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LETTER TO THE EDITOR

# Letter to editor regarding Results of histopathology, immunohistochemistry, and molecular clonality testing of small intestinal biopsy specimens from clinically healthy client-owned cats

Dear Editor,

I read with great interest the recent article by Marsilio and colleagues[1] describing gastrointestinal pathology findings in clinically healthy cats. These authors make an excellent case for redefining the notion of what constitutes a "normal" gastrointestinal tract in this patient population. I do believe, however, that some additional perspective is warranted on the points of discussion the authors raised on the use of clonality testing in small animals.

Clonality tests are typically regarded as moderately-to-highly sensitive and highly specific for detecting the presence of lymphoid neoplasia.[2,3] However, as the authors mention, clonality tests are not currently standardized in veterinary medicine, and the results of these tests must therefore be interpreted in the context of laboratory-specific sensitivity and specificity values.[4] The authors did not report these values for the laboratory that analyzed the samples described in their article nor do I believe that this laboratory posts these values in a public forum. Other laboratories, however, do so. For example, 1 laboratory cites a sensitivity of 65% and specificity of approximately 94% for its feline clonality assay on its website.[5] The positive likelihood ratio for this test, calculated as the sensitivity divided by the false-positive rate, is 0.65/0.06 or 10.83 (technically 10.83:1). This value reflects the odds of a positive result occurring in a patient with lymphoid neoplasia relative to the odds of such a result occurring in a patient without lymphoid neoplasia. This value would seemingly suggest that a positive test in any cat should be taken as highly suggestive of the presence of cancer.

In a study such as the 1 the authors conducted, however, where disease prevalence is likely to be low, sensitivity and specificity (and the positive likelihood ratio derived from these values) are not the only important measures of assay performance to be considered. The positive and negative predictive values of a test are perhaps more important in this situation, as low disease prevalence will dramatically affect these values. For example, the results of the authors' study suggested that the true prevalence of small-cell lymphoma (SCL) in clinically "healthy" cats is approximately 10% (2/20 cats). If the prior probability of SCL in this population is 0.1, then the prior odds of SCL can be calculated as 0.1/(1–0.1) or 0.11. The post-test odds of disease are simply the product of the prior odds and the positive likelihood ratio for the clonality assay, or 0.11 × 10.83, yielding a value of 1.20. The post-test probability of disease (a.k.a. positive predictive value) in this setting, then, is calculated by dividing the post-test odds by (1 + post-test odds), yielding a value of 0.54, or 54%.

For the sake of comparison, the same calculation can be made when the clonality assay is put to its intended use: confirming a cancer diagnosis in patients with suspected lymphoid neoplasia. In this population, disease prevalence is likely to be much higher than what Marsilio and colleagues observed. For instance, if the disease prevalence in this population is 90%, the positive predictive value of the test increases to 99%. Even if the disease prevalence drops to 60%, the positive predictive value remains very high at 94%. However, at very low disease prevalences, the positive predictive value of the test drops rapidly, to 73% at a disease prevalence of 0.2, and to 36% (ie, worse than a coin toss) at a prevalence of 0.05. Such a low disease prevalence in a population of apparently healthy older cats would not be inconceivable, given the authors' results. In this population of cats, therefore, clonality assays probably should not be run in the first place as they add no clarity to the patient's diagnostic assessment.

The authors concluded their article, "Although the sensitivity of molecular clonality testing is generally considered to be high, our results imply that further assessment of the specificity of this diagnostic modality may be warranted." I believe this statement is misguided. Most pathology laboratories performing clonality assays are careful to emphasize that the results of these tests should not be interpreted in a vacuum. They must be interpreted in light of histopathologic and immunophenotypic data.[6] The authors were careful to emphasize this point as well. I would add, however, that it is equally important that the results of clonality tests be interpreted in light of *clinical* findings. Failure to do so is likely to result in a misdiagnosis of neoplasia, a conclusion I believe is confirmed by the authors' results. Such a misdiagnosis may end up subjecting cats to additional unnecessary diagnostic tests, such as surgical collection of full-thickness intestinal biopsies. As diagnosticians, we must be constantly aware that our own performance—choosing the right test at the right time in the right patient—is just as important to making an accurate diagnosis as the

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performance of a diagnostic test itself. The apparent poor performance of the clonality assay in this group of clinically normal cats likely reflects not an inherent shortcoming of the assay but rather its inappropriate use in this patient population.

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