



Detect. Treat. Monitor. BECAUSE With approximately 84 million dogs¹ in the United States as well as around 75 Millions in Europe, there is no shortage

Our canine companions have moved from the backyard to the bedroom and have broadened our scope of how we define family; the human-animal bond has never been stronger. Your pet matters to us, and our work is guided by preserving that human-animal bond and keeping your family together.

of people who know how wonderful owning a dog can be. Dogs don't ask for much; a treat, a long walk, a bit of food off your plate, and in return they provide companionship, unconditional love, and

scientifically proven comfort².

Cancer is an emotional word; it is a disease we have all been touched by. **The incidence of cancer in dogs, approximately 1 in 4**³, is the same as that in humans. As with human cancer patients, early detection and treatment are crucial to achieving the best clinical outcome⁴.

The Nu.Q[®] Vet Cancer Test was developed with the goal of providing an accessible and affordable screening test to aid in early detection. We believe that cancer screening should be woven into wellness exams, becoming as routine as clinical chemistry or fecal tests.

We deliver a gold standard product without gold standard pricing.

Why? Because together, we can give your pet the best chance at successful treatment.





Consider earlier testing in case of a family history of cancer!

The Nu.Q[®] Vet Cancer Test is a simple, affordable, easy to use screening blood test for all dogs (7 years and older) and younger dogs (4 years and older) with an increased risk of developing cancer in their lifetimes including:

> Labrador Retriever French Bulldog **Golden Retriever** German Shepherd Beagle Rottweiler Boxer Pembroke Welsh Corgi **Great Dane** Miniature Schnauzer Siberian Husky Bernese Mountain Dog Mastiff Irish Wolfhound **Flat Coated Retriever** Scottish Wolfhound

"Earlier detection can save lives, it can also improve the quality of life of the dog and the quality of time with its owner."

In the USA there is no word more dreaded than cancer, particularly when it comes to the health of your pet. Nevertheless, approximately 6 million pet dogs⁵ are diagnosed with cancer each year.

Many diseases can be detected and treated before they become serious, cancer is one of them. Cancer screening tests (mammogram, colonoscopy, HPV DNA test) have become commonplace in human medicine as part of our annual physical exams. However, in the veterinary market there are few cancer screening tests available.

Earlier detection can save lives, it can also improve the quality of life of the dog and the quality of time with its owner. Yet as of today, many dogs are diagnosed at an advanced stage when they are showing signs of illness. With the Nu.Q[®] Vet Cancer Test, pet parents can now have their dogs screened for cancer during wellness visits. In a case series presented at ACVIM 2022, the Nu.Q[®] Vet Cancer Test was shown to detect 76% of systemic cancers; lymphoma (77%), hemangiosar-coma (82%), and histiocytic sarcoma (54%), and was able to identify approximately 50% of all cancers researched at 97% specificity⁶.

Lymphoma is the most common form of canine cancer and together with hemangiosarcoma make up approximately one-third of all cancers.

Alongside other routine blood work and imaging, the Nu.Q[®] Vet Cancer Test may help find cancer at an early stage, before symptoms appear, allowing for a better chance at effective treatment.

Our mission is to provide an affordable screening test to assist pet owners and their veterinarians allowing them to be more proactive about catching cancer early. We strive to protect the lives of pets, and the families who love them.

How to Submit a Sample



How to Interpret the Results





Low Suspicion result <50 ng/ml

Nu.Q[®] Vet Cancer Test results at the green level indicate that this patient has low risk for active neoplasia in the classes of tumors screened for by the Nu.Q[®] test.

Continue routine annual or bi-annual screening.

YELLOW LEVEL

Moderate Suspicion result 51-80 ng/ml

This patient's results are in the "gray zone" of moderate suspicion and further testing should be considered.

Patient may have low-levels of circulating nucleosomes due to certain early-stage neoplasia.

Repeat testing with a fasted sample after 2 – 4 weeks or when convenient to evaluate trends in results if patient is otherwise healthy.*

ORANGE LEVEL

High Suspicion result >81 ng/ml

This patient has risk for active neoplasia in the classes of tumors screened for by the Nu.Q[®] test, warranting further screening for the presence of neoplasia. This may include additional laboratory testing such as repeating the Nu.Q[®] test in 2 weeks, radiographs, ultrasound, fine needle aspirates and/or biopsies, depending on the clinical presentation and physical examination findings for this patient.*

The Nu.Q[®] Vet Cancer Test identifies patients who may have cancer, however, not all neoplastic conditions are detectable using elevated plasma nucleosomes.

Localized tumors are least likely to cause elevated plasma nucleosomes, and this test is not able to differentiate severe/systemic inflammation from cancer.

If there is a suspicion of cancer, we recommend that you perform confirmatory diagnostics to confirm the suspicion of cancer.



*Other considerations result >81 ng/ml

Inflammatory diseases such as immune mediated disease, systemic inflammation, sepsis, and trauma can also cause elevated nucleosome levels. This test will not differentiate between patients sick with systemic inflammatory mediated illness from those sick with cancer.

How does the **NU** · **Q** Cancer Test work?^{vet}

Chromosome

Nucleosome (DNA wrapped histones)

DNA strand

DNA is compacted within a cell's nucleus in the form of nucleosomes which are bead like structures comprised of DNA coiling around a histone protein core.

When a patient (human or canine) has cancer, nucleosomes from those cancer cells are released into the blood and can be measured using antibodies that are specific to nucleosomes.

By measuring and analyzing nucleosomes, our Nu.Q[®] Vet Cancer Test can identify patients who may have a cancer. This must then be confirmed by follow up procedures – for example, a biopsy or scan. Please refer to the Nu.Q[®] Vet Pathway (page 4).





OTIS 12 YEAR-OLD MN CATAHOULA MIX

Presenting for an annual recheck and doing well at home Temperature, pulse, respiration within normal limits





- Physical Exam
 - Moderate dental tartar
 - Grade 1 murmur (new)
- Mild arthritic changes
- to hips and elbows
- As part of Wellness Exam, you run blood work (minimum database) as well as the Nu.Q[®] Vet Cancer Test

ORANGE LEVEL Nu.Q[®] Vet Cancer Screening Test: 618.4 ng/mL HIGH SUSPICION

INTERPRETATION

Plasma nucleosome concentrations in the orange level are consistent with an increased risk of cancer in healthy animals over the age of 1 year, and all genders.

The Nu.Q[®] Vet Cancer Test identifies patients who may have cancer, however, confirmatory diagnostics should be used to confirm the suspicion of cancer.

Please refer to the Nu.Q[®] Vet Pathway (page 4) for procedures that may be included in the diagnostic process.

NOTE

Nucleosome spikes may occur when patients have not been fasted for a minimum of 4 hours, as well as conditions such as immune-mediated disease, systemic inflammation, sepsis, and trauma.

- Laboratory CBC:
- Tests



- Stress leukogram
- Slightly low platelets at 192,000 /µl
- Chemistry panel:
 Mild elevation in globulins 4.6g/dl
- Heartworm test and Fecal float:
 Negative
- Urine analysis: - No significant findings

WHAT TO DO NEXT?

Was Otis fasted? Yes, he fasted so proceed to a full work up.

Imaging • Chest radiograph

• Abdominal ultrasound



Refer / advanced imaging like full body CT

RESULT



Unfortunately, there are numerous metastatic lesions on thoracic radiographs. Further investigation also determines a 6cm cavitated splenic mass- hemangiosarcoma is the most likely cause.



- Exam
- Physical Exam

 Firm painful mass associated with right carpus
 - Toe touching lame
 - Rest of physical exam unremarkable

Suspicion – Osteosarcoma

- Owner agrees to minimum database, Nu.Q[®] Vet Cancer Test, and sedation for carpal radiographs
- GREEN LEVEL Screening Test: 32.7 ng/mL LOW SUSPICION

INTERPRETATION

Plasma nucleosome concentrations in the green level are consistent with those found in healthy animals over the age of 1 year, and all genders.

The Nu.Q[®] Vet Cancer Test identifies patients who may have cancer, however, not all neoplastic conditions are detectable using elevated plasma nucelosomes.

Please refer to the Nu.Q[®] Vet Pathway (page 7) for procedures that may be included in the diagnostic process.

NOTE

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

Laboratory • CBC:

Tests - Stress leukogram - Mild increase in platelets: 479,000 /µl



- 479,000 /μlChemistry panel:
 - Mild elevation in Alk Phos: 283 U/l
 - Urine analysis:
 - Few lipid droplets
 - 2+ protein
 - USG 1.040

WHAT TO DO NEXT?

Pathology

- and a start
- If appropriate start anti-fungal therapy
- Perform FNA or biopsy to get definitive diagnosis

RESULT

Suspicion: Osteosarcoma.



Unfortunately, mass found.

HANK 10 YEAR-OLD GREAT DANE MIX

Presenting for 3-day history of right forelimb lameness Temperature, pulse, respiration within normal limits

BELLE 5 YEAR-OLD GOLDEN RETRIEVER FS

Presenting for annual wellness exam Temperature, pulse, respiration within normal limits



Exam • Ph



- Physical Exam
 - Owner reports doing well at home, a little more tired since they got
 - a new puppy - No significant findings
- Owner agrees to minimum database and Nu.Q[®] Vet Cancer Test

Laboratory • CBC:

Tests



- Mildly low platelets at 190,000 /µl
- Otherwise, normal
- Chemistry panel:
 - Mild elevation in Alk Phos-143 U/l
- Urine analysis: - No significant findings

ORANGE LEVEL Nu.Q[®] Vet Cancer Screening Test: 187.6 ng/mL *HIGH SUSPICION*

INTERPRETATION

Plasma nucleosome concentrations in the orange level are consistent with an increased risk of cancer in healthy animals over the age of 1 year, and all genders.

The Nu.Q[®] Vet Cancer Test identifies patients who may have cancer, however, confirmatory diagnostics should be used to confirm the suspicion of cancer.

Please refer to the Nu.Q[®] Vet Pathway (page 4) for procedures that may be included in the diagnostic process.

NOTE

Nucleosome spikes may occur when patients have not been fasted for a minimum of 4 hours, as well as conditions such as immunemediated disease, systemic inflammation, sepsis, and trauma.

WHAT TO DO NEXT?

Pathology •

- Owner agrees to an abdominal ultrasound and chest film
- Cranial mediastinal mass on chest rads
- Perform FNA to get definitive diagnosis

RESULT

Unfortunately, the fine needle aspirate showed a monomorphic population of large immature lymphocytes. Diagnosis of lymphoma was made.

Exam

- Physical Exam
 Moderate dental tartar
 - Moderate dental tartar
 Mass over left flank that is soft
 - and moveable
 - No other significant findings

• Also, as part of Wellness Exam, you run blood work (minimum database) as well as the Nu.Q[®] Vet Cancer Test

As Dr. Sue Cancer Vet says, "see something, do something!" so #WhyWaitAspirate. If size of a pea and been there more than a month then aspirate.

YELLOW LEVEL Nu.Q[®] Vet Cancer Screening Test: 58.2 ng/mL MODERATE SUSPICION

WHAT TO DO NEXT?

Was Percy fasted? No, repeat the test the following morning.

INTERPRETATION

Plasma nucleosome concentrations in the yellow level have a number of contributing factors.

If the patient has not been fasted, and is otherwise healthy, we recommend repeating the test at your earliest convenience.

If the patient has been fasted, and is otherwise healthy, we recommend testing in 2-4 weeks.

NOTE

Nucleosome spikes may occur when patients have not been fasted for a minimum of 4 hours, as well as conditions such as immune-mediated disease, systemic inflammation, sepsis, and trauma.

Consider patient age and breed in your decision to retest.

If there is a suspicion of cancer, we recommend that you perform confirmatory diagnostics to confirm the suspicion of cancer.

FOLLOW-UP RESULT

GREEN LEVEL LOW SUSPICION

INTERPRETATION

Plasma nucleosome concentrations in the green level are consistent with those found in healthy animals over the age of 1 year, and all genders.

The Nu.Q[®] Vet Cancer Test identifies patients who may have cancer, however, not all neoplastic conditions are detectable using elevated plasma nucelosomes.

Please refer to the Nu.Q[®] Vet Pathway (page 4) for procedures that may be included in the diagnostic process.

Cytology Result Lipoma - Great news! Next step Repeat test at next Wellness visit.

PERCY 8 YEAR-OLD FS BICHON FRISE

Presenting for an annual recheck and doing well at home Temperature, pulse, respiration within normal limits



"AT 97% SPECIFICITY THE NU.Q® VET CANCER TEST WAS ABLE TO DETECT APPROXIMATELY 50% OF ALL CANCERS RESEARCHED, AND 76% OF SYSTEMIC CANCER (LYMPHOMA, HEMANGIOSARCOMA, AND HISTIOCYTIC SARCOMA)."

The following case series was peer-reviewed, published, and presented at the 2022 American College of Veterinary Internal Medicine (ACVIM)^{7,8,9}.

- Samples were either collected at the Texas A&M Small Animal Teaching Hospital (AUPs CA 2019-0211 and 2017-0350) or from the NCI Division of cancer Treatment and Diagnosis Biorepository.
- A total of 662 dogs (134 healthy and 528 with cancer) were included in this study.

- A variety of breeds, weights and cancer stages were represented in the dataset.
- 7 cancers evaluated in this study:
 - Lymphoma
 - Malignant melanoma
 - Hemangiosarcoma
 - Mast cell tumors
 - Osteosarcoma
 - Histiocytic sarcoma
 - Soft tissue sarcoma
- Localized tumors are least likely to cause elevated plasma nucleosomes, if cancer is suspected and the Nu.Q[®] score is low, continue the cancer evaluation (please refer to the Nu.Q[®] Vet Pathway (page 4) for additional information).





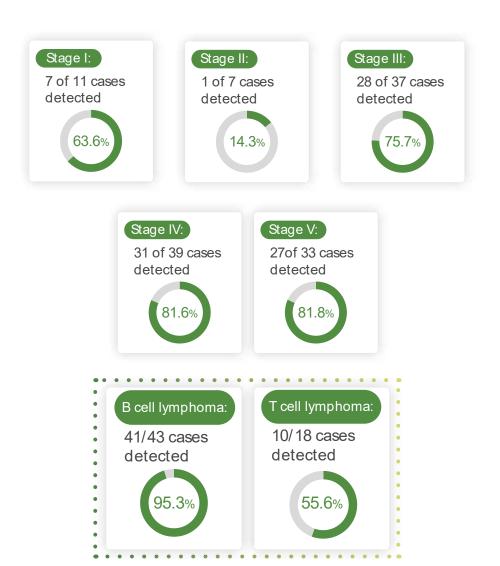
At 97% specificity, the Nu.Q® Vet Cancer Test was able to detect 77% of lymphoma

Diagnosis by Disease Type/Stage: Lymphoma

At 97% specificity, the Nu.Q[®] Vet Cancer Test was able to detect 82% of hemangiosarcoma

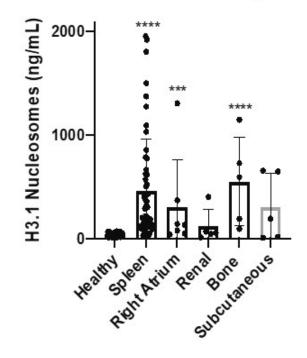


Diagnosis by Disease Type/Stage: Hemangiosarcoma





Nucleosome Concentration by HSA location





POINT OF CARE TEST

By providing results within minutes, point-of-care testing will expedite the clinical decision-making process. A future where veterinarians can detect, treat, and monitor in-clinic using the Nu.Q[®] Vet Cancer Test is what we work towards. To potentially save lives through early cancer screening is why we work.

- Volition Veterinary has entered a licensing agreement with Heska to offer the Nu.Q[®] Vet Cancer Test in clinic, at the point of care.
- We are in the process of developing the point of care test, and anticipate a launch in the first half of 2023.

DIFFERENTIAL DIAGNOSIS

- We are currently working on developing additional assays to add to the Nu.Q[®] test to better differentiate inflammatory and other conditions from cancer.
- Studies are underway at several leading university hospitals to collect data comparing a variety of concomitant conditions including inflammatory conditions, immune mediated disease and endocrinopathies.

CATS

 Volition Veterinary is committed to the saving the lives of all your furry family members through early detection and have begun research on a Nu.Q[®] Vet Cancer Test for our feline friends. We hope to report data in the coming months.









DISEASE PROGRESSION AND TREATMENT MONITORING



DISEASE PROGRESSION AND TREATMENT MONITORING^{10,11}

Abstracts presented at the 2022 European Society of Veterinary Oncology (ESVONC) Congress and 2021 Veterinary Cancer Society Meeting show:

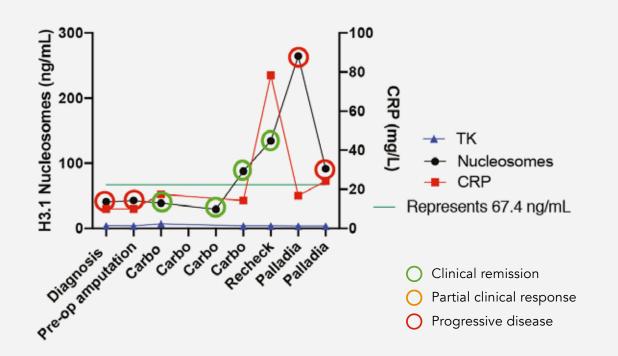
- Nucleosome concentrations i.e., "Nu.Q[®] Vet results", during treatment in Lymphoma patients changed week to week, and appeared to mirror disease state.
- Most patients achieving clinical remission showed healthy plasma nucleosome levels i.e., "a Nu.Q[®] Vet test result" in the low, healthy dog range.
- The Nu.Q[®] Vet Cancer Test may therefore be a useful tool to monitor disease response progression.

• Circulating nucleosome levels, i.e., "Nu.Q[®] Vet results" may serve as a more sensitive measurement of both residual disease and of clinical progression out remission.

NOTE:

due to the other possible causes of elevated plasma nucleosomes (high Nu.Q[®] Vet result), it is recommended to have 2 consecutive elevations before altering treatment or protocol.

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



THE CHART

- Poppy was diagnosed with osteosarcoma and at diagnosis her Nu.Q nucleosome levels were normal. This is a finding which we typically see in osteosarcoma patients.
- As time progresses during the first recheck we start to see a slow rise in Nu.Q[®] nucleosome concentrations.
- Nu.Q[®] nucleosome concentrations continued to rise. When they peaked ultimately, chest x-rays were performed and evidence of progression of the disease was noted.

POPPY 7 YEAR OLD FS ST. BERNARD



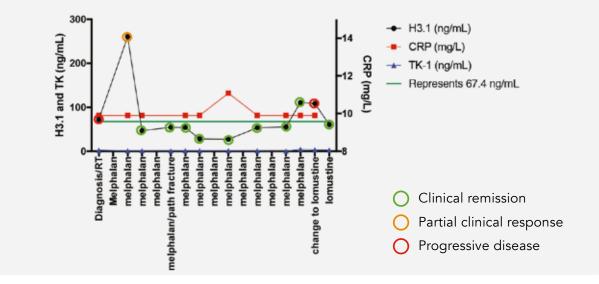
Poppy suffered from osteosarcoma and was treated with amputation, <u>carbopla</u>tin and palladia treatment.

BUSTER 9 YEAR OLD MALE NEUTERED CHOW CHOW MIX

Multiple Myeloma was the diagnosis of Buster. Regular follow-up examinations revealed low nucleosome concentrations during treatment.

At some point, nucleosome levels rose and some time later deterioration of the clinical signs warranted a change in the treatment protocol.



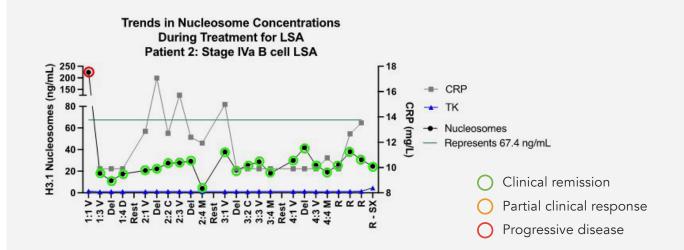


THE CHART

Buster was diagnosed with multiple myeloma. His Nu.Q[®] nucleosome concentration was just above the normal reference interval at the time of diagnosis.

We treated him with radiation and chemotherapy for bone lesions immediatedly. This lead to a significant increase of Nu.Q[®] nucleosome levels which you can see in the yellow circle in the graph.

Over the treatment period with melphalan Buster's Nu.Q[®] nucleosome concentrations remained stable below the reference interval. Three weeks before we saw a progression of disease we noted an increase in the Nu.Q[®] nucleosome concentration above the reference interval. At Buster's next appointment we also saw clinically progressive disease (last red circle) and switched the therapy to lomustine. This resulted in a significant drop of the Nu.Q[®] nucleosome concentrations at the last time point. CRP concentrations remained low during most of the time when Buster was monited. There was no increase in CRP concentration at the time of diagnosis nor after the initial radiation and begin of chemotherapy. A mild rise in the CRP concentration was obvious in the middle of the treatment period without a change in the clinical signs. The final progressive disease which was noted clinically, was not reflected by a change in the CRP concentration either.



THE CHART

- 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.
- V=vincristine, C = Cytoxan (sometimes given at home rarely in protocol).
- D=doxorubicin, Mitoxantrone (this is her second CHOP, and due to the cumulative life-time max dose of Doxorubicin being achieves – she was switched to mitoxantrone midway through)
- Del= treatment delay (due to low WBC counts), R= recheck

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.

FINAL OUTCOM

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

ROXY 6 YEAR-OLD FS ROTTWEILER

Roxy presented to Texas A&M university in December in 2019 with generalized lymphadenopathy.

Out of remission - she had previously been diagnosed with lymphoma 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.



FREQUENTLY ASKED QUESTIONS

CLINICAL

1. When should I have my dog tested? The Nu.Q[®] Vet Cancer Screening Test is best suited to be performed with annual wellness checks for healthy, older dogs (7 years and older) as well as for younger dogs (4 years and older) with an increased risk for developing cancer in their lifetimes, such as those with familial history and/or certain breeds, e.g., Labrador Retriever, French Bulldog, Golden Retriever, German Shepard, Beagle, Rottweiler, Boxer, Pembroke Welsh Corgi, Great Dane, Miniature Schnauzer, Siberian Husky, Bernese Mountain Dog, Mastiff, Irish Wolfhound, Flat Coated Retriever, Scottish Wolfhound.

2. What does the Nu.Q[®] Vet Cancer Test measure?

The Nu.Q[®] Vet Cancer Test measures the level of nucleosomes that are circulating in the blood. When a patient has cancer, nucleosomes from those cancer cells are released into the blood and can be measured using antibodies that are specific to nucleosomes.

3. Is there any risk to having this test done?

One of the advantages to the Nu.Q[®] Vet Cancer Test is that it is non-invasive, only requiring a peripheral blood draw. Since it is only a blood draw there is no significant risk to the dog and no required down time.

4. Will this test tell me what kind of cancer my dog has?

The Nu.Q[®] Vet Cancer Screening Test does not provide a definitive cancer diagnosis. The primary purpose is to screen for the possible presence of cancer. If there is a suspicion of cancer, it is recommended to continue down the diagnostic pathway to confirm and locate the patient's cancer. Refer to the section on interpreting results for additional information.

5. What types of cancer has the Nu.Q[®] Vet Cancer Test been able to detect?

The Nu.Q[®] Vet Cancer Test was shown to detect 76% of systemic cancers (lymphoma (77%), hemangiosarcoma (82%), and histiocytic sarcoma (54%) at 97% specificity.

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

In additon, the Nu.Q[®] Vet Cancer Test may have benefits in the monitoring of cancer patients over time.



CLINICAL

6. Can I run this test on a sick patient or does the patient need to be healthy?

Inflammatory diseases such as immune mediated disease, systemic inflammation, sepsis and trauma can also cause elevated nucleosome levels. This test will not differentiate between patients sick with systemic inflammatory mediated illness from those sick with cancer. For this reason, we do not recommend running the test in patients that could have these types of diseases.

Chronic inflammatory conditions, systemic inflammation that is being treated medically and not ,flaring', hypothyroidism, renal disease, osteoarthritis, mild or moderate pyoderma, or other such minor illnesses are less likely to impact the results of the Nu.Q[®] Vet Cancer Screening Test.

7. Are there any medications that interfere with the Nu.Q[®] Vet Cancer Test results?

Prednisone decreases the nucleosome concentrations in the sample and therefore dogs should be off prednisone for 10-14 days prior to pulling a Nu.Q® sample. Dexamethasone can also decrease the Nu.Q[®] score and should be given at least 48 hours prior to testing if the sample cannot be pulled before the injection is given.

Though we have not done exhaustive studies of all medications and their potential for interference, the common medications listed below have not been shown to have any effect on test results as long as there is no significant systemic inflammation: Trazadone, Diazepam, Thyroid Supplements, NSAIDs, Joint Supplements, Apoquel, Cytopoint*.

8. Do pre-existing or other clinical conditions impact the results of the Nu.Q[®] Vet Cancer Test?

The Nu.Q[®] Vet Cancer Test measures elevated nucleosomes that occur with rapid cell death which may be an indication of cancer. However, nucleosome spikes may also occur in inflammatory diseases such as immune mediated disease, systemic inflammation, sepsis, and trauma. For this reason, we do not recommend running the test in patients that could have these types of diseases. Chronic inflammatory conditions, systemic inflammation that is being treated medically and not 'flaring', hypothyroidism, renal disease, osteoarthritis, mild or moderate pyoderma, or other such minor illnesses do not impact the results of the Nu.Q[®] Vet Cancer Test. Additionally, a patient who has not been fasted a minimum 4 hours may have elevated nucleosome levels.

9. Can I still use the sample if the patient has not been fasted?

Dogs who have not been fasted for 4 hours may have slightly elevated levels when compared to fasted samples less that 4 hours in the same dog. If your dog has not been fasted, they may end up in the moderate to high risk zone even though they are healthy. If this is the case, please fast your dog for 4 hours and repeat the test at a later date. If the level remains elevated, then additional testing may be necessary.

10. Will the Nu.Q[®] Vet Cancer Test tell me what type of cancer my dog has?

The Nu.Q[®] Cancer Test does not provide a definitive cancer diagnosis. The primary purpose of the test is to screen for cancer and if there is a suspicion of cancer your veterinarian should continue down the diagnostic pathway to confirm and locate the cancer. Refer to the How to Interpret the Results of the Nu.Q[®] on page 4 for additional information.

11. Is the baseline Nu.Q[®] Vet level prognostic?

The Nu.Q[®] Vet Cancer Test is not prognostic for cancer. A "high" Nu.Q[®] Vet value does not indicate advanced disease or a shorter survival time.

A study of 25 dogs with lymphoma presented at 2022 ESVONC conference demonstrated that all dogs with elevated nucleosome concentrations (high-very high Nu.Q[®] Vet result) achieved normal concentrations (low Nu.Q[®] Vet result) while in clinical remission. The dogs with the highest values did not have shorter survival times than dogs with lower values.

12. Are special tubes needed? The Nu.Q[®] Vet Cancer Test does not require any special equipment to collect and submit a sample. Please refer to the "How to Submit a Sample" section for specific instructions. It is important that the dog is fasted for a minimum of four hours prior to the test.

13. How much blood is needed?

The Nu.Q[®] Vet Cancer Test requires at least 1ml of K2-EDTA plasma to process the sample.



FREQUENTLY ASKED QUESTIONS

PRACTICAL

1. How do I order the test?

Contact scil via *info-int@scilvet.com* to find out which lab closest to you offers the test!

Ask your local lab if they can already provide the test for your patients!

2. How do I process the sample?

The venous blood sample can be taken from a peripheral vein or the jugular vein. The blood sample must be collected in a K2-EDTA tube. Keep the sample always refrigerated (in refrigerator at 0-4 °C), and centrifuged a 1600xg for 10 minutes within 2 hours of sampling. Transfer the plasma by carefully aspirating it (take care to not touching the buffy coat, that separates the plasma from the cell layer), and pipetting it in a small test tube without additives (i.e. Eppendorf tube). Keep the sample refrigerated until shipment, which must take place within 72 hours of collection. Ship the sample on ice (0-4°C).

3. What kind of tubes can I send the plasma sample in?

The blood should be taken in a K2-EDTA tube. Spin it and transfer the plasma (without touching the buffy coat) into a clean tube without additives (i.e. Eppendorf tube).

4. What is the minimal required volume of plasma for the assay?

Though we prefer a larger sample in case repeated assays are needed, the minimum volume requirement for the assay is 1mL K2-EDTA--plasma (smaller volumes may be accepted on a case-bycase basis).

5. What if I let the tube sit for more than 60 minutes before spinning?

This will likely falsely increase the nucleosome levels in the sample. Please redraw the sample and spin it down at 1600xg for 10 minutes within 60 minutes of the blood draw.

6. Can I use serum instead of plasma?

Ideally the test should be performed on plasma and we do not recommend the use of serum as nucleosome levels are much less stable in serum.

However, if serum is the only thing that you have the test can be performed but the sample MUST be centrifuged within 20 minutes of the blood draw and the serum removed immediately. Serum levels are often slightly higher than plasma levels, so if your patient has a result in the "gray zone", then you may need to retest the patient using plasma to confirm the elevation in nucleosomes.





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. Consider earlier testing in case of a family history of cancer!

> Labrador Retriever French Bulldog **Golden Retriever German Shepherd** Beagle Rottweiler Boxer Pembroke Welsh Corgi **Great Dane** Miniature Schnauzer Siberian Husky Bernese Mountain Dog Mastiff Irish Wolfhound **Flat Coated Retriever** Scottish Wolfhound



"CANCER IS AN EMOTIONAL WORD; IT IS A DISEASE WE HAVE ALL BEEN TOUCHED BY.

THE INCIDENCE OF CANCER IN DOGS, APPROXIMATELY 1 IN 4, IS THE SAME AS THAT IN HUMANS. AS WITH HUMAN CANCER PATIENTS, EARLY DETECTION AND TREATMENT ARE CRUCIAL TO ACHIEVING THE BEST CLINICAL OUTCOME."

- Accessible with no special kits required, this test can be run at any time with any patient.
- Affordable a routine blood draw, no sedation required.
- **Reliable** at 97% specificity, detects 76% of systemic cancers.

Alongside other routine blood work and imaging, the Nu.Q[®] Vet Cancer Test may help find cancer at an early stage, before symptoms appear, allowing for a better chance at effective treatment.

Our mission is to provide an affordable screening test to assist pet owners and their veterinarians allowing them to be more proactive about catching cancer early;

We strive to protect the lives of pets, and the families who love them.

Because

nu.a vet Cares

7. What if my sample is hemolyzed after centrifugation?

Mild or moderate hemolysis will not interfere with the test. In humans, haemoglobin levels of 500 mg/dL were shown to not interfere with the assay. However, if your sample has 3+ or higher hemolysis you may want to redraw a new sample.

ENARY Because we care **NU**.G vet

References

1. Larkin, M. (2021). Pet population still on the rise, with fewer pets per household. American Veterinary Medical Association. Retrieved 9 July 2022, from https://www. avma.org/javma-news/2021-12-01/pet-population-still-rise-fewer-pets-household.

2. Pendry, P., & Vandagriff, J. (2019). Animal Visitation Program () Reduces Cortisol Levels of University Students: A Randomized Controlled Trial. AERA Open, 5(2), 233285841985259. https://doi.org/10.1177/2332858419852592

3. Cancer in Pets. American Veterinary Medical Association. (2022). Retrieved 9 July 2022, from https://www.avma.org/resources/pet-owners/petcare/cancer-pets.

4. IS MY DOG AT RISK FOR CANCER?. American Animal Hospital Association. Retrieved 9 July 2022, from https://www.aaha.org/your-pet/pet-owner-education/ask-aaha/ canine-cancer/.

5. COP - Pet Owners - What is Comparative Oncology | Center for Cancer Research. Ccr. cancer.gov. Retrieved 9 July 2022, from https://ccr.cancer.gov/Comparative-Oncology-Program/pet-owners/what-is-comp-onc.

6,9,12. Wilson-Robles, H., Bygott, T., Kelly, T., Miller, 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. BMC Veterinary Research, 18(1). https://doi. org/10.1186/s12917-022-03429-8

7. 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. BMC Veterinary Research, 17(1). https://doi.org/10.1186/s12917-021-02991-x

8. 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. BMC Veterinary Research, 17(1). https://doi.org/10.1186/s12917-021-02934-6

9. Wilson - Robles, H. (2022). Utility of Serial Plasma Nucleosomes Concentrations for Monitoring Treatment Response and Disease Progression In Canines with Hematopoietic Malignancies. Presentation, Syracuse, Italy.

10. 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

11. Flory, A., Kruglyak, K., Tynan, J., McLennan, L., Rafalko, J., & Fiaux, P. et al. (2022). Clinical validation of a next-generation sequencing-based multi-cancer early detection "liquid biopsy" blood test in over 1,000 dogs using an independent testing set: The CANcer Detection in Dogs (CANDiD) study. PLOS ONE, 17(4), e0266623. https://doi. org/10.1371/journal.pone.0266623

Volition 🐝 Veterinary



- **Publications** .
- Clinical cases
- **Educational Videos**
- More information



SCIL ANIMAL CARE COMPANY

Dina-Weissmann-Allee 6 Germany - 68519 Viernheim Tel.: +49 (0) 6204 78 90 - 0 Fax: +49 (0) 6204 78 90 - 200 info-de@scilvet.com www.scilvet.com



Veterinary Medicine Medical Technology **Further Education** Career