FACT SHEET 2

AXIOM Veterinary Laboratories Ltd

'The Quality Clinical Pathology Service'

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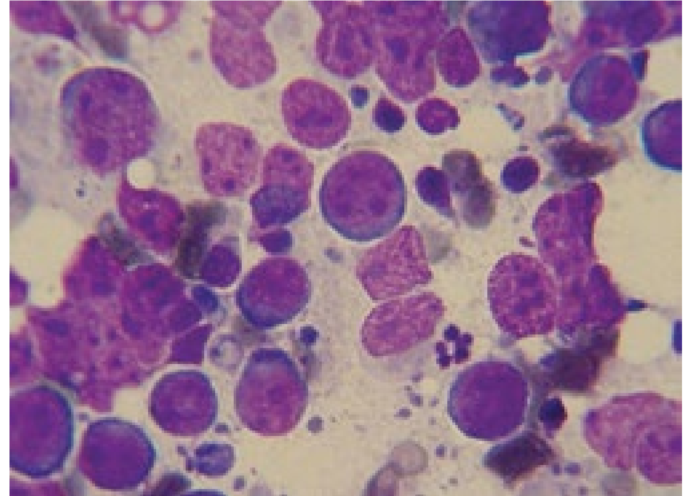
Canine Lymphoma Phenotyping and Clonality Analysis



This fact sheet is intended to give you information regarding ancillary testing on canine lymphoma cases at Finn Pathologists or any of the laboratories within our labs division.

Background

Canine lymphoma is a common disease, and the diagnosis of it and lymphoid leukemia in advanced stages is generally uncomplicated, however some presentations of the disease can be a diagnostic challenge. In certain situations, lymphoma can be difficult to distinguish from a benign reactive proliferation of lymphocytes particularly in chronic indolent forms of the disease.[1] Several adjunct diagnostic methodologies have been developed, over the years, to improve the diagnosis in these cases. In addition, these techniques have led to the improved ability to often accurately differentiate the T versus B cell lineage, and identify the clonality and phenotype of canine lymphomas. This has improved the prognostic indications related to phenotype, as well as the developing of more specific variations in treatment methods related to T or B cell lineage.

There are currently 4 primary methods of ancillary diagnostic techniques to attempt further confirmation of canine lymphoma, and potential clonality evaluation; after lymph node cytopathology and/or node biopsy and histopathology analysis has been performed.

1. Immunocytochemical staining of Fine needle aspirate (FNA) cytology smear samples (ICC for lymphoma).
2. Polymerase chain reaction (PCR) for antigen receptor rearrangements (PARR).
3. Immunohistochemical staining of tissue biopsy samples (IHC for lymphoma).
4. Flow cytometry staining and analysis of lymphocytes in blood or FNA from nodes.

General information regarding these techniques includes:

- IHC and ICC tests are performed in our labs, while Flow cytometry and PARR are send away tests with longer turn around for results.
- IHC, ICC, and Flow cytometry all require immunostaining of the T and B cell receptors on the lymphocytes, with counts and percentages of positive cells analysed (Actual counts in Flow testing, and estimated percentages in IHC/ICC) to help attempt to determine phenotype and clonality. PARR utilizes a fundamentally different methodology, polymerase chain reaction, to amplify the variable regions of immunoglobulin genes and T-cell receptor genes to detect the presence of a clonal lymphocyte population. Therefore PARR does not rely on the quality, freshness or intactness of the lymphocytes being analyzed. In addition, previously stained slides as well as small sample sizes can often be tested effectively with this technique.
- IHC with biopsy and histopathology of nodes can be very helpful in confirming a diagnosis in difficult lymphoma cases.
- IHC and ICC are generally equally accurate in determining clonality and lineage of T and B cell lymphomas, depending on the size and quality of the samples submitted.[5]

- ICC has a 70-80% accuracy, in our hands, for determining clonality in already confirmed lymphoma cases; depending on the quality of the samples submitted. This test requires unstained cytology smears for this level of accuracy.
- Flow cytometry has a very high accuracy for neoplastic lymphocytes in the blood (leukemias / Stage V lymphomas), and high accuracy for node aspirates in special preservative solution. *Note, however the accuracy and effectiveness of this test decreases dramatically and quickly over time; therefore ideally samples are best analysed within 1-2 hours after collection. When this sample is sent in, note that it often requires a minimum of 24 hours to be processed and analysed; therefore the actual accuracy is often decreased significantly.[2]
- PARR has a 91% accuracy rate for identifying T or B cell clonality and a very low false negative rate. Rare false positives have been identified in cases of Ehrlichial infections.[1,3]
- PARR Assessment and demonstration of clonality generally provides the most accurate diagnostic tool for canine lymphoma diagnosis. PARR using DNA from cytologic specimens and peripheral blood provides a minimally invasive method to confirm neoplasia, monitor treatment and assess recurrence or remission by demonstrating clonality, while concurrently providing the lymphocyte phenotype.[4]

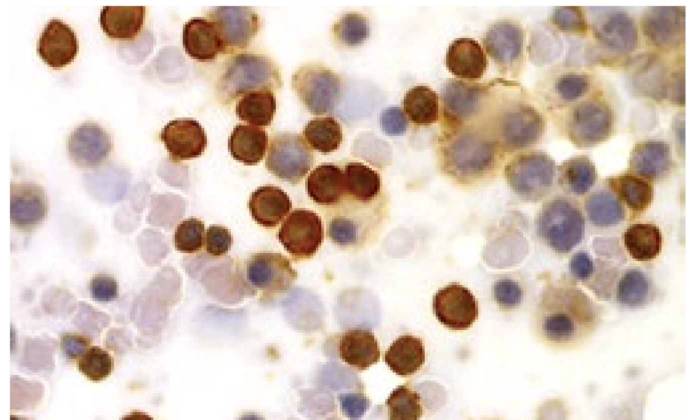
Consultation with a clinical oncologist is always advisable if considering further tests and/or treatment options for these tumours.

ICC

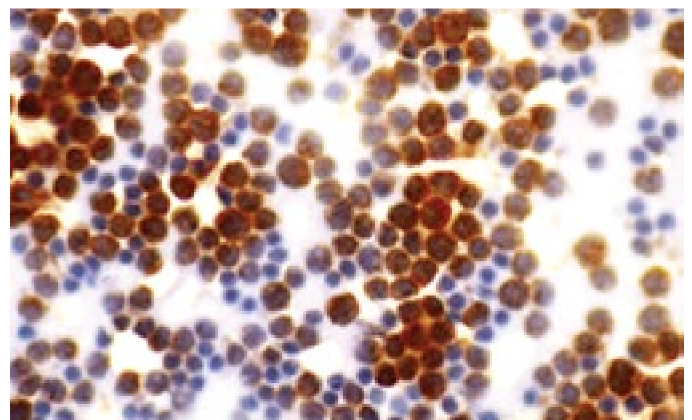
T and B cell receptors on neoplastic lymphocytes will be clonal and all of the same type; therefore we can take advantage of this by labeling these receptors with specific antibodies that will bind to conserved portions of these receptor molecules on T and B cell receptors. These monoclonal antibodies also have attached loci for an immunologically specific stain so that when these cells are exposed to the appropriate staining process, they will be labeled with a prominent brown marker. Note that due to variability in the cells and the quality of the slides, the staining uptake may be variable with sometimes weak positive staining or strong positive staining and all variations in between. Also, if many of the cells are broken / lysed or not intact, then the staining may not be effective or may have more uniform diffuse background staining which makes the interpretation challenging. This is why there are approximately 20-25% of these cases that will be equivocal, and not give a clear indication of T or B cell type.

In addition, one of the factors we have learned that contributes to more challenging and equivocal results on this staining test, is when the slides have already been stained for initial cytology examination. If we try and de-stain and then re-stain with these immunologic specific markers, the quality of the staining decreases fairly dramatically and we do not recommend attempting this.

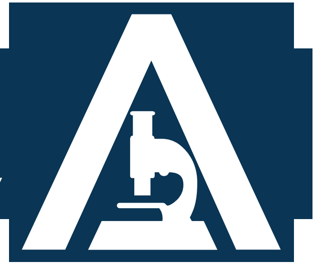
Therefore, we recommend unstained aspirates of ideally at least 2 nodes, with 2-4 slides submitted for the ICC test. These should be air dried and sent to the lab for processing with the submission form checked / listed for ***ICC special stains for Lymphoma.***



CD3 – Approximately 20% strong Positive T cells with 10-20% weak Positive – Equivocal Result but less likely a T cell phenotype here; potential Double Positive Phenotype (T+B cell) if high percentage of B cell Positive staining also seen.



CD79a – Approximately Greater than 80% strong Positive – Likely indicates a B Cell Phenotype.



The turn around time for these results will be 7-10 days, and generally, the pathologist that initially saw your case and diagnosed lymphoma will likely be the one to write an ADDENDUM report added to your previous report with the results of the ICC testing, or a new report may be issued as well.

PCR / PARR

This test may be run on any sample type including aspirates from nodes into EDTA tubes, Unstainied ***or*** stained slides from previous node FNAs, or on tissue samples from node biopsies. This test cannot be run on the animals blood; ***unless*** there are neoplastic lymphocytes circulating in the peripheral blood such as seen with Stage V lymphoma or with Lymphocytic Leukemia cases.

The turn around time for these results will be 12-14 days, and generally, the pathologist that initially saw your case and diagnosed lymphoma will likely be the one to write an ADDENDUM report added to your previous report with the results of the PARR testing.

IHC

This test may be run or requested on tissue biopsies from wedge or entirely removed enlarged lymph nodes, and added on after initial histopathology diagnosis of lymphoma. Similar to ICC, the tissue sections are stained with the immunologic specific stains for the T and B cell markers.

The turn around time for these results will be 7-10 days, and generally, the pathologist that initially saw your case and diagnosed lymphoma will likely be the one to write an ADDENDUM report added to your previous report with the results of the IHC testing, or a new report may be issued as well.

FLOW CYTOMETRY

This test is more designed as an ancillary test primarily for lymphocytic leukemias or Stage V lymphomas, with the cells labeled in the blood sample submitted. This test has more recently been adapted to work on FNAs of enlarged lymph nodes, provided that the aspirates are immediately placed into a special preservative solution in special tubes to help keep the cells healthy and intact for labeling. The cells are then run through the flow cytometer. Similar type T and B cell markers are used to label the receptors on the lymphocytes, however the marker used is a fluorescent antibody marker which will be counted by the automated cell counter in the flow cytometer. More specific percentages of T versus B cell positive cells are then calculated and the interpretation of the lymphocyte phenotype is recorded and reported.

The turn around time for these results will be 14-17 days, and generally, the pathologist that initially saw your case and diagnosed lymphoma will likely be the one to write an ADDENDUM report added to your previous report with the results of the Flow Cytometry testing.

Additional references

1. Vet Pathol. 2003 Jan;40(1):32-41. *Diagnosis of canine lymphoid neoplasia using clonal rearrangements of antigen receptor genes.* Burnett RC1, Vernau W, Modiano JF, Olver CS, Moore PF, Avery AC.

2. J Vet Intern Med. 2013 Nov-Dec;27(6):1509-16. doi: 10.1111/jvim.12185. Epub 2013 Sep 20. *Lymphoma immunophenotype of dogs determined by immunohistochemistry, flow cytometry, and polymerase chain reaction for antigen receptor rearrangements.* Thalheim L1, Williams LE, Borst LB, Fogle JE, Suter SE.

3. J Vet Intern Med. 2006 Mar-Apr;20(2):329-34.*Utility of polymerase chain reaction for analysis of antigen receptor rearrangement in staging and predicting prognosis in dogs with lymphoma.* Lana SE1, Jackson TL, Burnett RC, Morley PS, Avery AC.

4. Can Vet J. 2010 Jan; 51(1): 79–84. *Clonality and phenotyping of canine lymphomas before chemotherapy and during remission using polymerase chain reaction (PCR) on lymph node cytologic smears and peripheral blood.* Dilini N. Thilakaratne, Monique N. Mayer, Valerie S. MacDonald, Marion L. Jackson, Brenda R. Trask, and Beverly A. Kidney

5. Pol J Vet Sci. 2010;13(4):661-8. *Practical aspects of immunocytochemistry in canine lymphomas.* Sapierzyński R

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