Volition i Veterinary The nuice Cancer Test:

Early Detection Can Save Lives



Section Table



Canine Cancer: At a Glance

Cancer Screening



Circulating **Nucleosomes**



Future Products



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in Development



Canine Cancer: At a Glance



Canine Cancer: At a Glance



Cancer Screening



Circulating **Nucleosomes**



Case

Studies

Future Products

in Development



FAQs

Number of pet dogs in the U.S.¹

Pets improve our health²

Volition



Approximately 84 million





76% of pet owners report improved health





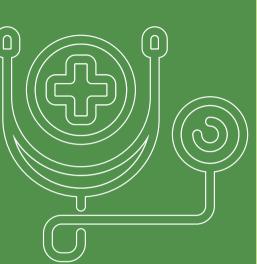
Say they would be more likely to maintain regular check-ups with their veterinarian

1.Larkin, M. (2021). Pet population still on the rise, with fewer pets per household. American Veterinary Medical Association. Retrieved 9 July 2022 . 2.Survey of U.S. Pet Owners | HABRI. HABRI. (2022). Retrieved 19 August 2022. 3.2022 Pet Ownership Statistics: 70 Fur Facts - Lemonade Lemonade Pet. (2022). Retrieved 19 August 2022

Human/Canine Cancer⁴



25-40% of all humans and dogs develop cancer



Dogs Diagnosed with Cancer Annually⁵



Approximately 6 million



Volition





4.Sarver, A., Makielski, K., DePauw, T., Schulte, A., & Modiano, J. (2022). Increased risk of cancer in dogs and humans: A consequence of recent extension of lifespan beyond evolutionarily determined limitations? 5.COP - Pet Owners - What is Comparative Oncology | Center for Cancer Research. Ccr.cancer.gov. Retrieved 9 July 2022



nu.a vet Cares



Cancer is an emotional word

It is a disease we have all been touched by



Early detection and treatment

Crucial to achieving the best clinical outcomes



Together We can give your pet the best chance at successful treatment





Cancer Screening Today



Canine Cancer: At a Glance

Cancer Screening



Circulating **Nucleosomes**





Case **Studies**

Future Products in **Development**

FAQs

What is Screening?



Screening refers to tests performed on healthy, asymptomatic patients who may have disease but do not yet have symptoms.



Screening tests have become a routine part of exams.

There are few cancer screening tests available for dogs.

S S

Some (human) cancers that screening tests aim to identify:

Breast (Mammogram)

Colorectal (Colonoscopy)

Cervical (Pap Smear)

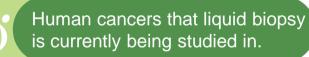
Prostate (PSA)

Types of Screening: Liquid Biopsy Veterinary

Liquid biopsy tests are revolutionizing oncology and large-scale human clinical studies involving hundreds of thousands of participants necessary for regulatory approval of a human screening test are in progress.



Blood is commonly sampled, urine and saliva are also used.



Lung Cancer⁶

Colorectal Cancer⁷

Breast Cancer⁸



Liquid biopsy can be inexpensive and non-invasive.



Liquid biopsy is an *accessible* and *affordable* option for vets.

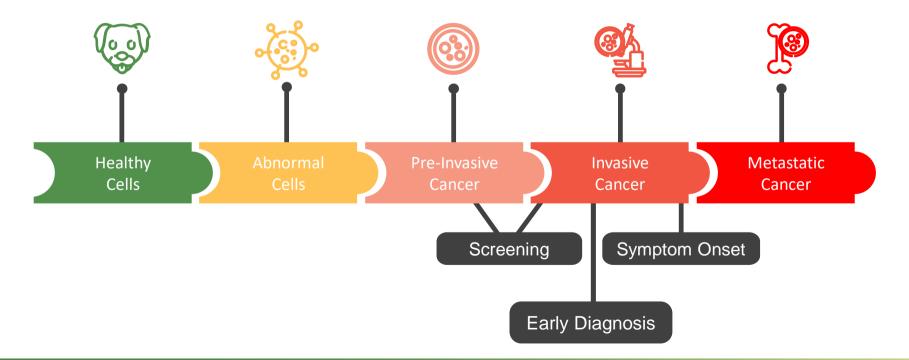
6. Li et al. 2022. Liquid biopsy in lung cancer: significance in diagnostics, prediction, and treatment monitoring.

7. Mazouji et al. 2021. Updates on Clinical Use of Liquid Biopsy in Colorectal Cancer Screening, Diagnosis, Follow-Up, and Treatment Guidance.

8. Chen & Zhao. 2019. Next-generation sequencing in liquid biopsy: cancer screening and early detection.

The Value of Screening

Timeline of cancer development in the body, and where screening occurs within the process



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Veterinary

Who to Screen (and When)

Screening is recommended for healthy dogs as part of annual check-ups



All dogs (7 years and older)

Dogs with an increased risk due to breed

Liquid biopsy screening can be integrated into annual wellness visits.

Liquid biopsy has been studied in aggressive and common cancers.

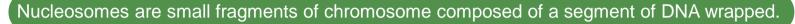
Screening asymptomatic patients could help identify cancer earlier.

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Veterinary

Circulating Nucleosomes in Cancer^{9,10,11,12}

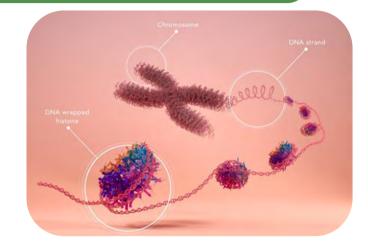


Cancer and cell death release nucleosomes into the blood.

Nucleosomes can be measured using antibodies.

·;))

Measuring nucleosome levels can prognostic and diagnostic markers for disease.



The Nu.Q[®] Test is a proprietary epigenetic immunoassay platform that determine levels of circulating nucleosomes.

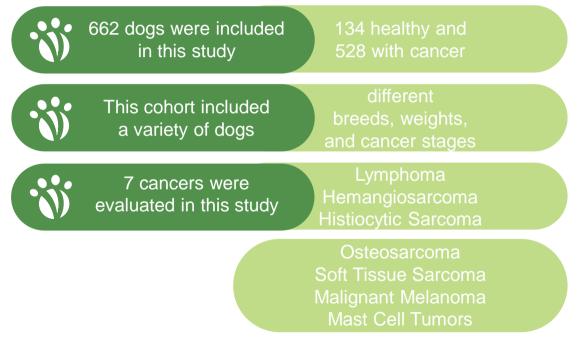
- 9. Holdenrieder, S., Stieber, P., von Pawel, et al. (2004). Circulating Nucleosomes Predict the Response to Chemotherapy in Patients with Advanced Non–Small Cell Lung Cancer
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- 11. Stoetzer, O., Fersching, et al. (2013). Prediction of response to neoadjuvant chemotherapy in breast cancer patients by circulating apoptotic biomarkers nucleosomes, DNAse, cytokeratin-18 fragments and survivin. 12. Fahmueller, Y., Nagel., et al. (2012). Predictive and prognostic value of circulating nucleosomes and serum biomarkers in patients with metastasized colorectal cancer undergoing Selective Internal Radiation Therapy.

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Clinical Evidence^{13,14,15}



Nucleosome Concentrations in Healthy Dogs and Dogs With Cancer



Dolan, C., Miller, T., Jill, J., Terrell, J., Kelly, T., Bygott, T., & Wilson-Robles, H. (2021). Characterizing circulating nucleosomes in the plasma of dogs with lymphoma.
Wilson-Robles., H., Miller, T., Jarvis, J., Terrell, J., Kelly, T., Bygott, T., & Bougoussa, M. (2021). Characterizing circulating nucleosomes in the plasma of dogs with hemangiosarcoma.
Wilson-Robles, H., Bygott, T., Kelly, T., Miller, P., & Matsushita, M. et al. (2022). Evaluation of plasma nucleosome concentrations in dogs with a variety of common cancers and in healthy dogs



The **NU·Q vet** Cancer Test

A simple, affordable, easy-to-use blood test





Lymphoma – 77%

Hemangiosarcoma – 82%

Histiocytic Sarcoma – 54%

Localized tumors are least likely to cause elevated plasma nucleosomes

The Nu.Q[®] Vet Cancer Test

The Nu.Q[®] Vet Cancer Test is recommended for **all** dogs over the age of 7, and younger dogs aged 4 and older with an increased risk of cancer such as:





Labrador Retriever

French

Golden Bulldoa **Retriever**



German Shepard



Great Dane Miniature Schnauzer





Bernese **Mountain Dog**



Beagle



Rottweiler



Boxer

Pembroke Welsh Corgi



Mastiff Irish Wolfhound



Huskv

Retriever



Scottish Wolfhound



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Veterinary





How to Submit a Sample

Samples will be run through the Texas A&M GI Laboratory

To create an account



3.

Patients should be fasted (minimum 4 hours).



Immediately fill EDTA tube with blood



Spin the sample in-house 1600xg for 10 minutes within one hour of sampling.



Remove plasma

place in non-additive tube (be careful to not disturb buffy coat).



Ship overnight on ice

Sunday to Thursday Reduced FedEx shipping is available through the GI lab website

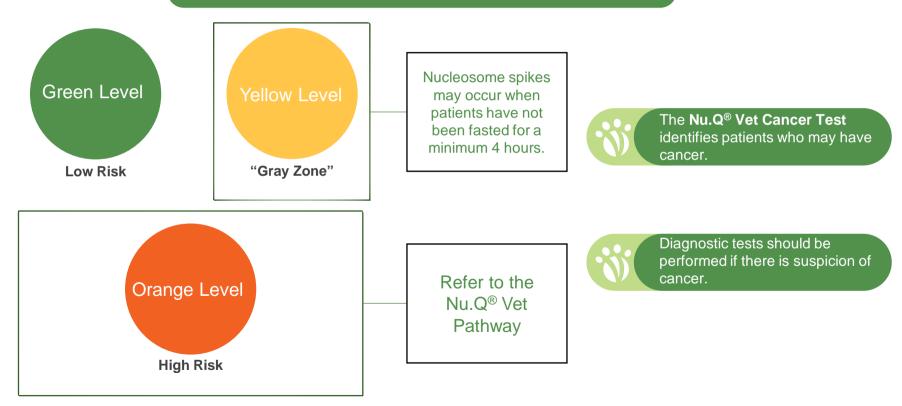
Diagnostics should be used confirm the suspicion of cancer.

Please refer to the Nu.Q[®] Vet Pathway for procedures that may be included in the diagnosis/staging process.

The Nu.Q[®] Vet Cancer Test Results Key



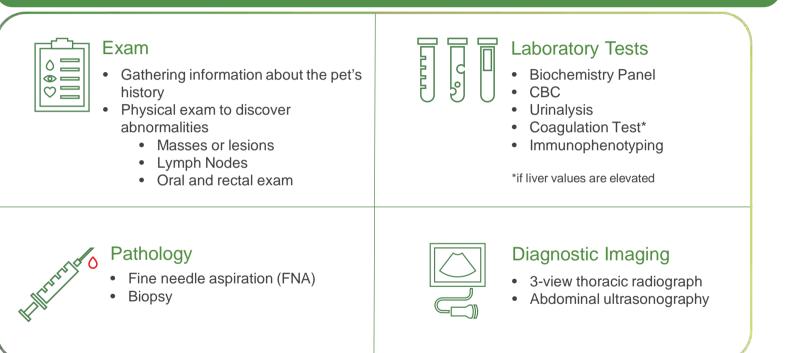
Colors correspond with Nu.Q[®] levels, which may identify cancer is present:



The Nu.Q[®] Vet Pathway



The pathway to diagnosis and staging may include some of the following:

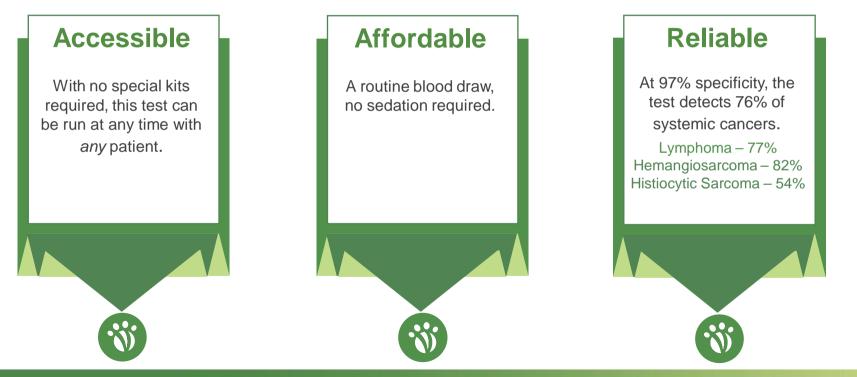


Advanced Imaging (such as MRI or CT scan) may be utilized in some cases

Summary



The Nu.Q[®] Vet Cancer Test is recommended for all dogs over the age of 7, and younger dogs aged 4 and older with an increased risk of cancer as a part of annual wellness exams.





Circulating Nucleosomes



Canine Cancer: At a Glance

Cancer Screening



Circulating Nucleosomes



in **Development**

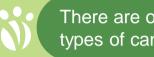


Case **Studies**

Future Products

Canine Lymphoma: At a Glance^{16,17} Veterinary

Among the most common cancers diagnosed in dogs.



There are over 30 described types of canine lymphoma.

Performing a biopsy is the best way to diagnose

Some are life threatening, others are managed as chronic disease.



Chemotherapy is the most effective therapy for most types.

Originates in lymph nodes before spreading to organs.

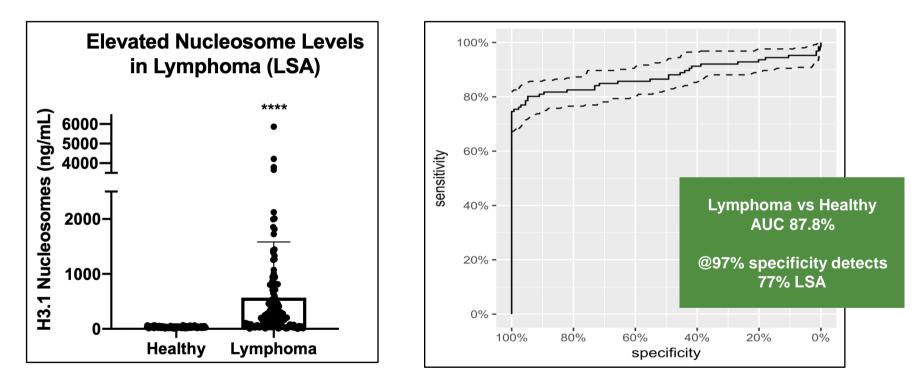
``)

Staging tests are required to determine how far lymphoma has spread.

16. Medicine, P. (2022). Canine Lymphoma. Purdue University College of Veterinary Medicine.

17. Comazzi, S., Marelli, S., Cozzi, M., Rizzi, R., Finotello, R., & Henriques, J. et al. (2018). Breed-associated risks for developing canine lymphoma differ among countries: an European canine lymphoma network study.

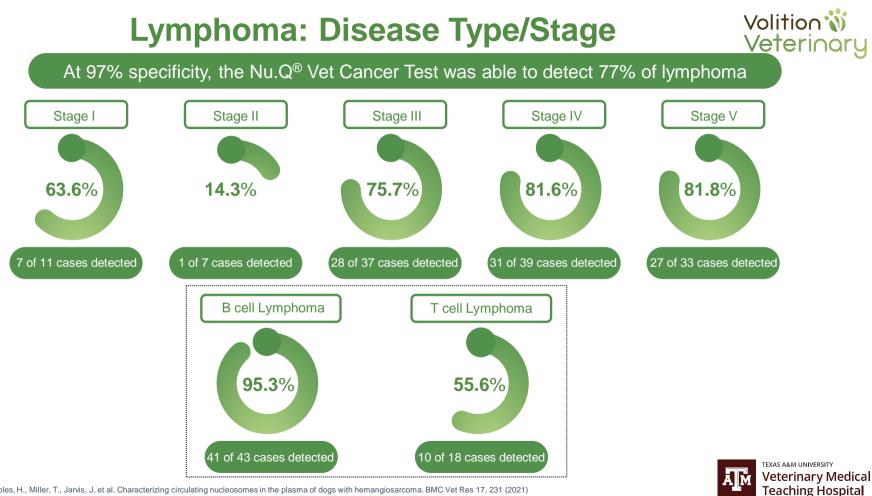
Circulating Nucleosomes in Dogs with Lymphoma



Dolan, C, Wilson-Robles, H et al. Characterizing Circulating Nucleosomes in the plasma of dogs with lymphoma BMC Vet Res 17, 276 (2021)

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Veterinary



Wilson-Robles, H., Miller, T., Jarvis, J. et al. Characterizing circulating nucleosomes in the plasma of dogs with hemangiosarcoma. BMC Vet Res 17, 231 (2021)



Hemangiosarcoma: At a Glance¹⁸

A highly malignant cancer arising from cells that normal create blood vessels. It is often diagnosed via biopsy.



In most cases, the cause of hemangiosarcoma is unknown.



Superficial skin tumors appear as a red to purple colored region of skin



Any breed can be affected.

Can also appear as a bump that may bruise or bleed.

Clinical signs depend on location of disease.

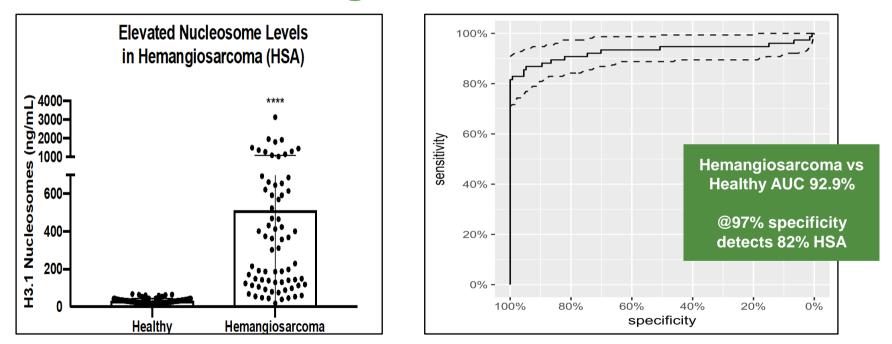


Impossible to tell if malignant or benign from appearance or feel.

18. NC State Veterinary Hospital: Canine Hemangiosarcoma - NC State Veterinary Medicine. NC State Veterinary Medicine. (2022). Retrieved 17 August 2022

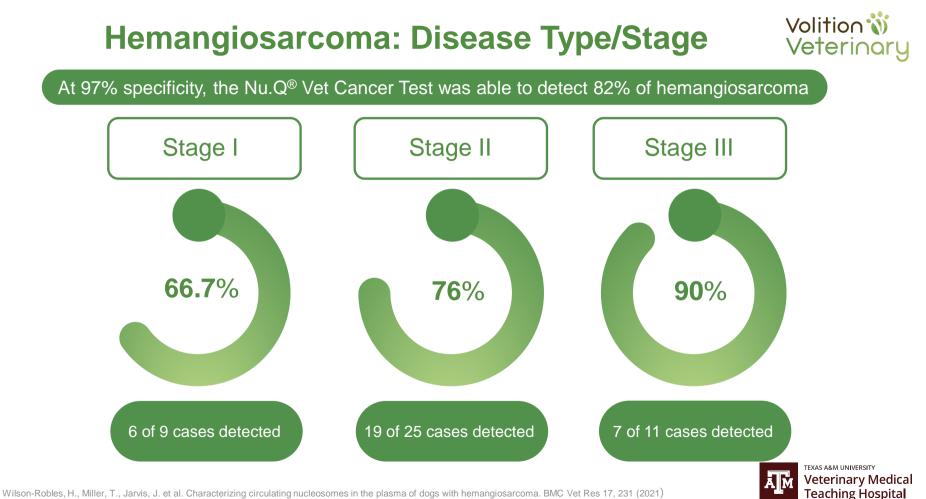


Circulating Nucleosomes in Dogs with Hemangiosarcoma





Wilson-Robles, H., Miller, T., Jarvis, J. et al. Characterizing circulating nucleosomes in the plasma of dogs with hemangiosarcoma. BMC Vet Res 17, 231 (2021)



Clinical Staging System for Hemangiosarcoma

Primary Tumor (T)

- T0: no evidence of tumor
- T1: Tumor <5 cm diameter & confined to primary tissues
- T2: Tumor >5 cm or ruptured, invading SQ tissues
- T3: Tumor invading adjacent structures, including muscle

Regional LN (N)

- N0: no regional LN involvement
- N1: regional LN involvement
- N2: distant LN involvement

Distant metastasis

- M0: no distant metastasis
- M1: Distant metastasis

Stage	т	Ν	Μ
1	T0 or T1	NO	M0
II	T1 or T2	N0 or N1	M0
Ш	T2 or T3	N0, N1 or N2	M1

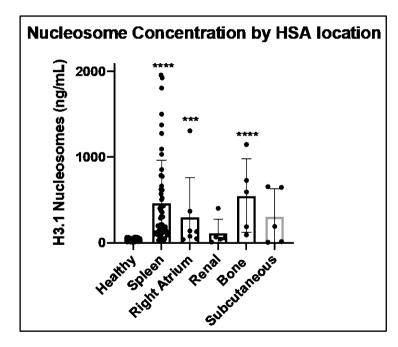


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Veterinary



Nucleosome Concentration by Hemangiosarcoma Location



TEXAS ARM UNIVERSITY Veterinary Medical Teaching Hospital

Case Studies





Canine Cancer: At a Glance



Cancer Screening



Circulating **Nucleosomes**



Case **Studies**



in Development

FAQs

Case 1: Otis

12-year-old MN Catahoula mix

History of subcutaneous hemangiosarcoma removed 2 years ago

Presenting for an annual recheck

• Doing well at home

Physical exam:

- Moderate dental tartar
- Grade 1 murmur (new)
- Mild arthritic changes to hips & elbows





Case 1: Otis Wellness Exam



Plan: routine blood work & Nu.Q® Vet Cancer Test

- Stress leukogram
- Slightly thrombocytopenia: **192,000**

Chemistry panel:

• Mild hyperglobulinemia: 4.6

Urinalysis:

No significant findings

HW test and Fecal float:

• Negative





Nu.Q[®] Vet Cancer Test :

• Cancer Suspicion: High Risk

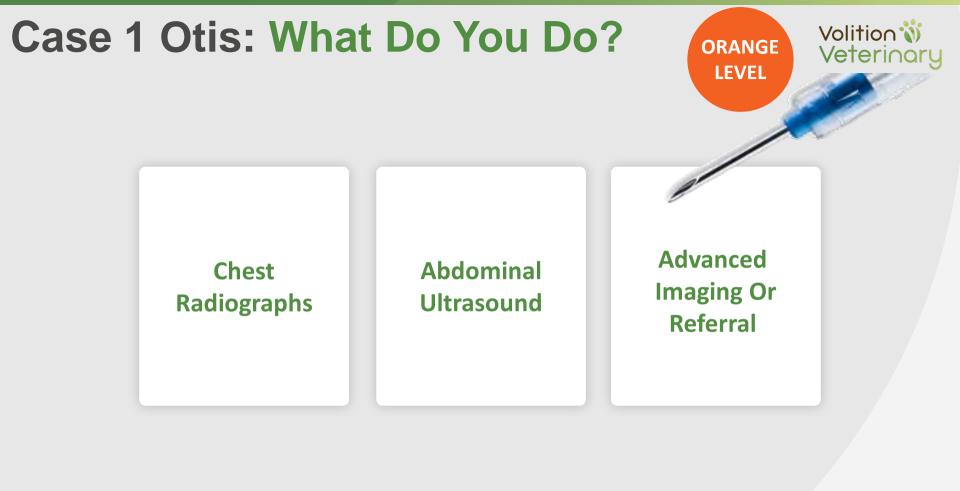
Interpretation:





- Plasma nucleosome concentrations in the orange level are consistent with common canine cancers including lymphoma and hemangiosarcoma.
- This test is not able to differentiate severe/systemic inflammation from cancer.
- Additional tests such as a CBC, chemistry, urinalysis, cytology/biopsy, and/or imaging may be needed to confirm or deny the suspicion of cancer.

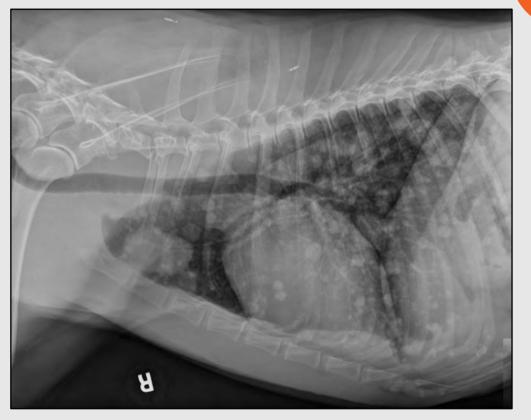
NOTE - Dogs that have not been fasted may have artificially elevated nucleosome levels and should be retested after fasting



Case 1: Otis

ORANGE





Case 2: Belle

5-Year-Old FS Golden Retriever



Presenting for annual wellness exam

- Doing well at home
- Mild lethargy since they got a new puppy

Physical exam: **No significant findings**





Case 2: Belle Wellness Exam

Plan: routine blood work & Nu.Q[®] Vet Cancer Test

CBC:

- Slightly thrombocytopenia: **134,000**
- Otherwise, normal

Chemistry panel:

• Mild elevation in Alk Phos: 243 mg/dL

Urinalysis:

No significant findings





Case 2: Belle



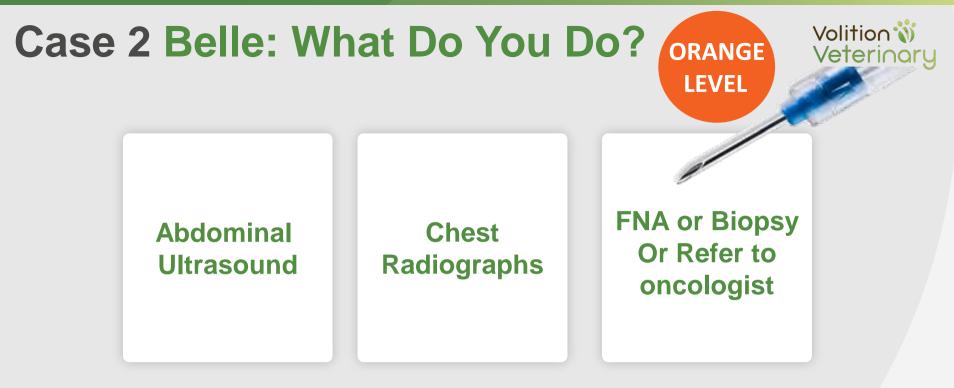
Nu.Q[®] Vet Cancer Test:

• Cancer Suspicion: High Risk

Interpretation:



- Plasma nucleosome concentrations in the orange level are consistent with common canine cancers including lymphoma and hemangiosarcoma.
- Test is not able to differentiate severe/systemic inflammation from cancer.
- Work up case to confirm or deny the suspicion of cancer.
 - CBC, Chemistry, Urinalysis, cytology/biopsy, and/or imaging



- AUS: splenomegaly with severely mottled appearance ("moth eaten")
- AUS-guided splenic aspirates: high grade lymphoma

Case 3: Hank

10-year-old MN Great Dane Mix

Presenting for 2-week history of progressive left forelimb lameness



Physical exam:

- Firm painful mass associated with left carpus
- Toe touching lame to non-weight bearing
- Rest of exam unremarkable







Case 3: Hank



Owner agrees to minimum database, **Nu.Q® Vet Cancer Test**, & sedation for carpal radiographs

CBC

- Stress leukogram
- Mild thrombocytosis: 479,000

Chemistry panel:

• Mild elevation in Alk Phos: 283 mg/dL

Urinalysis

- USG 1.040
- 2+ proteinuria



Case 3 Hank: Radiographs of Carpus





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Case 3: Hank

Nu.Q[®] Vet Cancer Test :

Cancer Suspicion: LOW RISK

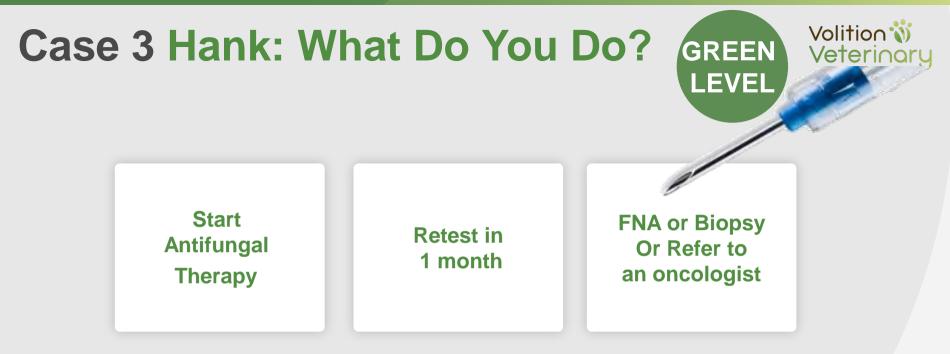
Interpretation:





- Plasma nucleosome concentrations in the green level are consistent with those found in healthy animals of over age of 1 year & all genders.
- Not all neoplastic conditions are detectable using elevated plasma nucleosome concentrations.

Additional tests may be needed to confirm or deny suspicion of cancer in your patient



Bone aspirate: sarcoma, ALP-stain positive

Consistent with osteosarcoma

NOTE: The current Nu.Q[®] Vet Cancer Test more reliably detects systemic cancers rather than soft tissue or localized cancers

Case 4: Percy

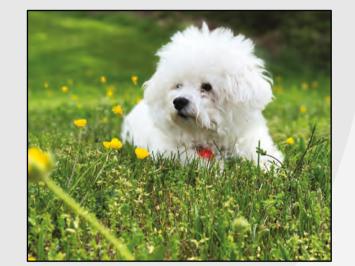
- 8-year-old FS Bichon Frise
- Presents for annual wellness check & doing well

Physical exam:

- Moderate dental tartar
- Dermal mass over left lateral flank
- No other significant findings

Plan: routine blood work & Nu.Q® Vet Cancer Test

• Owner declines aspirate





Case 4: Percy

Nu.Q[®] Vet Cancer Test :

Cancer Suspicion: "GRAY ZONE"

Interpretation:





- Plasma nucleosome concentrations at the yellow level can be seen in early-stage cancer or cancers with low levels of circulating nucleosomes including lymphoma and hemangiosarcoma.
- Test not able to differentiate severe inflammation from cancer.
- Additional tests may be needed to confirm or deny the suspicion of cancer.
- If patient is otherwise healthy, recommend repeating test in 2 4 weeks.
- If high suspicion of cancer or if Nu.Q[®] result remains elevated after retesting, recommend additional testing.

NOTE - Dogs that have not been fasted may have artificially elevated nucleosome levels and should be retested after fasting

Case 4 Percy: What Do You Do?

Upon questioning owner, you determine Percy was not fasted

- Owner forgot to pull food & she was fed breakfast
- What do you do with these results?



Retest in 1 month

Fast & retest later this week & FNA mass Refer for a full work up with oncologist

Case 4: Percy



- Repeat test and FNA: Owner agreed to fast & bring dog back in am
- Nu.Q[®] Vet Cancer Test :
- Cancer Suspicion: LOW RISK



Nu.Q[®] Interpretation:

- Green level consistent with in healthy animals of over age of 1 year
- Not all neoplastic conditions are detectable using elevated plasma nucleosome concentrations.
- Do you have clinical suspicion of cancer? Work up

Cytology Result: "Epithelial hyperplasia or benign neoplasia with mild mixed inflammation" - GREAT NEWS!

Next steps - Repeat Nu.Q[®] test at next Wellness visit

Future Products in Development





Canine Cancer: At a Glance



Cancer Screening



Circulating Nucleosomes



Case Studies

Future Products in Development

FAQs

Future Products in Development



Point of Care Test

Volition has entered a licensing agreement with Heska.

We anticipate a launch in early 2023.



We are currently working on developing additional assays to add to the Nu.Q[®] test to better differentiate other conditions from cancer.



We have begun research on a Nu.Q[®] Vet Cancer Test for our feline friends. We hope to report data in the coming months.



Future Products in Development^{19,20}



Disease Progression and Treatment Monitoring^{16,17}

Most patients achieving clinical remission showed healthy plasma nucleosome levels i.e., "a Nu.Q[®] Vet test result" in the low, healthy dog range.

The Nu.Q[®] Vet Cancer Test may therefore be a useful tool to monitor disease response progression.

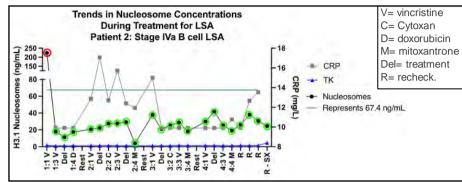
Further studies are ongoing to examine the role Nu.Q[®] Vet can play in Disease Progression and Treatment Monitoring.

19.Wilson - Robles, H. (2022). Utility of Serial Plasma Nucleosomes Concentrations for Monitoring Treatment Response and Disease Progression In Canines with Hematopoietic Malignancies. Presentation 20.Wilson-Robles, H. Miller, T, Miller, P, Jarvis, J, Butera, T, Matsushita, M, Terrell, J, Kelly, TK. VCS 2021. Evaluation of plasma nucleosome concentrations as a tool for treatment and disease monitoring in cancer bearing dogs.

Disease Progression and Treatment Monitoring: Case Studies

Roxy 6 year-old FS Rottweiler





Roxy presented to TAMU in December 2019 with generalized lymphadenopathy.



Out of remission – she had previously been diagnosed with LSA in 2018 and treated with CHOP.



Cytology confirmed LSA, flow cytometry confirmed B cell lymphoma with high MHC class II expression.

She was induced with CHOP Chemotherapy.

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Disease Progression and Treatment Monitoring: Case Studies

The Chart

Roxy

Both CRP and Nu.Q[®] are high at diagnosis, TK-1 is not.

By week 3 (2nd time point) – Nu.Q[®] is back within the reference range and she is deemed to be in **clinical remission.**



This is her second CHOP, and due to the cumulative life-time max dose of Doxorubicin being achieved

She was switched to mitoxantrone midway through.

Interpretation

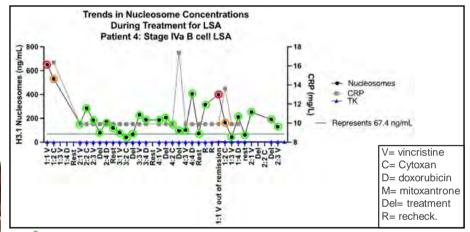
CRP fluctuates throughout the protocol and the TK-1 is consistently low. The Nu.Q[®] value correlates better with her response and disease progress.

Result

Last recheck, she was noted to have a 7cm cavitated splenic mass on ultrasound restaging of her lymphoma. The Nu.Q[®] was in the normal range. She had a splenectomy, and the mass was determined to be benign.

Disease Progression and Treatment Monitoring: Case Studies





Abbigail presented to TAMU in 2020 with submandibular lymphadenopathy.



Cytology diagnosed lymphoma; flow cytometry confirmed B cell lymphoma with high MHC class II expression.



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Disease Progression and Treatment Monitoring: Case Studies

The Chart

- Abbigail had one of the highest Nu.Q[®] values we have seen and continues to remain high – though she does occasionally dip below the normal range.
- She is 2.5 years and on her 3rd CHOP so prognosis is not affected by this value. Her high Nu.Q[®] value is her "normal" range.
- *
 - To get Abbigail back to her "normal" Nu.Q[®] range, an entire cycle of CHOP was needed:
 - Vincristine = weeks 1&3 Cytoxan = week 2 Doxorubicin = week 4

Abbigail

Abbigail gets below reference range after 2 cycles.

Interpretation

- After a rest or delay, her Nu.Q® value increases demonstrating that we have minimal control of her disease with chemotherapy.
- At her last Doxorubicin appointment, the Nu.Q[®] value began to increase – but she is responsive to Doxorubicin, so the value comes back down.

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Disease Progression and Treatment Monitoring: Case Studies

Result

- Abbigail's Nu.Q[®] value remains low at her 1st monthly recheck.
- 2nd monthly recheck, the Nu.Q[®] value is high but still deemed to be in clinical remission based on PE and restaging
- 3rd monthly recheck she is clearly out of remission and the Nu.Q[®] value is even higher.
- CHOP is reinstituted and her Nu.Q[®] value decreases again, and she goes back into remission again.
- **
 - CRP fluctuates throughout treatment and the TK-1 is consistently low. The Nu.Q[®] value correlates better with her response and disease progresses.

Abbigail

Materials Available for Download







Race Approved Webinars



Frequently Asked Questions



Canine Cancer: At a Glance

Cancer Screening



Circulating Nucleosomes



Case **Studies**



in **Development**

FAQs



FAQs

Will this test tell me if my dog has cancer?

No, the release of nucleosomes into the blood is common to many different types of cancers.

Additional tests are necessary to diagnose cancer and determine the source of the circulating nucleosomes What types of cancer has the Nu.Q[®] Vet Cancer Test been able to detect?

At 97% specificity, the Nu.Q[®] Vet Cancer Test was shown to detect 76% of systemic cancers

Lymphoma – 77%

Hemangiosarcoma – 82%

Histiocytic Sarcoma – 54%

Data also suggests the Nu.Q[®] Vet Cancer Test can detect some instances of Mast Cell tumors, Osteosarcoma, Oral Melanoma, and Soft Tissue Sarcoma.





Do pre-existing or other clinical conditions impact results?

Nucleosome Spikes May Occur in the Following



- Systemic Inflammation
- Sepsis
- Trauma

Diseases That Do Not Impact Results

- Chronic Inflammatory Conditions
- Systemic Inflammation (being treated regularly, not flaring)
- * Hypothyroidism
- Nenal Disease
- Steoarthritis







What Should I Do if My Patient has <u>NOT</u> Been Fasted?

Dogs that have not been fasted may have slightly elevated Nu.Q[®] levels. What to do?



Repeat the test at a later date.



Ensure patient has been fasted minimum 4 hours.



If levels remain high, additional testing may be necessary.

References



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- 3. Sarver, A., Makielski, K., DePauw, T., Schulte, A., & Modiano, J. (2022). Increased risk of cancer in dogs and humans: A consequence of recent extension of lifespan beyond evolutionarily determined limitations?. Aging And Cancer, 3(1), 3-19. https://doi.org/10.1002/aac2.12046
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