LYMPHOMA DIAGNOSIS

CANINE LYMPHOMA BLOOD TEST (cLBT)

SENSITEST

INNOVATION IN ANIMAL HEALTHCARE

Avacta ANIMAL HEALTH
Lymphoma is the most common malignancy affecting dogs, accounting for approximately 20% of all canine tumours and having an estimated annual incidence of approximately 25 per 100,000 dogs. The occurrence of lymphoma increases with age, having a median age of onset around seven years old. It can be seen in any dog, although some breeds show a particularly high incidence including Golden Retrievers, Boxers, German Shepherds and West Highland Terriers. It is well established that lymphoma is one of the more treatable cancers and that earlier diagnosis and treatment will give a better prognosis.

Canine lymphoma is a complex disease which results from mutations in lymphoreticular cells. There are basically 5 types of lymphoma resulting from the location of malignant growth - multi-centric, mediastinal, gastro-intestinal, cutaneous, extra nodal and central nervous system. The disease usually presents as painless swellings in the peripheral lymph nodes, however, non-peripheral disease can seriously complicate diagnosis.

Typical presenting signs are:

- Lymphadenopathy
- PU/PD
- Hypercalcaemia
- Vomiting and Diarrhoea
- Weight Loss
- Loss of Appetite
- Lethargy/Loss of Stamina

It is a minimally invasive procedure that can be used as a precursor to histological diagnosis of the disease. Definitive diagnosis requires cytological examination of tissue from fine needle aspirate (FNA), or, preferably histological classification of a biopsy collected from the affected tissue. Once diagnosed, patients generally respond rapidly to chemotherapy, with peripheral lymphadenopathy being visibly reduced within seven days.

**BENEFITS OF cLBT**

- A simple blood test
- Can be used as part of blood work up at initial appointment
- Results available within 24 hours from receipt of sample
- Similar cost to fine needle aspirate (FNA) but less invasive
- Aids in earlier diagnoses
- No sedation required
- Differentiate between benign lymphadenopathy and malignant lymphoma
- Ideal as an alternative to non-diagnostic/inconclusive FNAs.

**NEW OPPORTUNITIES FOR YOUR PRACTICE**

The canine Lymphoma Blood Test (cLBT) is a blood test that simply requires 1ml of serum. The test measures the levels of C-reactive Protein (C-RP) and Haptoglobin (HAPT), then combines these readings with a unique algorithm to provide a new tool to assist in the diagnosis of lymphoma.
ASSISTING IN THE DIAGNOSIS OF LYMPHOMA

Lymphoma in dogs presents with a wide range of symptoms, however swollen lymph nodes (lymphadenopathy) is the strongest indication of the disease. Aspiration or biopsy of the affected lymph nodes remains the gold standard test for diagnosis of lymphoma and most practices will rely on fine needle aspiration (FNA) combined with cytological examination. However, FNA samples are often inconclusive or non-diagnostic. Additionally, the FNA approach is only applicable to cases presenting with peripheral lymphadenopathy. Since lymphoma can also affect the numerous internal lymph nodes illustrated in the following diagram, many cases cannot be accessed by FNA.

The cLBT provides a new and valuable tool to assist in the diagnosis of canine lymphoma. It has been used to confirm the veterinarian’s diagnosis in cases when FNA results are inconclusive. It can also help in decisions for further costly investigations in cases of suspect non-peripheral lymphoma.

Cases studies which illustrate how cLBT has been used in cases of inconclusive or inaccurate FNA are illustrated below:

DISCOVERING THE ACUTE PHASE PROTEINS

We have previously reported that mass spectrometry was able to discover multiple biomarkers capable of differentiating between dogs diagnosed with lymphoma from healthy dogs and those diagnosed with diseases presenting in a similar manner to lymphoma. Subsequently we demonstrated that two of the biomarkers discovered by mass spectrometry were the acute phase proteins (APPs), C-Reactive Protein (C-RP) and Haptoglobin (HAPT).

Other publications have also reported that C-RP levels increase in canine lymphoma and C-RP tests are now routine in the diagnostic work up of Non Hodgkins lymphoma in Humans. However, C-RP alone lacks specificity to assist in the diagnosis of lymphoma in dogs. By adopting a multi-variant analytical approach to develop algorithms employing both C-RP and HAPT values, we have been able to develop a new test which overcomes the limitations found by measuring single APPs.
THE ALGORITHM

By working with the University of Leicester, a multivariate algorithm was developed which draws on a database of samples (control and lymphoma), using the relative levels of C-RP and HAPT, the age and sex of the animal and the presence of lymphadenopathy. All samples in the database were confirmed as positive either by cytology or histopathology and all negative samples were confirmed to be free of the disease for a minimum of 6 months after the serum had been taken. The algorithm uses three distinct analytical processes to assess the risk of lymphoma in each dog being presented to it. The algorithm combines decision trees (as shown in Figure 1), k nearest neighbour analysis (KNN) and probability density function estimation (PDFE) [Scott, 1992]. Further information is available upon request or you can view online at http://arxiv.org/ftp/arxiv/papers/1305/1305.4942.pdf

SAMPLES USED FOR cLBT DEVELOPMENT

<table>
<thead>
<tr>
<th>Outcome</th>
<th>N</th>
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</thead>
<tbody>
<tr>
<td>TP</td>
<td>73</td>
</tr>
<tr>
<td>TN</td>
<td>144</td>
</tr>
<tr>
<td>FP</td>
<td>26</td>
</tr>
<tr>
<td>FN</td>
<td>9</td>
</tr>
<tr>
<td>Borderline</td>
<td>51</td>
</tr>
<tr>
<td>Sensitivity</td>
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</tr>
<tr>
<td>Specificity</td>
<td>84.7%</td>
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<tr>
<td>PPV</td>
<td>73.7%</td>
</tr>
<tr>
<td>NPV</td>
<td>94.1%</td>
</tr>
</tbody>
</table>

Table 1: Performance breakdown of the cLBT.

**Figure 1.** An example of a typical Classification and Regression Tree based algorithm.

**KEY:**
- TN = True Negative
- TP = True Positive
- FN = False Negative
- FP = False Positive
- PPV = Positive Predictive Value
- NPV = Negative Predictive Value
- N = Number of samples in each group.
CLBT USE IN PRACTICE

Diagnostic Testing – The cLBT is an aid to diagnosis to be used when you suspect lymphoma in patients showing classical symptoms such as generalised lymphadenopathy, PU/PD and lethargy. It can be used as an initial test for differentiating between benign lymphadenopathy and malignant lymphoma.

Hypercalcaemia – Lymphoma is generally considered to be the most common cause of hypercalcaemia in dogs occurring in approximately 40% of cases. A high proportion of these lymphomas tend to be non-peripheral which are difficult to diagnose from palpation or FNA alone. The cLBT becomes a useful reflex test when hypercalcaemia is flagged up, establishing a more rapid diagnosis.

Remission Monitoring – We have conducted a separate retrospective study for remission monitoring over a period of 4 years (2008-2012)* and the results show:

- both physical examination (vet diagnosis) and cLBT correlate extremely well
- cLBT can detect a lymphoma relapse up to 8 weeks in advance before clinical signs appear
- cLBT may be useful as a prognostic tool before treatment
- Evidence suggests patients that achieved a lower cLBT value during treatment tended to live longer.
- Objective remission monitoring can be carried out at first opinion practice

For more information on using the cLBT for remission monitoring, please see our ‘Lymphoma Remission Monitoring’ brochure.


SAMPLE COLLECTION

- Take 2ml of whole blood in a serum gel tube and allow to clot at room temperature.
- Centrifuge sample to obtain 1ml serum, and place into an appropriate transport tube (plain serum or serum gel tubes preferred).
- Wrap in cotton wool, insert into a bio-hazard bag and place in a SENSITEST submission box or other protective packaging. Sample can be chilled but not frozen.
- Send by post using the freepost envelope provided within the submission box (UK only).

RESULTS

Results will be made available by email (or facsimile/post if requested) within 24 hours of receiving the sample. The cLBT ranks the probability of lymphoma from 0 to 5, where 0 indicates dogs typically free of lymphoma and 5 equates to dogs typical with lymphoma. An example of a canine lymphoma test result is shown below. Our customer services team are on hand to respond to any enquiries by telephone or email.

Score Explanation
0.00-1.92: Low cLBT score (typical of dogs free of lymphoma)
1.95-3.28: Borderline cLBT score
3.29-5.00: High cLBT score (typical of dogs with lymphoma)

“We have confidence to consider the cLBT as part of our diagnostic procedure where we suspect, or wish to rule out lymphoma. As it is very difficult to diagnose lymphoma until it is quite apparent, it is good to know we now have a simple blood test that can help in ruling this condition in or out of our list of differentials”

Sarah Probert - Bridgnorth Veterinary Centre, Wolverhampton
A BETTER LEVEL OF CARE

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