VITICUSGROUP™
WVC ANNUAL CONFERENCE
FEBRUARY 18 - 21, 2024 | LAS VEGAS, NV

Canine Cancer Screening:

Making sense of the why, the when, and next steps

Andy Plum, DVM
Global Medical Affairs Specialist
IDEXX Laboratories

Financial Disclosure

I am a full-time employee of IDEXX, but do not believe that it will influence my presentation.

Learning outcomes

+ Understand the major risk factors for canine cancer development and describe the population of dogs who most likely benefit from cancer screening

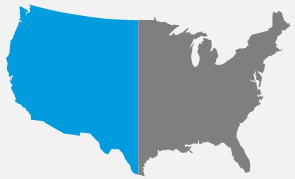
+ Understand the underlying biology of cancer development and how that has been leveraged to develop canine cancer screening tests

+ Become familiar with selected canine cancer screening tests and their published performance metrics

+ Identify the appropriate next steps after a positive cancer screening result.

Canine Cancer at a Glance

Canine Cancer:
Defining the problem



Approximately **84 million** dogs in the U.S.[1]

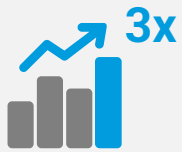
45% of households own dogs

1.46 dogs/household

One in four dogs in the U.S. will be diagnosed with cancer in their lifetime[2]

Somewhat similar as humans in the U.S. (1 in 2.5)[3]

Estimated that **6 million dogs** in U.S. are diagnosed with cancer annually[4]

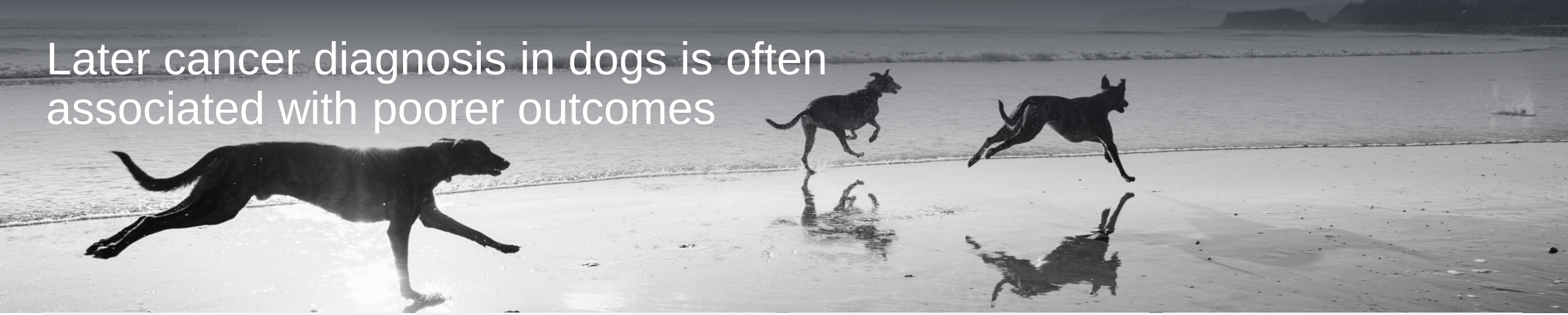


Cancer is the **leading cause** of death in adult dogs[5]

References

1. Larkin M. Pet population still on the rise, with fewer pets per household: Survey on pet ownership also indicates that pet owners value veterinarians' expertise. JAVMA News. Published on November 17, 2021. Accessed March 9, 2023. https://www.avma.org/javma-news/2021-12-01/pet-population-still-rise-fewer-pets-household#:~:text=Both%20dog%20and%20cat%20populations,38%25%20of%20dogs%20in%202020.
2. What are the most common types of cancers in dogs? How many dogs typically get cancer? Veterinary Cancer Society; 2021. Accessed March 9, 2023. www.vetcancersociety.org/pet-owners/faqs
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7–30. doi:10.3322/caac.21590
4. What is comparative oncology? National Cancer Institute Center for Cancer Research. Accessed March 9, 2023. ccr.cancer.gov/Comparative-Oncology-Program/pet-owners/what-is-comp-onc
5. Fleming JM, Creevy KE, Promislow DE. Mortality in North American dogs from 1984 to 2004: an investigation into age-, size-, and breed-related causes of death. *J Vet Intern Med*. 2011;25(2):187–198. doi:10.1111/j.1939-1676.2011.0695.x

Later cancer diagnosis in dogs is often associated with poorer outcomes



Lymphoma

- Later stage at diagnosis associated with lower survival time[1–3]
- Presence of clinical signs at time of diagnosis associated with shorter survival times and greater chance of relapse[2]

Hemangiosarcoma

- For dogs receiving splenectomy and adjuvant chemotherapy, median time to disease progression is longer for stage I versus stage II[4–5]

Osteosarcoma

- Larger tumors (which are associated with earlier metastasis) have a poorer prognosis[6]

Malignant melanoma

- Larger tumors and higher clinical stage are associated with shorter survival times[7–8]

References

1. Lautscham EM, Kessler M, Ernst T, Willimzig L, Neiger R. Comparison of a CHOP-LAsp-based protocol with and without maintenance for canine multicentric lymphoma. *Vet Rec*. 2017;180(12):303. doi:10.1136/vr.104077
2. Rassnick KM, Bailey DB, Malone EK, et al. Comparison between L-CHOP and an L-CHOP protocol with interposed treatments of CCNU and MOPP (L-CHOP-CCNU-MOPP) for lymphoma in dogs. *Vet Comp Oncol*. 2010;8(4):243–253. doi:10.1111/j.1476-5829.2010.00224.x
3. Sorenmo K, Overley B, Krick E, Ferrara T, LaBlanc A, Shofer F. Outcome and toxicity associated with a dose-intensified, maintenance-free CHOP-based chemotherapy protocol in canine lymphoma: 130 cases. *Vet Comp Oncol*. 2010;8(3):196–208. doi:10.1111/j.1476-5829.2010.00222.x
4. Treggiari E, Borrego JF, Gramer I, et al. Retrospective comparison of first-line adjuvant anthracycline vs metronomic-based chemotherapy protocols in the treatment of stage I and II canine splenic haemangiosarcoma. *Vet Comp Oncol*. 2020;18(1):43–51. doi:10.1111/vco.12548
5. Batschinski K, Nobre A, Vargas-Mendez E, et al. Canine visceral hemangiosarcoma treated with surgery alone or surgery and doxorubicin: 37 cases (2005–2014). *Can Vet J*. 2018;59(9):967–972.
6. Forrest LJ, Dodge RK, Page RL, et al. Relationship between quantitative tumor scintigraphy and time to metastasis in dogs with osteosarcoma. *J Nucl Med*. 1992;33(8):1542–1547.
7. Turek M, LaDue T, Looper J, et al. Multimodality treatment including ONCEPT for canine oral melanoma: A retrospective analysis of 131 dogs. *Vet Radiol Ultrasound*. 2020;61(4):471–480. doi:10.1111/vru.12860
8. Manley CA, Leibman NF, Wolchok JD, et al. Xenogeneic murine tyrosinase DNA vaccine for malignant melanoma of the digit of dogs. *J Vet Intern Med*. 2011;25(1):94–99. doi:10.1111/j.1939-1676.2010.0627

What is screening?

Screening refers to **tests performed on at-risk, asymptomatic patients who may have disease but do not yet have clinical signs**

- Have become a routine part of wellness visits for asymptomatic patients
  - Examples: Canine vector-borne disease testing; SDMA; fecal antigen

There are few cancer screening tests available for dogs

- Careful palpation of patient is essential, but many areas inaccessible
- CBC, chemistry profile, urinalysis changes can be insensitive and nonspecific
- Routine use of advanced imaging, like digital radiography, ultrasonography, and CT scans, may be impractical and expensive

Several human cancers have specific screening tests

- Breast (mammography)
- Colorectal (colonoscopy)
- Cervical (Pap smear)
- Prostate (PSA)

Breaking down the cancer screening process

Five Considerations for Canine Cancer Screening Success

Align on Expectations

Define your Screening Population

Know the Biology

Understand Test Performance

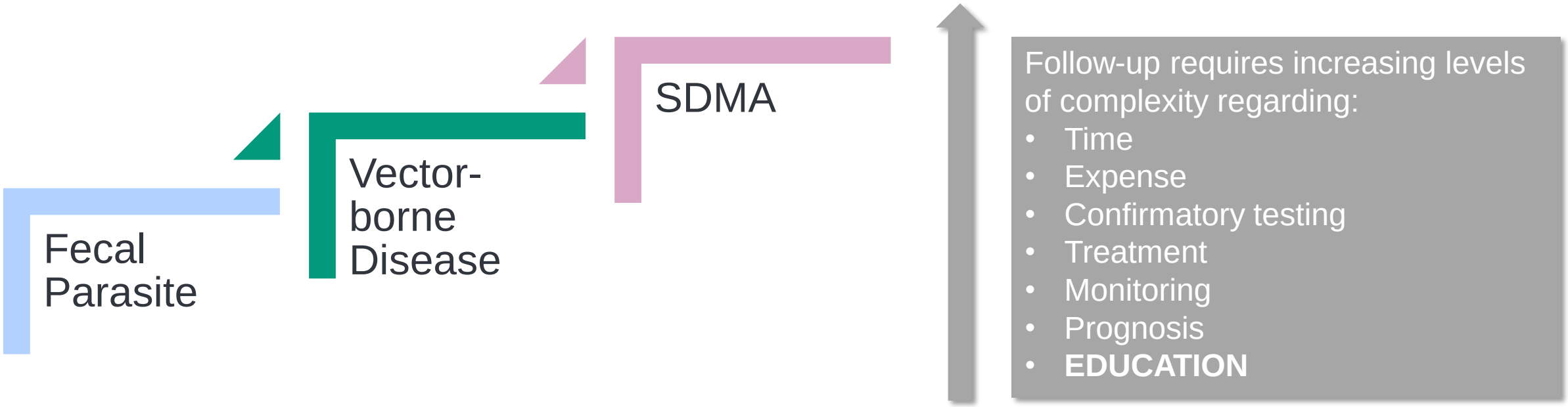
Complete the Diagnosis

Five Considerations for Canine Cancer Screening Success

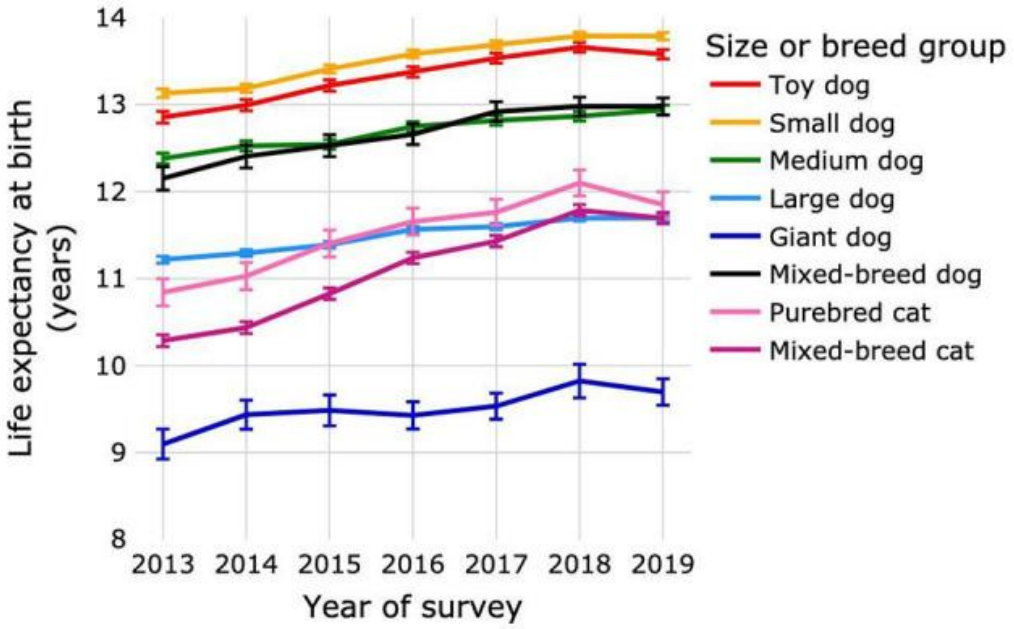
Align on Expectations

Are your goals in sync with those of the dog owner?

- “Begin with the end in mind”
  - What follows a positive test result in any disease screening scenario?



Now consider the complexity of adding cancer screening to the conversation!



- Finances: not starting from $0
  - Cancer screening would not be a one-time event
  - Wellness visit already includes exam fee, fecal, VBD screening, CBC/Chem/UA, vaccines, parasite preventives
- What would be the next steps after a positive screening test?
  - Potential for more invasive, expensive confirmatory testing
- Owner attitudes about treatments (radical surgeries, chemotherapy, etc.)
- Finding cancer may not guarantee happy ending

- **“Above all else, do no harm”**

Montoya M, Morrison JA, Arrignon F, Spofford N, Charles H, Hours MA, Biourge V. Life expectancy tables for dogs and cats derived from clinical data. Front Vet Sci. 2023 Feb 21;10:1082102. doi: 10.3389/fvets.2023.1082102. PMID: 36896289; PMCID: PMC9989186.

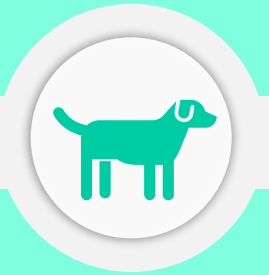
Five Considerations for Canine Cancer Screening Success

Align on Expectations

Begin with the end in mind

Do no harm

Define your Screening Population

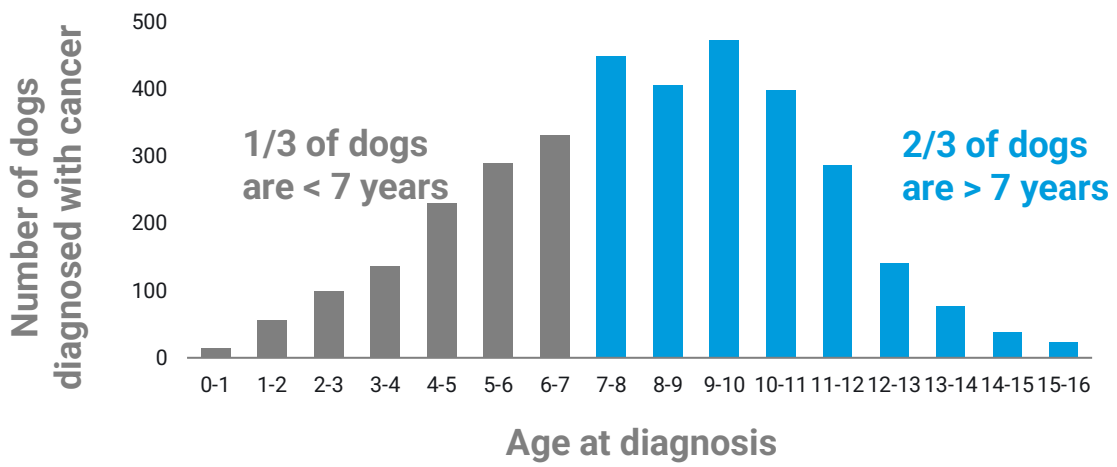


Canine cancer risk factors can guide patient selection

The average age at time of cancer diagnosis is 8.8 years[1]

Pure bred dogs have 1.9 times the relative risk of cancer when compared to cross-bred and mixed-breed dogs[2]

Study of 3,000+ canines diagnosed with cancer[1]



Common breeds with increased risk of cancer:[3,4]

- Beagle
- Bernese mountain dog
- Boxer
- Flat-coated retriever
- French bulldog
- German shepherd
- Golden retriever
- Great Dane
- Irish wolfhound
- Labrador retriever
- Mastiff
- Miniature schnauzer
- Pembroke Welsh corgi
- Rottweiler
- Scottish wolfhound
- Siberian husky

References

1. Rafalko JM, Kruglyak KM, McCleary-Wheeler AL, et al. Age at cancer diagnosis by breed, weight, sex, and cancer type in a cohort of over 3,000 dogs: determining the optimal age to initiate cancer screening in canine patients. Preprint. Posted online April 05, 2022. bioRxiv 486448. doi:10.1101/2022.03.30.486448
2. Some high, some low: Purebred cancer rates you need to know. Nationwide Pet Insurance. Published March 8, 2022. Accessed March 9, 2023. https://news.nationwide.com/some-high-some-low-purebred-cancer-rates-you-need-to-know/?utm_source=prpitch
3. Van Rooyen LJ, Hooijberg E, Reyers F. Breed prevalence of canine lymphoma in South Africa. J S Afr Vet Assoc. 2018;89(0):e1–e11. doi:10.4102/jsava.v89i0.1530
4. Edwards DS, Henley WE, Harding EF, Dobson JM, Wood JLN. Breed incidence of lymphoma in a UK population of insured dogs. Vet Comp Onc. 2003;1(4):200–206. doi:10.1111/j.1476-5810.2003.00025.x

Canine **cancer** screening: focused on the most at-risk population

Recommend for **all dogs over the age of 7**

- (Currently, the average age of dogs at time of cancer diagnosis is 8.8 years of age)

Recommend for **at-risk breeds** aged 4 and older with an increased risk of cancer, such as:[1,2]

- Beagle
- Bernese mountain dog
- Boxer
- Flat-coated retriever
- French bulldog
- German shepherd
- Golden retriever
- Great Dane
- Irish wolfhound
- Labrador retriever
- Mastiff
- Miniature schnauzer
- Pembroke Welsh corgi
- Rottweiler
- Scottish wolfhound
- Siberian husky

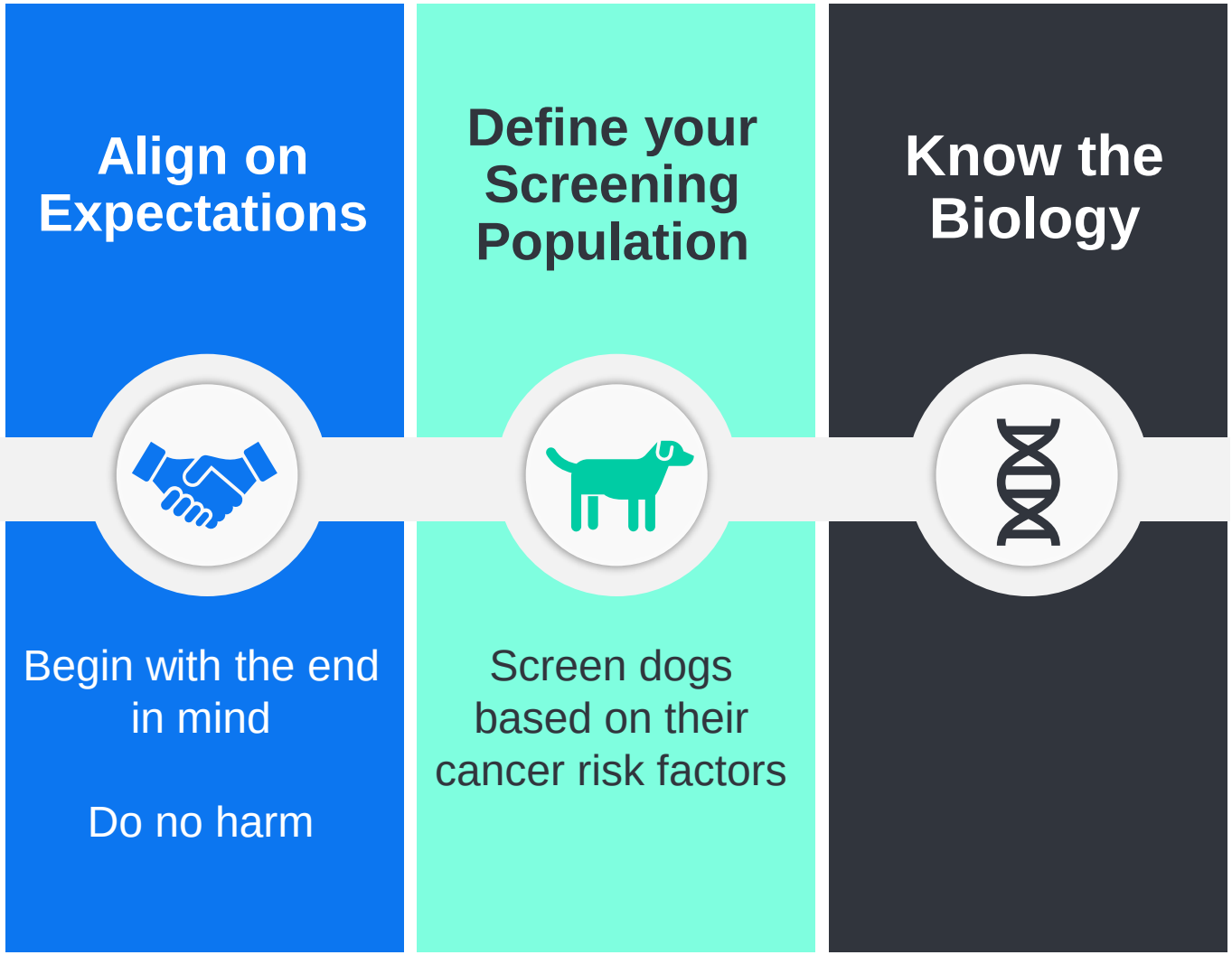
Ultimately, narrowing screening to those at-risk **increases the disease prevalence** in your **testing population**

- Higher prevalence increases the **positive-predictive value (PPV)**, meaning a positive result is more likely to be a true positive.

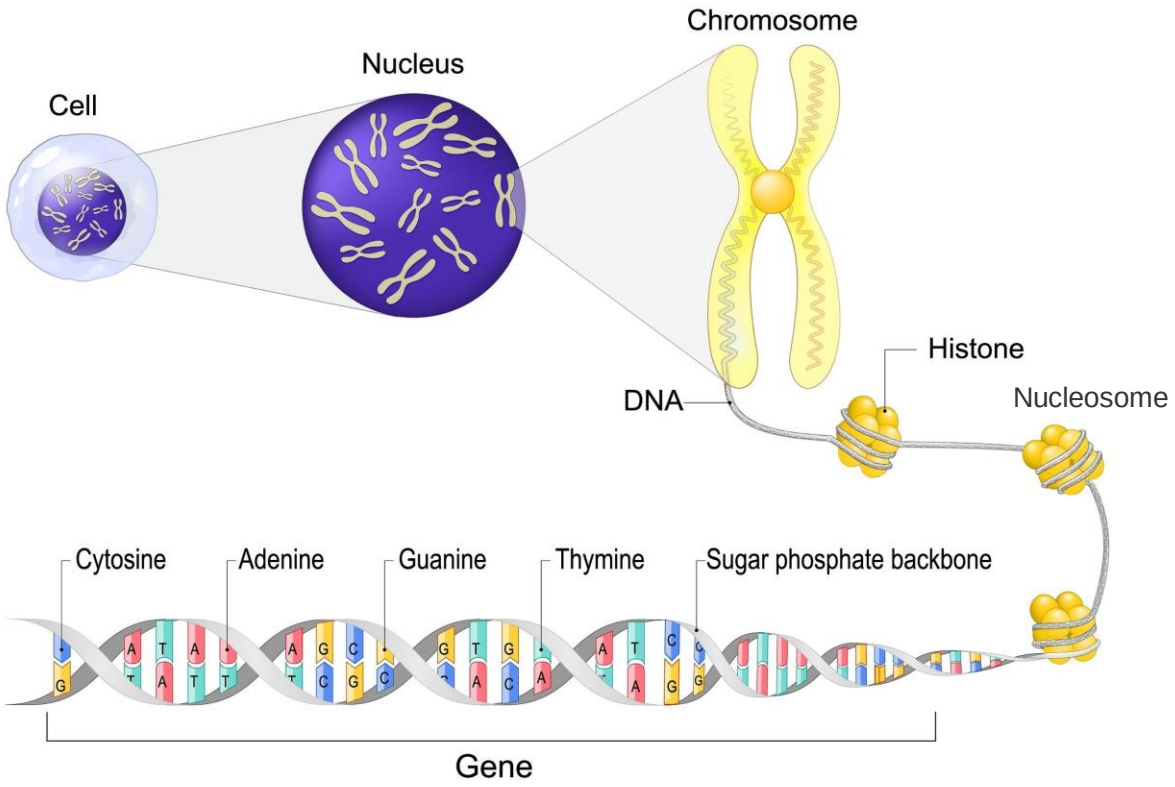
References

1. Van Rooyen LJ, Hooijberg E, Reyers F. Breed prevalence of canine lymphoma in South Africa. J S Afr Vet Assoc. 2018;89(0):e1–e11. doi:10.4102/jsava.v89i0.1530
2. Edwards DS, Henley WE, Harding EF, Dobson JM, Wood JLN. Breed incidence of lymphoma in a UK population of insured dogs. Vet Comp Onc. 2003;1(4):200–206. doi:10.1111/j.1476-5810.2003.00025.x

Five Considerations for Canine Cancer Screening Success



Cancer is a disease of the genome

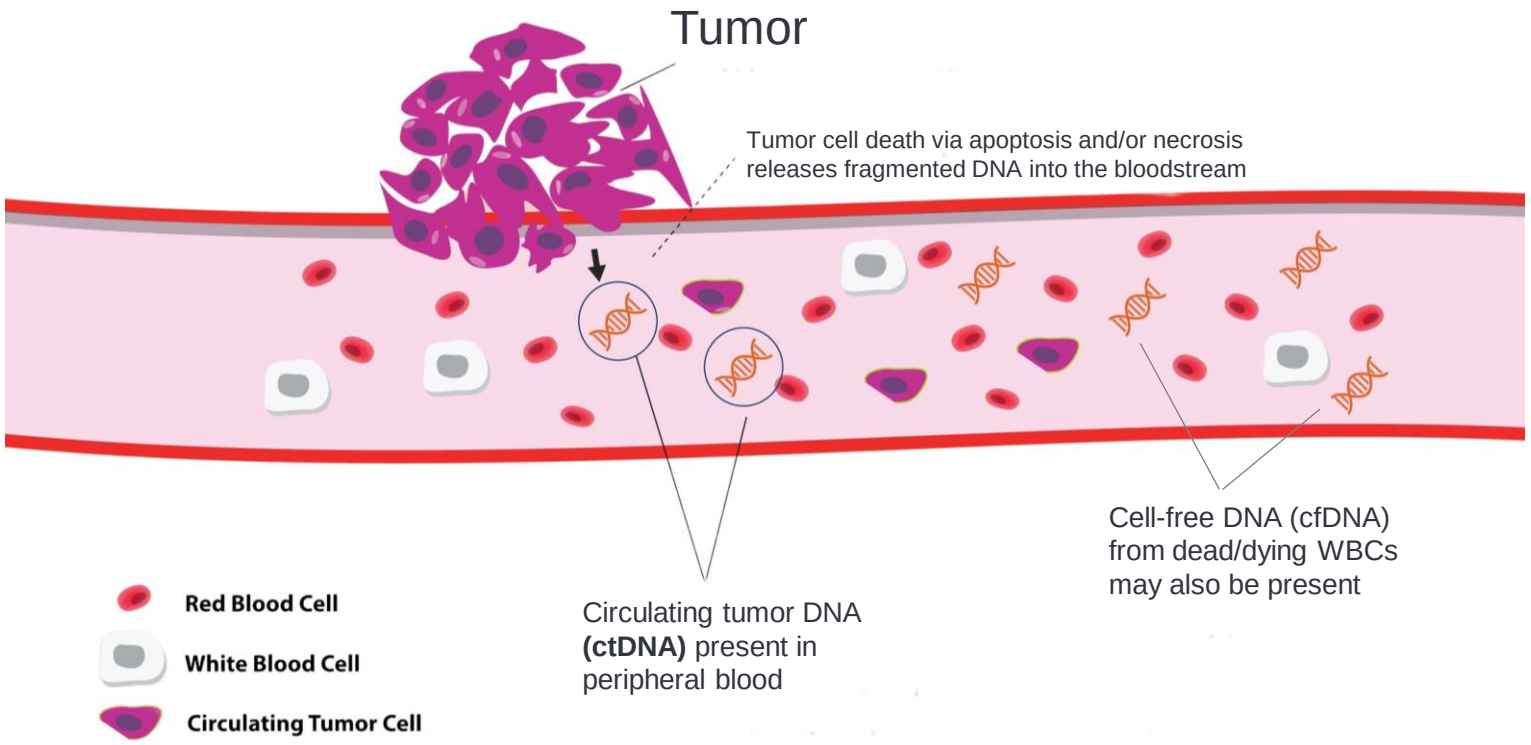


https://www.shutterstock.com/image-vector/cell-anatomy-nucleus-chromosomes-close-dna-2158340551

+ Uncorrected mutations confer a **growth** and/or **survival advantage** to affected cells

+ Mutations can affect germline or somatic cells
  + **Oncogenes** (“mash the gas pedal”)
  + **Tumor-suppressor genes** (“cut the brake lines”)

Liquid biopsy involves capturing circulating cell-free DNA



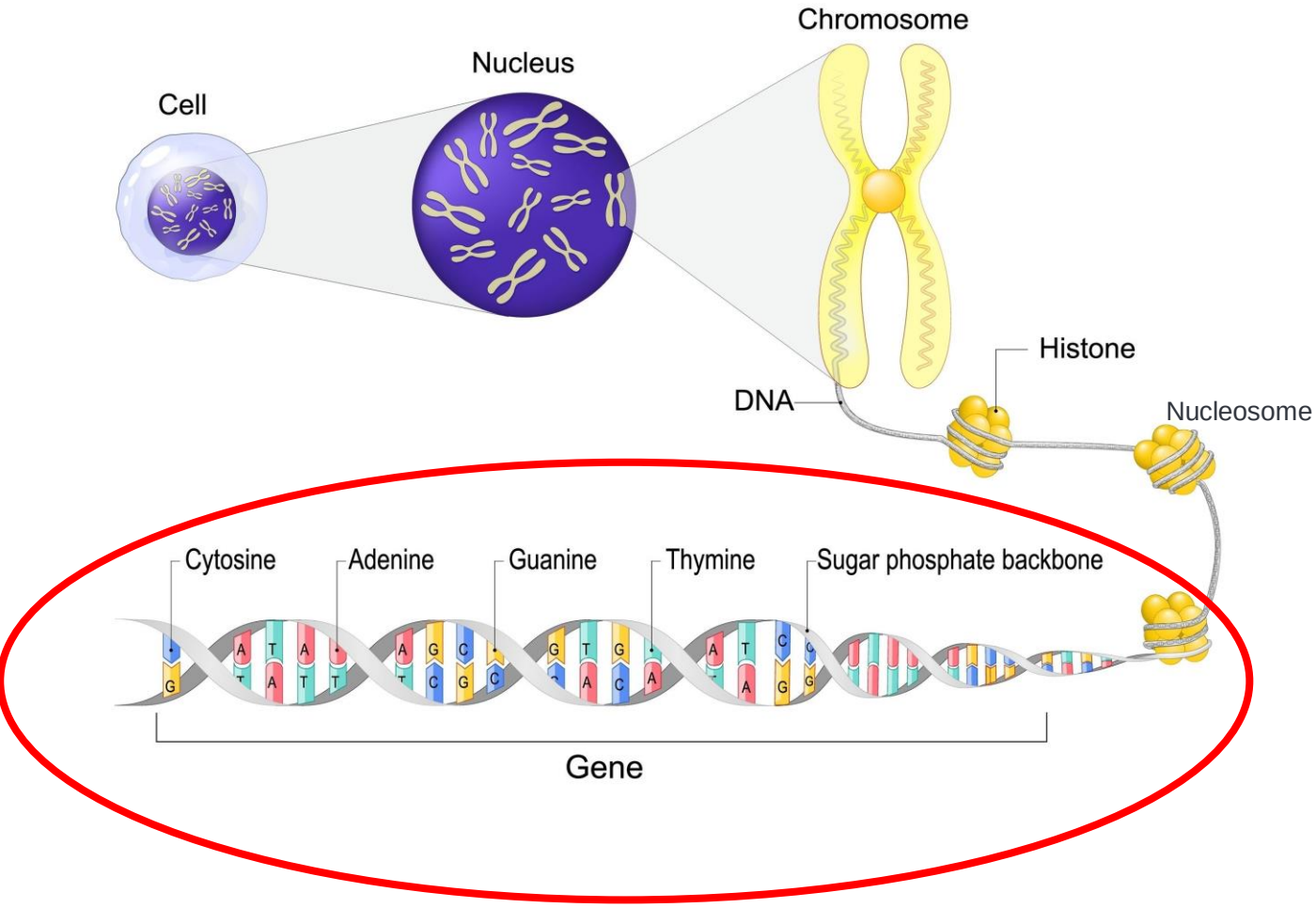
https://www.shutterstock.com/image-vector/circulating-tumor-dna-ctdna-primary-cell-2225391021

Factors affecting the amount of ctDNA in the peripheral blood:

- Size of tumor
- Rapid growth of tumor
- Local vs. systemic

As a result, the likelihood of detecting ctDNA increases when the dog has “more cancer”

OncoK9® detects cancer driver mutations in ctDNA

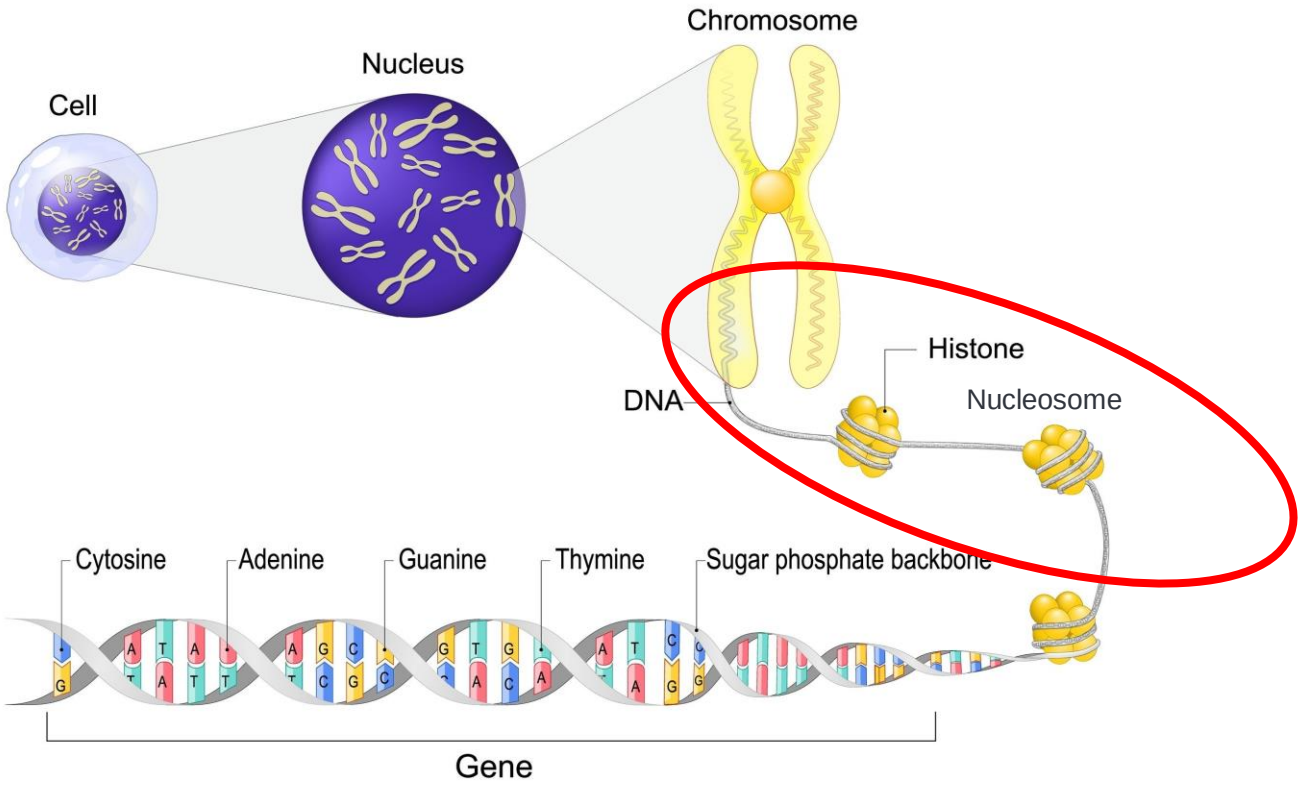


- Blood (two 7 mL DNA isolation tubes) is collected
- Millions of cfDNA fragments are isolated
- Next-generation sequencing (NGS) is used to interrogate the cfDNA
- Bioinformatic algorithms then compare against canine genome to identify mutations of significance

- Result is either “Cancer Signal Detected” or “Cancer Signal Not Detected”
- Sequencing of actual DNA mutations means high specificity for presence of cancer
- Provides no information on type of cancer present (except for ~40% of lymphoma/leukemia samples)

OncoK9® is a registered trademark of PetDx, Inc.

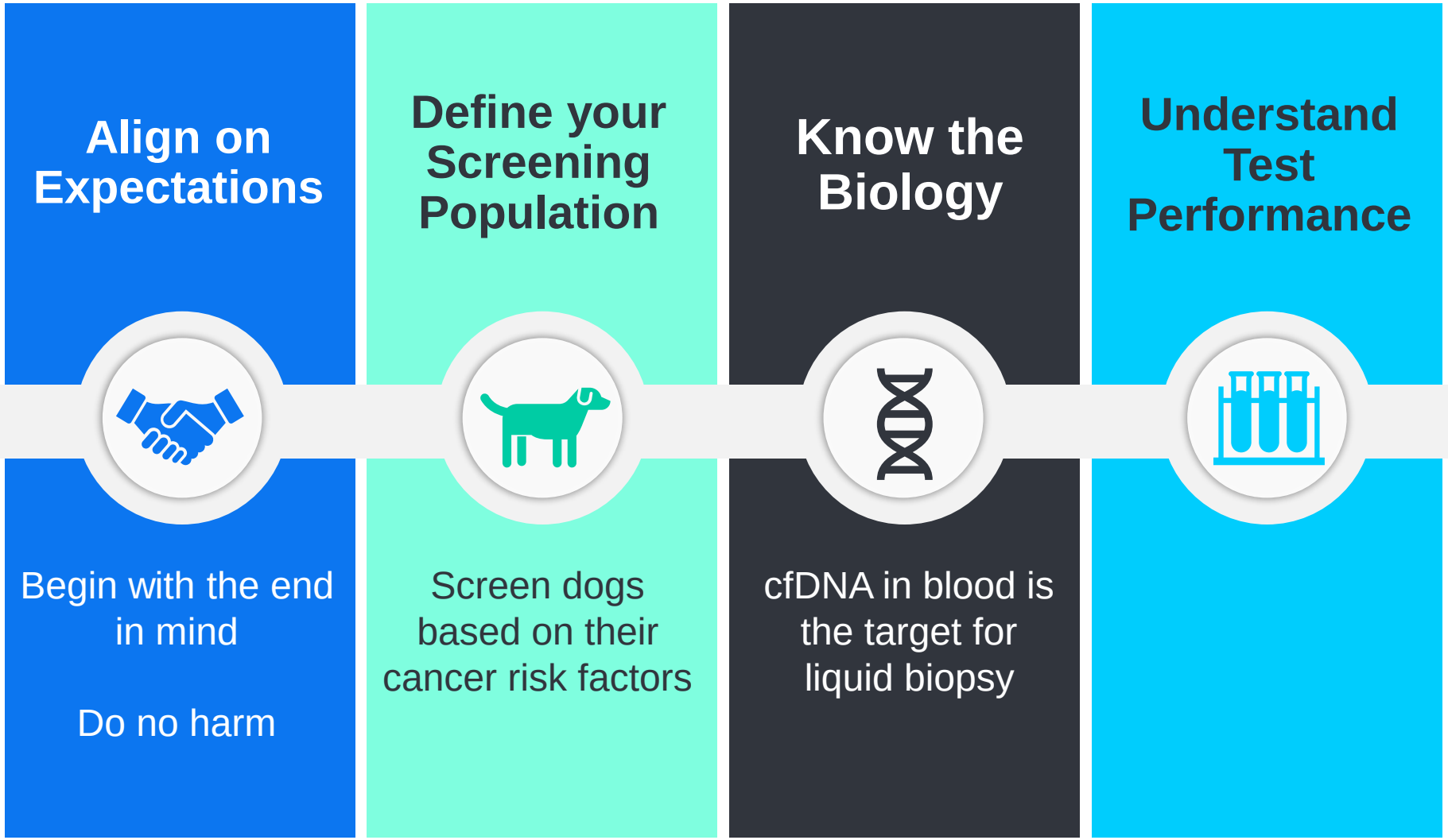
The Nu.Q® Vet Cancer test measures circulating plasma nucleosomes



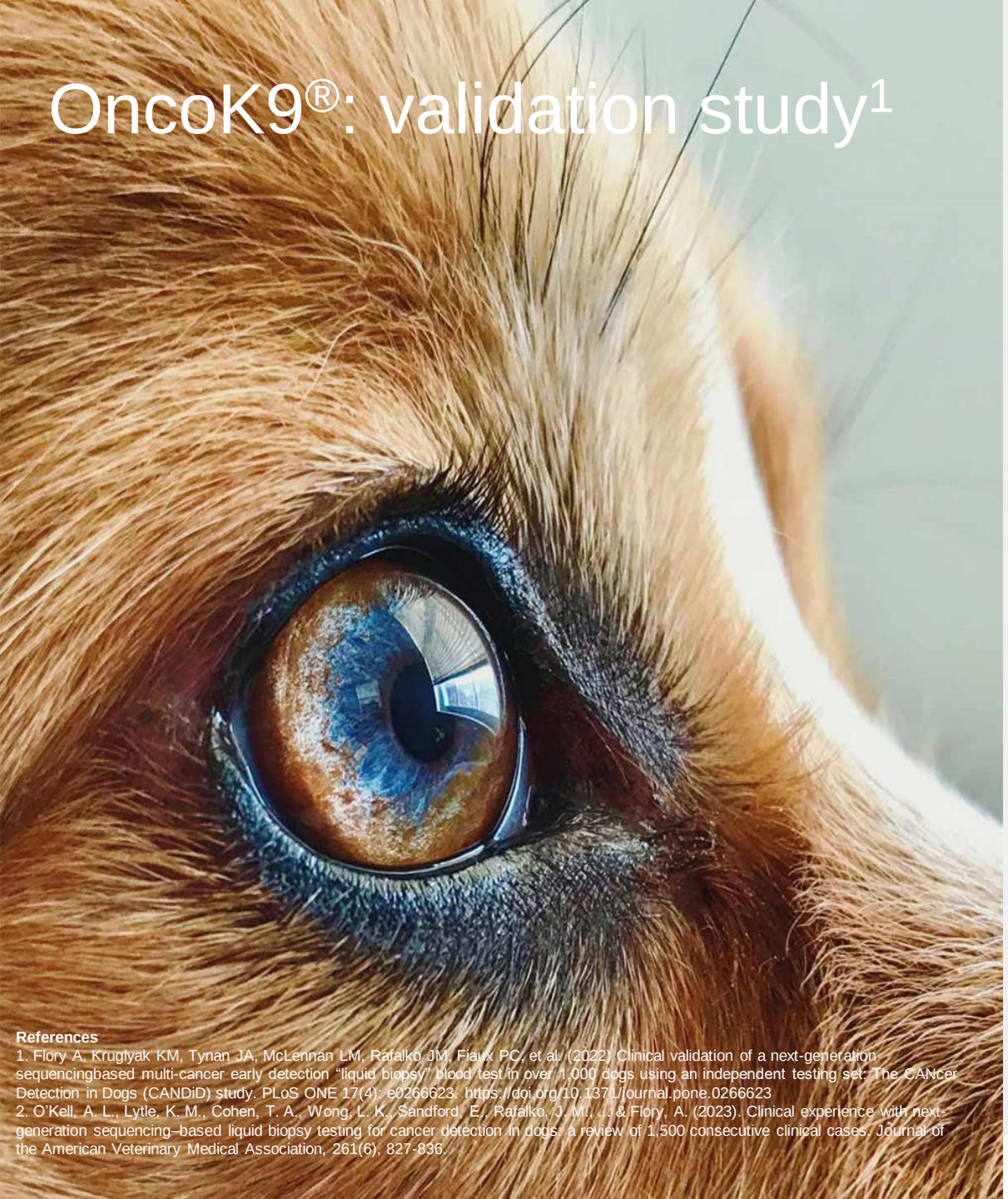
- Blood (2-5 mL) is collected into EDTA tube, centrifuged, and plasma is transferred to non-additive tube
- Nucleosome unit is captured by an ELISA using a specific antibody targeting the histone protein

- Result is a quantitative concentration that is categorized into low, medium, and high risk of cancer
- Provides no information on type of cancer present
- Not a specific marker of cancer, since other processes (e.g., inflammation, sepsis, fed state) can also increase nucleosome concentration

Five Considerations for Canine Cancer Screening Success



OncoK9®: validation study[1]



1100 canine samples included in study:

- 667 presumably cancer-free dogs
- 433 cancer-diagnosed dogs

Variety of dogs included

- Different breeds, weights, age, weight, sex, and cancer stages

All cancers with confirmed diagnosis of malignancy included in the study

Bioinformatic algorithms optimized on training set

- Locked-down algorithm then used on testing set to establish the clinical performance data

Similar performance results were documented in 2023 publication reviewing 1500 clinical cases[2]

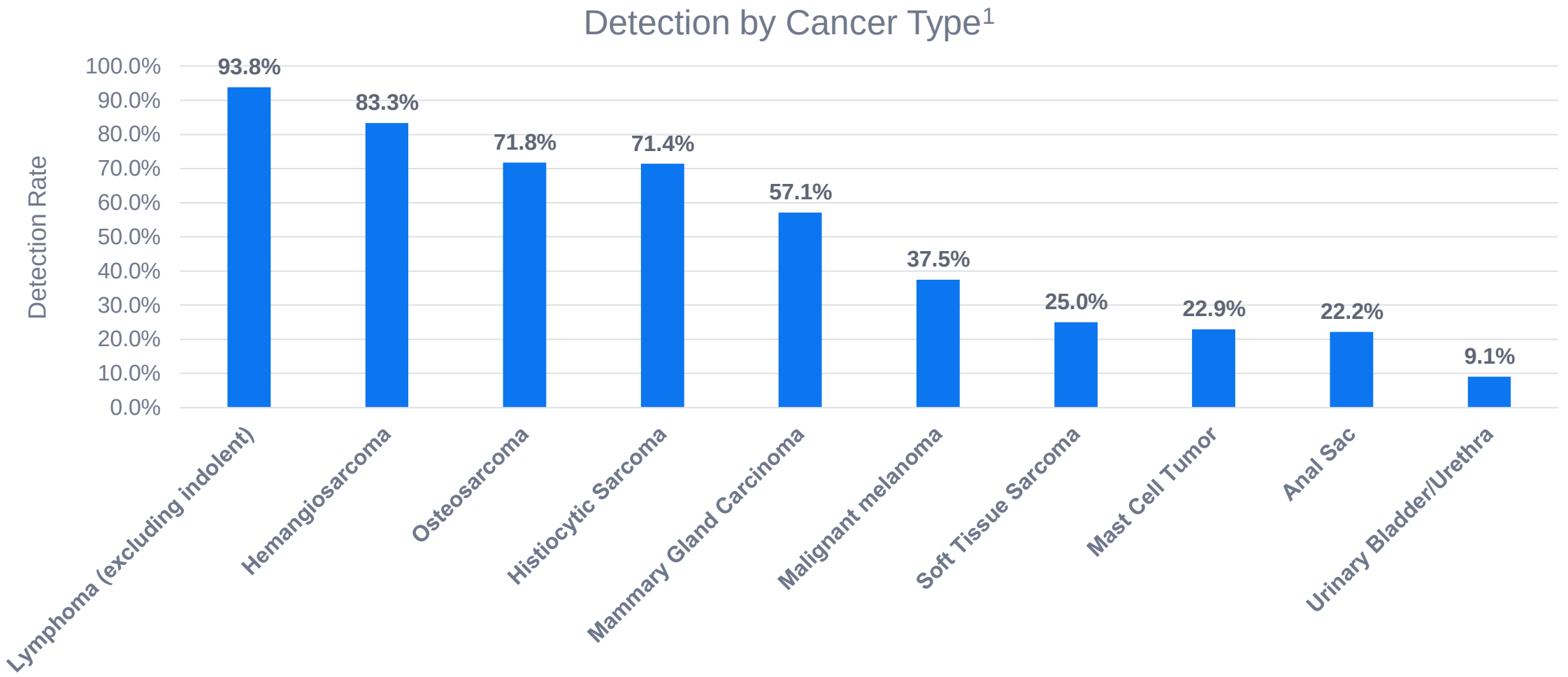
References

1. Flory A, Krugtyak KM, Tynan JA, McLennan LM, Rafalko JM, Fiatx PC, et al. (2022) Clinical validation of a next-generation sequencingbased 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
2. O'Kell, A. L., Lytle, K. M., Cohen, T. A., Wong, L. K., Sandford, E., Rafalko, J. M., ... & Flory, A. (2023). Clinical experience with next-generation sequencing–based liquid biopsy testing for cancer detection in dogs: a review of 1,500 consecutive clinical cases. Journal of the American Veterinary Medical Association, 261(6), 827-836.

OncoK9® Test Sensitivity

At 98.5% specificity versus presumably cancer-free population

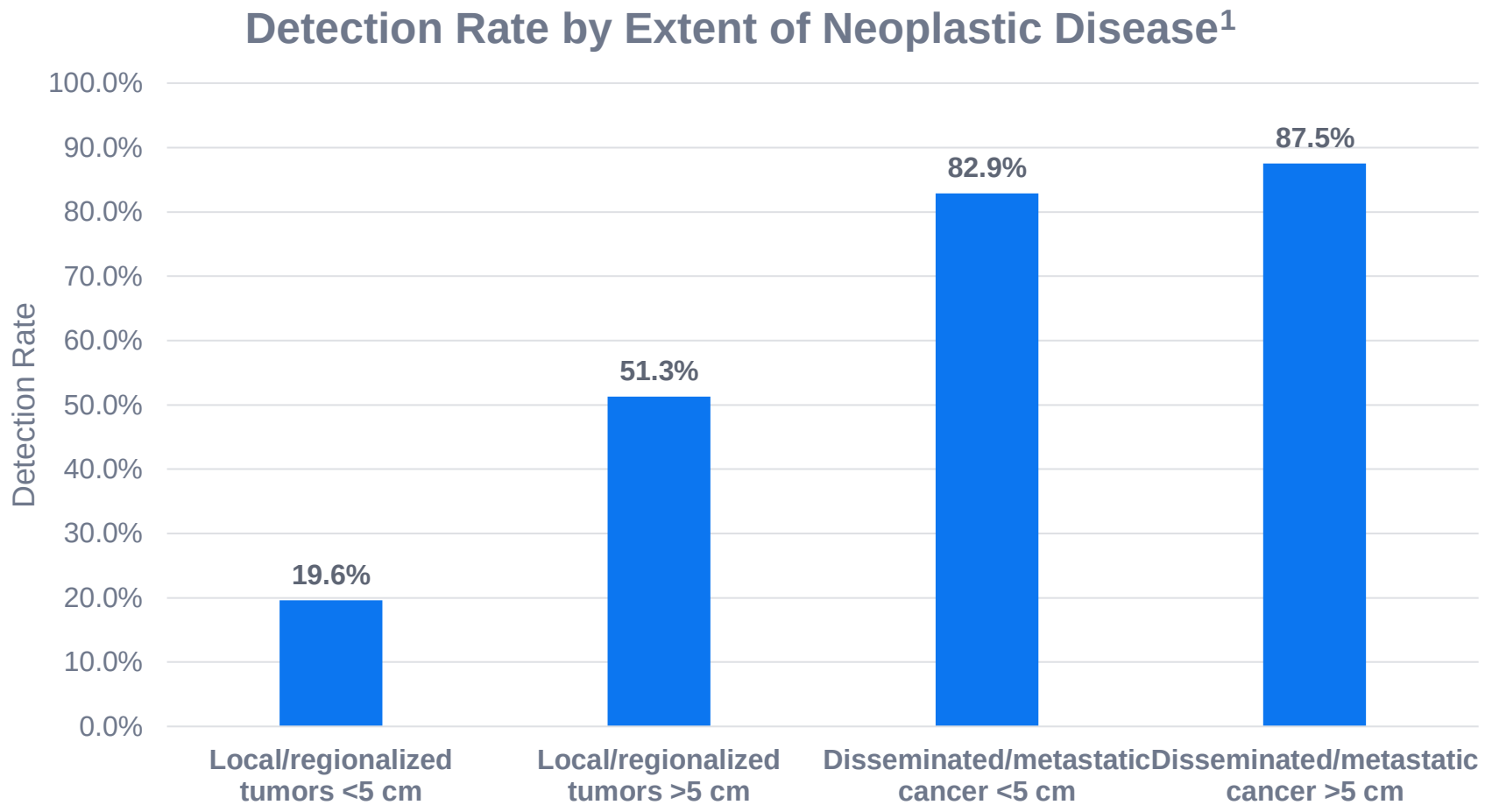
Sample of some of the 30+ cancer types detected by OncoK9®



Reference

1. Flory A, Kruglyak KM, Tynan JA, McLennan LM, Rafalko JM, Fiaux PC, et al. (2022) Clinical validation of a next-generation sequencingbased 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

Cancer detection rate increases with size of tumor and clinical stage



Reference

1. Flory A, Kruglyak KM, Tynan JA, McLennan LM, Rafalko JM, Fiaux PC, et al. (2022) Clinical validation of a next-generation sequencingbased 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

Nu.Q® Vet Cancer Test: validation studies[1]

662 dogs included in studies:

- 134 apparently healthy dogs (did not include dogs with inflammatory conditions)
- 528 dogs with cancer diagnosis

Variety of dogs included

- Different breeds, weights, age, weight, sex, and cancer stages

7 cancers included in studies:

- Lymphosarcoma
- Hemangiosarcoma
- Osteosarcoma
- Soft tissue sarcoma
- Mast cell tumor
- Melanoma
- Histiocytic sarcoma

Reference ranges for nucleosome concentrations established

- Cutoffs established for healthy versus cancer subjects

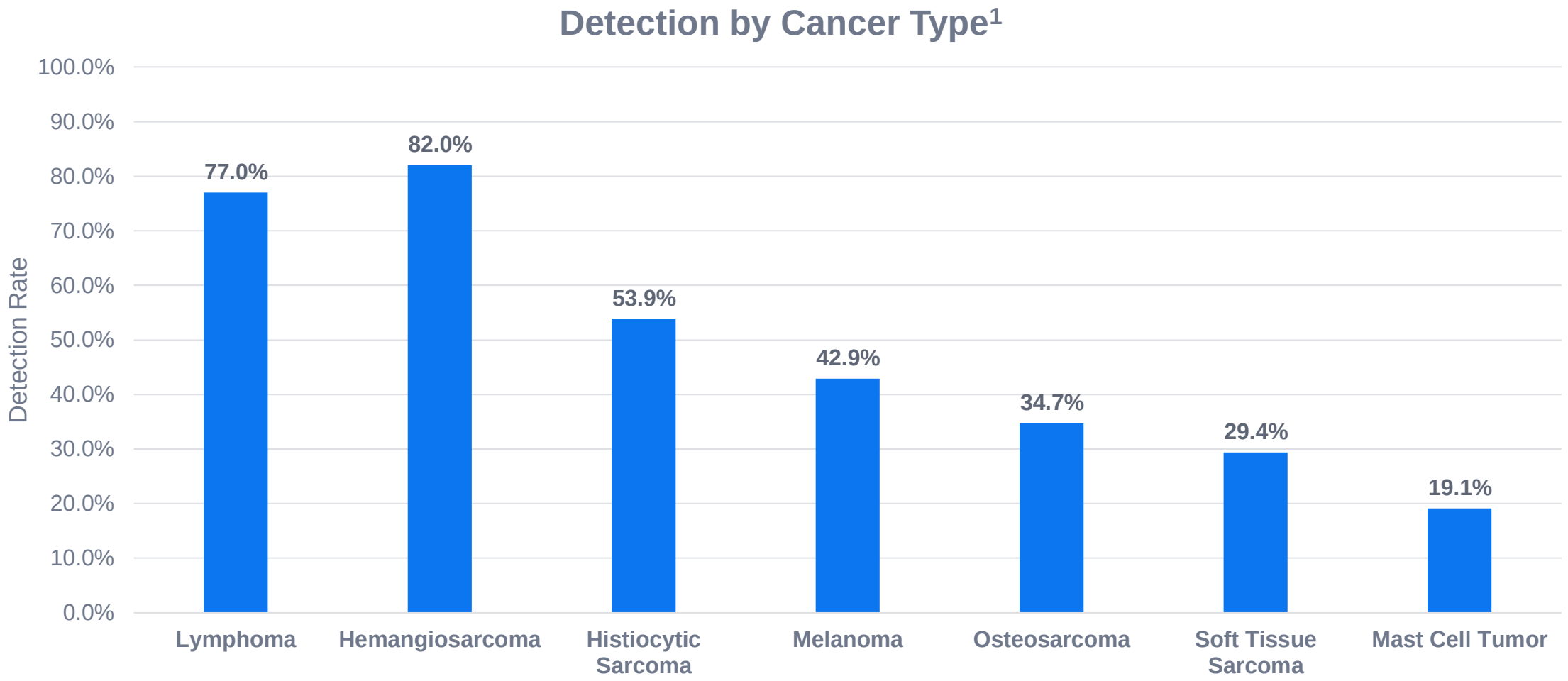
References

1. Dolan C, Miller T, Jill J, Terrell J, Kelly TK, Bygott T, Wilson-Robles H. Characterizing circulating nucleosomes in the plasma of dogs with lymphoma. *BMC Vet Res*. 2021;17(1):276. doi:10.1186/s12917-021-02991-x
2. Wilson-Robles H, Miller T, Jarvis J, et al. Characterizing circulating nucleosomes in the plasma of dogs with hemangiosarcoma. *BMC Vet Res*. 2021;17(1):231. doi:10.1186/s12917-021-02934-6
3. Wilson-Robles H, Miller T, Jarvis J, et al. Evaluation of nucleosome concentrations in healthy dogs and dogs with cancer. *PLoS One*. 2020;15(8):e0236228. doi:10.1371/journal.pone.0236228

IDEXX

Nu.Q® Vet Cancer Test Sensitivity

At 97% specificity versus a healthy control population

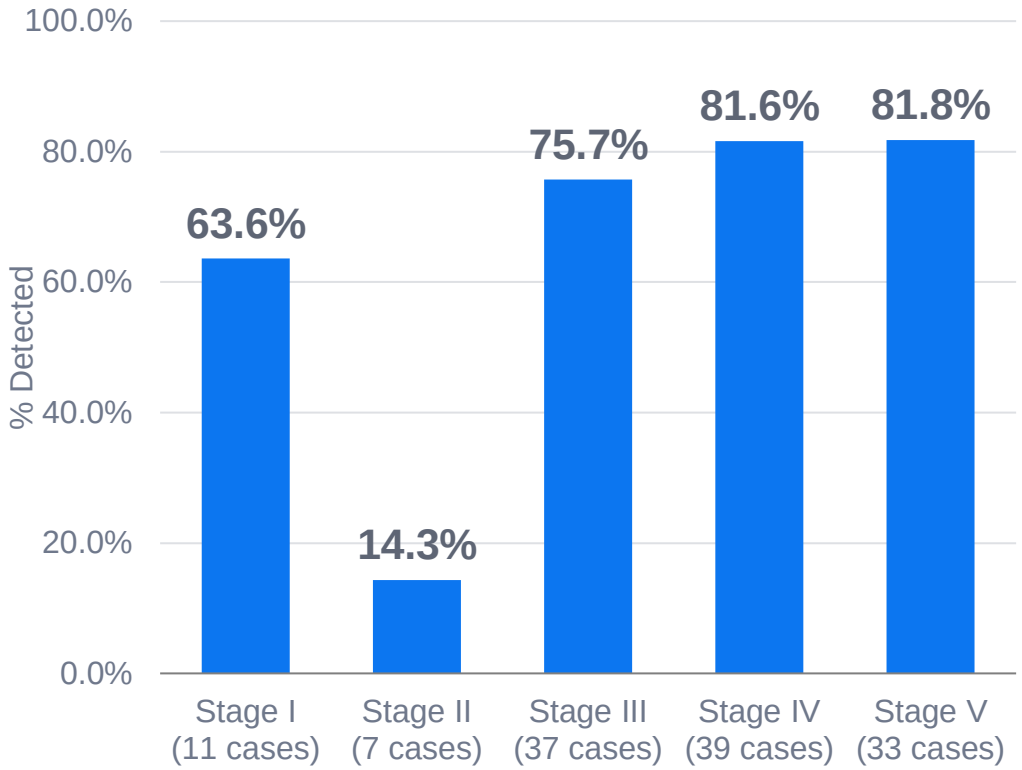


Reference

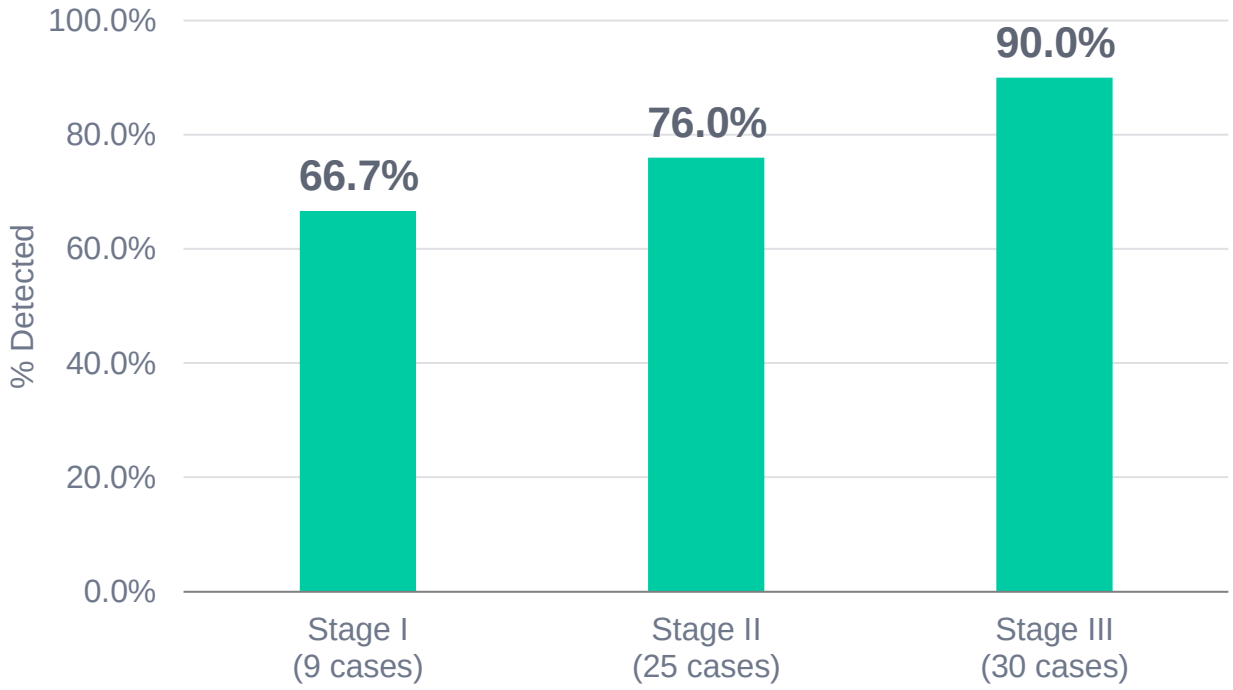
1. Wilson-Robles H, Miller T, Jarvis J, et al. Evaluation of nucleosome concentrations in healthy dogs and dogs with cancer. *PLoS One*. 2020;15(8):e0236228. doi:10.1371/journal.pone.0236228

Chances of elevated nucleosome concentrations increases with stage of cancer

Lymphoma detection by stage[1]



Hemangiosarcoma detection by stage[2]



Reference

1. Dolan C, Miller T, Jill J, Terrell J, Kelly TK, Bygott T, Wilson-Robles H. Characterizing circulating nucleosomes in the plasma of dogs with lymphoma. *BMC Vet Res*. 2021;17(1):276. doi:10.1186/s12917-021-02991-x
2. Wilson-Robles, H., Miller, T., Jarvis, J., Terrell, J., Kelly, T. K., Bygott, T., & Bougoussa, M. (2021). Characterizing circulating nucleosomes in the plasma of dogs with hemangiosarcoma. BMC Veterinary Research, 17(1), 231.

Five Considerations for Canine Cancer Screening Success

Align on Expectations

Begin with the end in mind

Do no harm

Define your Screening Population

Screen dogs based on their cancer risk factors

Know the Biology

cfDNA in blood is the target for liquid biopsy

Understand Test Performance

More advanced, systemic cancers have higher detection rates

Complete the Diagnosis

Before a cancer screening result, consider the information in hand

- Signalment
  - Are there identifiable cancer risk factors?
- History
  - What clues of cancer is the owner possibly providing?
- Physical exam
  - Honest assessment: Are you missing anything?
- Diagnostics
  - What is going to assist your assessment of this patient's health
    - CBC/Chemistry Panel/Urinalysis
    - Fecal Parasites
    - Vector-borne disease
    - Imaging

I have the result of my screening test, what's next?

OncoK9®

Test Result	Cancer Biology and Test Considerations	Potential Next Steps
Cancer Signal Not Detected	- No cancer-associated genomic mutations detected. Reduced likelihood cancer is present	- Retest as part of next wellness visit
	- Suspicion of cancer remains (patient factors, exam findings, other diagnostic findings, cancer type, etc.); not all cancer types detected	- Retest in 4 weeks to 6 months and/or: - Further cancer investigation warranted
Cancer Signal Detected	- Cancer-associated genomic alterations were detected in the DNA from the patient's blood sample. - Given high test specificity, likelihood of cancer is greatly increased.	- Further cancer investigation warranted

OncoK9® is a registered trademark of PetDx, Inc.

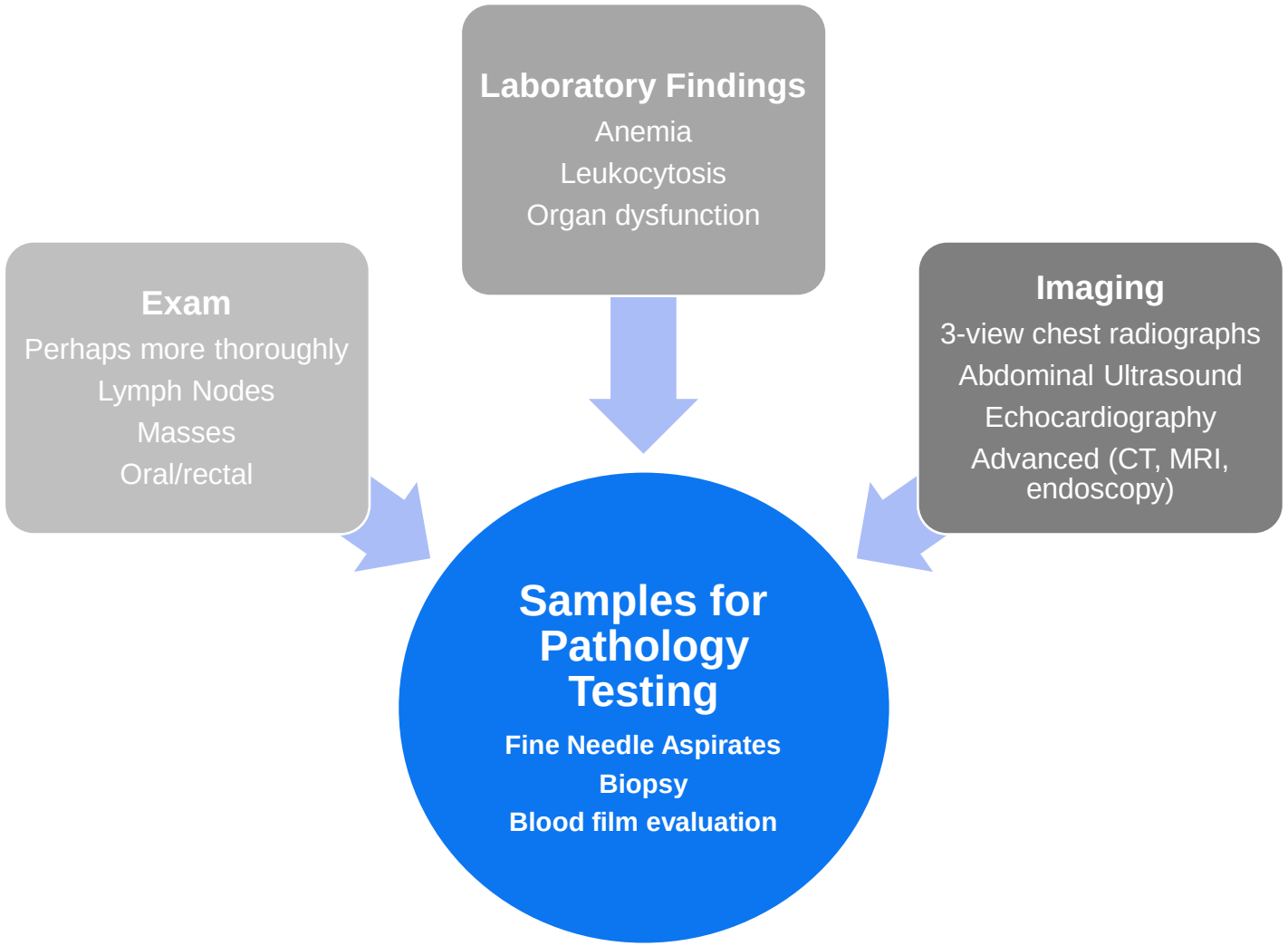
I have the result of my screening test, what's next?

Nu.Q® Vet Cancer Test

Test Result	Cancer Biology and Test Considerations	Potential Next Steps
Low Suspicion (<50 ng/mL)	- Nucleosome levels consistent with healthy patients	- Retest as part of next wellness visit
	- Suspicion of cancer remains (patient factors, exam findings, other diagnostic findings, cancer type, etc.); not all cancer types detected	- Retest in 2-4 weeks and/or: - Further cancer investigation warranted
Moderate Suspicion (50-80 ng/mL)	- Nucleosome concentrations seen with non-fasted patient and/or inflammatory conditions	- Confirm fasted sample; re-draw if necessary - rule-out/resolve inflammation; re-test in 2-4 weeks
	- Nucleosome concentrations consistent with early cancers	- Further cancer investigation warranted
High Suspicion (>80 ng/mL)	- Nucleosome concentrations consistent with levels found in cancer patients	- Further cancer investigation warranted

“Further Cancer Investigation Warranted”

Completing the diagnosis involves sampling for pathology testing



Tips for narrowing the search:

1. Consider screening test sensitivity in detecting specific types of cancer (larger/systemic cancers vs. smaller/local cancers

2. Consider common tumors affecting dogs and specific breed predilection (“common things happen commonly”)

Five Considerations for Canine Cancer Screening Success

Align on Expectations

Begin with the end in mind

Do no harm

Define your Screening Population

Screen dogs based on their cancer risk factors

Know the Biology

cfDNA in blood is the target for liquid biopsy

Understand Test Performance

More advanced, systemic cancers have higher detection rates

Complete the Diagnosis

Use all information in hand to find, sample, and definitively diagnose

Don’t just keep up. Stay ahead with the IDEXX Education Newsletter.

Scan the code to stay in the know and receive updates about:

- Upcoming live events
- Recently archived content and exciting on-demand education
- Articles and tips from peers and industry experts