aligned to the merged annotated digital image of the H&E stained tissue section. This composite image is used to specifically target the data acquisition from the exact areas annotated by the veterinary pathologist. Typically, about 20 measurements from various areas throughout the lesion are collected from each tissue biopsy.

Interpretation of HGMS data is based on a classification algorithm that differentiates between SCL and IBD. The algorithm assigns a classification based on statistical analysis of the proteomic fingerprints obtained from the mass spectrometer. Classifications are determined based on the relative abundance and overall distribution of 18 mass spectral peaks corresponding to targeted molecular content. The molecular profile of the patient specimen is reported to the ordering veterinarian classifying a patient specimen as likely IBD, indeterminant, or SCL.

Figure 2: General overview of the HGMS workflow that LymphoPro Feline Dx employs to distinguish between SCL and IBD.

Method Validation

LymphoPro Feline Dx has been validated for feline duodenal biopsies that were considered to be either SCL or IBD. Algorithm training was performed with 39 specimens, composed of 22 SCL and 17 IBD cases. Algorithm validation was performed with an independent cohort of 54 specimens, composed of 30 SCL and 24 IBD cases, and resulted in a sensitivity of 86.7% and specificity of 91.7%. An indeterminant rate of 3.7% was observed among specimens analyzed. For the purposes of determining sensitivity and specificity, indeterminant specimens were included in the calculations.

Specimens used for both algorithm training and validation were reviewed by a panel of five (5) to seven (7) expert veterinary specialists, including pathologists, oncologists, and internists. Only specimens that received a consensus diagnosis were included in the algorithm training and validation. For each case, panel diagnosis was based on clinical presentation, histopathological evaluation, and IHC results. PARR was ordered for each case, and in the cases where PARR could be performed, this data was incorporated into the panel review. Attempts were made to obtain the clinical outcome for each case, and, when available, this outcome data was assessed to further confirm the diagnosis.

Comparative Study

During method validation, the results of the LymphoPro Feline Dx algorithm and PARR test were compared to the consensus diagnoses of the review panel. In total, 52 biopsies yielded data from both tests, including 28 SCL and 24 IBD cases. The LymphoPro Feline Dx algorithm exhibited significantly improved accuracy, including sensitivity, specificity, and indeterminant rate, as compared to PARR.

Specimens (n=52)	LymphoPro Feline Dx	PARR
Accuracy	90.4%	61.5%
Sensitivity	89.3%	85.7%
Specificity	91.7%	33.3%
Indeterminant	1.9%	13.5%

Table 1: Overall accuracy, sensitivity, specificity and indeterminant rate resulting from a cohort of 52 specimens analyzed by both LymphoPro Feline Dx and PARR.

Summary

Differential diagnosis of feline SCL and IBD biopsies poses a significant and persistent challenge to the veterinary community. LymphoPro Feline Dx serves as an improved, objective adjunct to histopathology in the diagnosis of challenging feline GI cases by

addressing the limitations of existing secondary tests.

HGMS profiling of lymphocyte populations, combined with the use of classification algorithms, provides a spectral fingerprint for the targeted analysis of feline duodenal biopsies.

Recent method validation and comparative study data illustrate the ability of LymphoPro Feline Dx to provide more accurate diagnostic results and enhanced veterinary insight into the active state of lymphocytes while requiring less tissue material and providing faster results.

LymphoPro Feline Dx was developed in collaboration with Dr. Jörg Steiner (Texas A&M University) and Dr. Sina Marsilio (University of California-Davis).

References

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