Practical Oncology for the Veterinary Practitioner

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HESKA

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OUTLINE

- **Fine needle biopsy and cytology**
  - Cutaneous/SQ and percutaneous
- **Incisional vs excisional biopsies**
  - Indications for each
- **Molecular diagnostics for lymphoid cancers**
  - Flow cytometry and PARR

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FINE NEEDLE BIOPSY & CYTOLOGY

- Techniques
- Advantages and limitations
- Percutaneous sampling – prerequisites and risks
- Diagnostic yield and accuracy

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FINE NEEDLE BIOPSY:

- ASPIRATION
- FENESTRATION

- **Cutaneous/subcutaneous**
  - Including perianal/rectal
- **Percutaneous**
  - US-guided

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22-23G needles

12cc syringe

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FINE NEEDLE BIOPSY AND SLIDE PREP

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FINE NEEDLE BIOPSY OF ANAL SAC MASS

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FNA/FNF + CYTOLOGY

ADVANTAGES

- Inexpensive
- Expedient
- Noninvasive
- No special equipment or skills required
- Performed in awake or sedated patients
- Low morbidity
- High diagnostic yield

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FNA/FNF + CYTOLOGY
LIMITATIONS

- Small sample
- Poorly exfoliating neoplasm
- Cell lysis
- Hemodilution
- Necrosis
- Concurrent inflammation
- Lack of tissue architecture
- Cannot determine tumor grade

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PERCUTANEOUS FNA

- Intra-thoracic and intra-abdominal lesions
- Masses/nodules
- Staging purposes
  - Suspected metastatic lesion
  - Liver and spleen cytology for round cell tumor staging

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PERCUTANEOUS FNA
RISKS

Seeding

- Smith 1991: 0.009% risk in people
  - Pancreatic carcinoma
- Rare in vet med
  - No studies assessing risk
  - Urothelial carcinoma

Hemorrhage

- Léveillé et al. 1993: 195 dogs, US-guided biopsy or FNA
  - <2% major complication (bile peritonitis and hemorrhage); 5.6% minor localized hemorrhage
- Watson et al. 2011: 38 dogs, splenic FNA and needle core bx
  - No hemorrhage

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PERCUTANEOUS FNA
PREREQUISITES

US guidance by trained personnel	
Platelet count >100,000/uL	PT/PTT if concern for coagulopathy
Chemistry	• Liver enzymes and pseudo function tests • Globulins
Sedation	Opioid of your choice (e.g. butorphanol) + • Dexmedetomidine – 3-10mcg/kg IV or 250-500mcg/m2 IM • Alfaxalone – 0.5 – 2mg/kg IV, titrate to effect

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PERCUTANEOUS FNA
PROCEDURE

1) Remove gel & Sterile prep → 2) 1.5 inch, 22-23 G needle with 6-12mL syringe attached → 3) Non-aspirate technique → If poor sample, apply aspirate technique

4) 3-4 samples → 5-7 slides → 5) Rescan abdomen → 6) Quality control

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CYTOLOGY
DIAGNOSTIC YIELD AND ACCURACY

- Bonfanti et al. 2004
  - Retrospective, comparing cytology vs histopathology of masses from thorax and abdomen in dogs and cats
  - 152 samples
  - ~87% retrieval rate for diagnostic sample
  - ~90% agreement for differentiating inflammation vs neoplasia
  - Positive predictive value for neoplasia detection 100%
  - Negative predictive value for neoplasia detection ~55%

Takeaways:
- High diagnostic yield and accuracy overall and low false positives
- Cannot rule out neoplasia with cytology

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CYTOLOGY
DIAGNOSTIC YIELD AND ACCURACY

- Recognize limitations
  - Set expectations with client
  - Keep in mind with interpretation

- Quality control
  - Look yourself, 1-2 slides with Diff-Quick stain
  - Submit 5-7 slides

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TISSUE BIOPSY

- Indications for incisional vs excisional

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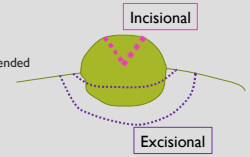
BIOPSY TECHNIQUES

- Incisional
  - Lesion cannot be removed with appropriate margins
  - Diagnosis will impact client decisions or what treatment is recommended
  - Examples
    - Oral tumors
    - Feline injection site sarcomas and soft tissue sarcomas in dogs
    - Large cutaneous tumors
- Excisional
  - Can completely excise the tumor with appropriate margins
  - Result will not impact client decisions
  - Examples
    - Cutaneous tumors



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MOLECULAR DIAGNOSTICS FOR LYMPHOID NEOPLASIA

Flow Cytometry

PARR

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LYMPHOPROLIFERATIVE DISEASES
BACKGROUND

- > 50 different types of lymphoma/leukemia in humans
- Derived from different stages of lymphocyte development

Precursor | B cell | T cell

Table 1. Summary of Canine Malignant Lymphoma Revised From the Revised European-American Classification of Lymphoid Neoplasms/ World Health Organization Classification of Lymphoid Neoplasms

B Cell Neoplasms
- Precursor B cell neoplasms
- Precursor B lymphoblastic leukemia/lymphoma
- Mature (peripheral) B cell neoplasms
- B cell chronic lymphocytic leukemia/prolymphocytic Leukemia/small lymphocytic lymphoma
- B cell prolymphocytic leukemia
- Lymphoplasmacytic lymphoma
- Splenic marginal zone B cell lymphoma
- Plasma cell myeloma/plasmacytoma
- Extranodal marginal zone B cell lymphoma of mucosa-associated lymphoid tissue type
- Nodal marginal zone lymphoma
- Follicular lymphoma
- Mantle cell lymphoma
- Diffuse large B cell lymphoma*
- Mediastinal large B cell lymphoma
- Burkitt's lymphoma/Burkitt's cell leukemia
- Provisional entity: high-grade B cell lymphoma Burkitt's-like*
- Primary effusion lymphoma

T Cell and Putative Natural Killer Cell Neoplasms
- Precursor T cell neoplasm
- Precursor T lymphoblastic Lymphoma/leukemia
- Mature (peripheral) T cell and natural killer cell neoplasms
- T cell prolymphocytic leukemia
- Large granular lymphocyte leukemia (LGL)
- Aggressive natural killer (NK) cell leukemia
- Peripheral T cell lymphomas, unspecified*
- Adult T cell lymphoma/leukemia
- Intestinal T cell lymphoma (± enteropathy associated)
- Hepatosplenic γδT cell lymphoma
- Subcutaneous panniculitis-like T cell lymphoma
- Mycosis fungoides/Sezary syndrome
- Anaplastic large cell lymphoma, T and null cell primary cutaneous type
- Peripheral T cell lymphoma not otherwise specified
- Angioimmunoblastic T cell lymphoma
- Angiocentric T cell lymphoma

Valli VE, et al. Vet Pathol. 2011

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LYMPHOPROLIFERATIVE DISEASES
CYTOLOGY VS BIOPSY

Cytology

- Diagnosis of "lymphoma" in most cases
- Quick
- Inexpensive
- Non-invasive

Biopsy

- Gold standard
- Tissue architecture
- Immunohistochemistry
- Grade
- Anesthesia
- Time to healing and results
- Cost

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LYMPHOPROLIFERATIVE DISEASES
CLASSIFICATION

Why?

- Prognosis
- Treatment
- Other reasons

How?

- Histopathology with IHC
- Flow cytometry

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FLOW CYTOMETRY

- Indications
- Sampling
- Interpretation

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FLOW CYTOMETRY

- Evaluates cell size, CD markers for immunophenotyping, and additional prognostic markers
- Indications
  - Characterization of lymphoma or leukemia
  - Lymphoma is highly suspected
    - Dogs only
    - Cats – use PARR instead
  - Lymphocytosis
    - Ideally lymphocyte count >10,000/uL

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FLOW CYTOMETRY
SAMPLES

- Liquid biopsy, LIVE cells suspended

- Lymph node
- Other organ

→ Flow buffer

- Whole blood
- Bone marrow
- Body cavity effusion

→ At least 1mL in a purple top tube

All samples should be sent via overnight shipping and kept cold, but not frozen

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FLOW CYTOMETRY VIDEO

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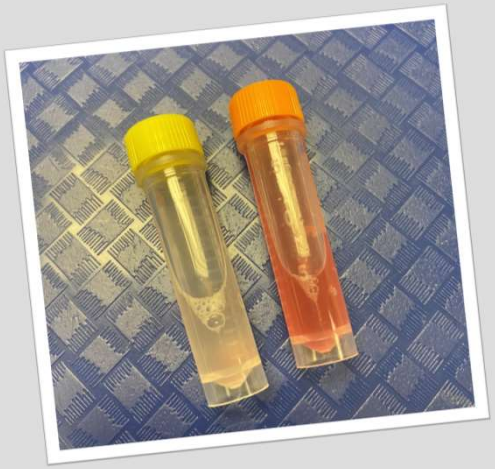

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CSU HEMATOPATHOLOGY LAB FLOW CYTOMETRY BUFFER RECIPE

- Place 1mL of saline into no-additive, plain tube
- Add 0.1mL of serum from the patient or other healthy animal of same species to the plain tube with saline
- CSU hematopathology lab website:

https://vetmedbiosci.colostate.edu/chl/

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FLOW CYTOMETRY
RESULTS

Clinical Hematopathology

COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES
COLORADO STATE UNIVERSITY

PATIENT INFORMATION
Patient: [redacted]
Species: DOG Breed: MIXED BREED
[redacted]

CLINIC INFORMATION
AUBURN UNIVERSITY SAC
519 HOERLEIN HALL
AUBURN UNIVERSITY, AL, 36849
[redacted]

SAMPLE DETAILS
[redacted]
Sample Type: ASPIRATE
Site Sampled: LYMPH NODE
Collected: [redacted]
Received:
Completed:

TEST RESULTS

Flow cytometry

CD4 LYMPHOCYTOSIS

INTERPRETATION

The flow cytometry study revealed a homogeneous population of CD4 T cells. A minor proportion of these CD4 T cells have lost expression of the T cell antigen CD5. These findings are diagnostic for T cell lymphoma/leukemia. Data from our laboratory indicates that peripheral nodal lymphoma with this phenotype has a median survival of 150 to 180 days from diagnosis when treated with a multi-drug protocol (Avery et al, JVIM, 28:538, 2014).

FLOW CYTOMETRY RESULTS

[redacted] %Dead cells: 73 [redacted]

	Small to intermediate sized cells	Intermediate to large sized cells	Reference interval for small cells in a canine lymph node
%Cells in gate:	21	79	
T cell subset: CD4	29	82	21 - 45
T cell subset: CD8	2	0	6 - 20
Pan T cell: CD3	34	73	35 - 74
Pan T cell: CD5	34	73	35 - 74
B cell: CD21	52	1	26 - 58
B cell/Plasma cell: CD21-CD22+	0	0	0
Monocytes: CD14		1	<5
Neutrophils: CD4+CD5-		4	<5
Precursor/Acute leukemia: CD34+MHCII-	0	0	0
T zone cells: CD5+CD45-	0	0	0
Aberrant T cell: CD4+CD8+	0	0	0

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PARR

- PCR for Antigen Receptor Rearrangement
- Clonality assay
- Amplifying the unique, hypervariable portion of DNA
  - T cell receptor
  - Immunoglobulin
- If ***clonal*** → most consistent with ***lymphoid neoplasia***
- If ***polyclonal*** → most consistent with ***reactive disease***

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PARR
SAMPLE

- Cytology slides
  - Stained or unstained slides
  - 4-5 highly cellular slides
- Tissue biopsy (formalin fixed, paraffin embedded)
  - Scrolls from tissue block
  - Tissue block
- Fluid
  - Blood, bone marrow, body cavity effusion in EDTA
  - Flow cytometry samples

Special shipping and handling is not necessary

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Clinical Hematopathology

COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES
COLORADO STATE UNIVERSITY

PATIENT INFORMATION
[redacted]
Species: CAT Breed: DOMESTIC LONG HAIR
[redacted]

CLINIC INFORMATION
AUBURN UNIVERSITY SAC
519 HOERLEIN HALL
AUBURN UNIVERSITY, AL, 36849
[redacted]

SAMPLE DETAILS
[redacted]
Sample Type: ASPIRATE
Site Sampled: MEDIASTINUM
Collected: [redacted]
Received:
Completed:

TEST RESULTS

PCR for antigen receptor rearrangements (PARR)

Immunoglobulin gene: POLYCLONAL
T cell receptor gene: CLONAL

INTERPRETATION

The PARR assay revealed a clonally rearranged T cell receptor gene. This finding is supportive of T cell neoplasia. The specificity of a positive result with the PARR assay has been determined on blood and lymph nodes from cats with no clinical, cytologic or histologic evidence of lymphoma (n = 21 samples). No false positive results were detected in these samples (Rout, 2019 Vet Clin Path 48 S1:45-58). The specificity of the assay when used on non-lymphoid tissue has not yet been determined. The specificity of the assay in cases where lymphoma is suspected but not confirmed has also not been determined.
The same sized PCR product was seen in both samples, indicating the same tumor in both sites.
Mediastinal T cell lymphoma is most commonly seen in FeLV positive cats.

Canine PARR detects T cell receptor gamma chain rearrangements, immunoglobulin heavy chain and incomplete immunoglobulin heavy chain rearrangements. Feline PARR detects these, as well as light chain rearrangements (kde and lambda).

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PARR
LIMITATIONS

- Clonality assay
  - Not ideal for immunophenotype
  - Agreement with IHC 69% [1]
    - VS agreement of FC – 94% [1]
- Sensitivity and specificity for clonal result for lymphoid neoplasia
  - Variable depending on the lab
  - Usually high

[1]Thalheim L, et al. J Vet Intern Med. 2013

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PARR
LIMITATIONS

- False negatives
  - Low number of neoplastic cells
  - PARR inhibitor in the sample
  - Clonal segment is not detected with primer set used
  - NK cell origin
- False positives
  - Ex - Ehrlichia, Lyme dz

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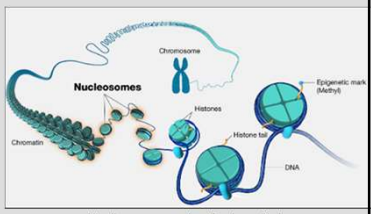
NU.Q VET CANCER TEST

- Cancer screening test
- Measures nucleosome levels in plasma of dogs
- Elevated nucleosome levels can indicate cancer
  - Other causes – inflammation, heavy exercise, surgery/trauma
- Low levels expected in healthy dogs
- Nu.Q is estimated to detect 77% of lymphoma cases, 82% of hemangiosarcoma, ~50% of histiocytic sarcoma and malignant melanoma[1]
  - 35% osteosarcoma, 30% of soft tissue sarcoma, 20% mast cell tumor[1]



https://www.genome.gov/genetics-glossary/Nucleosome

[1]Wilson-Robles HM, et al. BMC Vet Res. 2022

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NU.Q VET CANCER TEST

- Cancer screening test for healthy dogs
- Recommend start screen dogs at 7 years old[1]
  - Earlier if high risk individual based on breed, increased body weight (i.e. giant breeds), and family history[1]
- Fasted sample
- 2-5mL of blood
- Labs – Heska, IDEXX, Texas A&M GI lab
- Coming soon – in-clinic, point of care test from Heska

[1]Rafalko JM et al. PLoS One. 2023

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SUMMARY

Fine needle biopsy and cytology
- Essential oncology diagnostic
- Recognize limitations

Incisional vs excisional biopsies
- Incisional – histopath results will change what you or the owner do

Molecular diagnostics for lymphoid cancers
- Flow cytometry – characterization of lymphoid cancer
- PARR – clonality assay

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QUESTIONS?

Remember to
download the CE certificate
in the handouts panel of
the webinar control panel.
NOTE: CE certificate not available
for watching the recording.

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events@heska.com

Thank you for joining us!

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