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Abstract

Liquid biopsy refers to testing blood, urine, or other body fluids to identify disease markers. This technology can detect DNA fragments released by tumors or proteins associated with various tumor types and is widely used for cancer screening in humans. Liquid biopsy tests do not replace gold-standard diagnostic methods such as tissue biopsy; rather, they offer a complementary approach to the identification and monitoring of cancer patients. This article explores the diagnostic capabilities of liquid biopsy tests in veterinary medicine.



ONCOLOGY

Liquid Biopsy: The Future of Cancer Detection in Dogs?

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Liquid biopsy entails sampling biological fluids rather than tissue to detect or monitor disease via biomarkers. Several liquid biopsy tests exist; selection depends on the condition of interest. In humans and dogs, liquid biopsy plays a role in cancer diagnosis.^{1,2} An example in human medicine is the prostate-specific antigen test, which uses blood samples to screen for prostate cancer.

While significant advances have been made in diagnostic techniques for detecting various tumor types, controversy remains regarding the clinical utility of specific liquid biopsy tests in human and veterinary medicine.²

OVERVIEW OF LIQUID BIOPSY TESTS

Liquid biopsy tests used for diagnosing cancer rely on the ability to isolate cellular components of tumor cells, called biomarkers, from various fluids. Not all liquid biopsy tests report the same information. Some report the presence or absence of a biomarker, which may or may not have clinical significance related to a cancer diagnosis. Other tests further interrogate the markers for more specific signals that support a cancer diagnosis (e.g., the presence or absence of genetic mutations associated with specific cancers). Therefore, commercially available veterinary liquid biopsy tests do not replace a tissue-based diagnosis. Instead, they provide a

Take-Home Points

- Liquid biopsy tests detect cancer biomarkers in biological fluids (e.g., blood, urine).
- Liquid biopsy tests can be used as noninvasive screening tests to aid in diagnosing and monitoring cancer in dogs.
- Liquid biopsy tests can produce false-positive and false-negative results, and their sensitivity and specificity vary.
- Liquid biopsy test results do not replace the need for a confirmative diagnosis of cancer obtained by tissue biopsy or other standard-of-care clinical assessment methods.
- Liquid biopsy tests do not provide the information needed for grading and staging cancer.
- Complete staging should be performed when a liquid biopsy test result is suggestive of cancer. Further consultation or referral to a specialist can be considered.
- The significance of a positive liquid biopsy test with negative staging tests is unclear. Further research and clinical experience are necessary to understand how best to manage such patients.



noninvasive alternate method for cancer screening and aid in diagnosis as well as monitoring.¹ These tests complement information gathered from the physical examination, clinical history, and other diagnostic tests.

In veterinary medicine, liquid biopsy is marketed primarily as a cancer screening tool for senior dogs and high-risk breeds. Results can complement baseline information from senior blood panels by identifying cancer biomarkers that may not be apparent in the blood panel alone. These tests can also be beneficial in high-risk breeds for early detection and monitoring of cancer, as well as research purposes.¹ In patients with a known history of cancer, detecting tumor-derived components has the potential to indicate residual disease following a curative-intent intervention (such as surgery) or tumor recurrence.¹ However, the presence of cancer biomarkers does not always align with treatment response, and, whether used for screening or monitoring, liquid biopsy test results must be interpreted in conjunction with other clinical findings and diagnostic test results.³ The following are descriptions of liquid biopsy tests currently commercially available in veterinary medicine.

URINE TESTS

CADET *BRAF*

What is it?

CADET *BRAF* (Antech, [antechdiagnostics.com](https://www.antechdiagnostics.com)) detects a mutation in the *BRAF* gene of cancer cells shed in the urine of dogs with urothelial carcinoma (UC).⁴ This test can detect as few as 10 mutation-bearing cells in a sample. The biological sensitivity of this assay is 85% and the specificity is >99%; the high specificity means it is unlikely to have a false-positive result.⁴ In dogs with an initial negative *BRAF* result, a second test (CADET *BRAF-PLUS*) is typically automatically run. This test detects copy number changes in DNA sequences consistent with UC in dogs lacking *BRAF* mutations. Combined, the *BRAF* and *BRAF-PLUS* have an overall reported sensitivity of 95%. It is important to note that the overall performance of this test is being refined through ongoing research efforts that aim to further define its use for the diagnosis and monitoring of canine UC.^{4,5}

Clinical Applications

CADET *BRAF* is frequently used to support a

diagnosis of UC in dogs with a bladder or prostatic mass and associated clinical signs.⁴ This test can also confirm a diagnosis of UC in dogs with cytological evidence of a urinary tract carcinoma (e.g., following evaluation of urine cytospin, fine-needle aspiration of a bladder or prostatic mass).⁴ An additional application involves screening high-risk breeds (e.g., Scottish terriers, Shetland sheepdogs, West Highland White terriers), in which it offers the potential to detect emerging UC before clinical signs related to the tumor become apparent.⁶ These cases are challenging for veterinary oncologists, as it is unclear whether therapeutic intervention would be beneficial or affect disease progression at this time. The test can also be used to screen for cancer in dogs with recurrent or complicated urinary tract infections without obvious evidence of a bladder mass.

What Samples Are Necessary and How Are They Submitted?

A free-catch urine sample of 40 mL must be collected in the provided CADET *BRAF* collection cup. It does not require refrigeration. Collection may be performed over several days to achieve the optimal quantity of urine. The test is not affected by blood, protein, or bacteria in the urine.

Oncotect

What is it?

Volatile organic compounds (VOCs) are produced and emitted through the metabolism of cancer cells or the body's immune system. In humans, distinct urinary VOC patterns have been associated with different cancer types and stages.⁷ Oncotect ([oncotect.co](https://www.oncotect.co)) capitalizes on the strong olfactory capacity of the nematode *Caenorhabditis elegans* to detect VOCs in urine samples from dogs.⁸ The premise of this test is based on preclinical data that showed *C. elegans* can quantitatively detect the presence of signature VOCs in both culture media of neoplastic cell lines and urine samples obtained from human cancer patients.⁹ Similar results were seen in a pilot study using urine samples from dogs with various cancers. In that study, the worms were more strongly attracted to urine samples with cancerous cells than samples without.⁸

This test does not identify a specific type of cancer but rather provides information about a patient's risk of cancer. The risk is based on a scale that considers the

magnitude to which *C elegans* is attracted toward the urine sample. The sensitivity of identifying at least a moderate cancer risk in a confirmed cancer patient is 85% and the specificity is 90%.⁸ Oncotect was evaluated in the urine samples of dogs with lymphoma, melanoma, hemangiosarcoma, and mast cell tumors.

Clinical Applications

Oncotect is marketed as a cancer screening test that can be used every 6 to 12 months in dogs aged 7 years and older or in high-risk breeds as young as 5 to 6 years old. It can be considered for dogs in which cancer is suspected. Further diagnostic tests should be considered in dogs with a moderate- or high-risk result to confirm the presence of cancer. The role of urinary tract inflammation and its effect on VOCs and *C elegans* chemotaxis is unknown. Therefore, results should be interpreted cautiously.

What Samples Are Necessary and How Are They Submitted?

Oncotect provides a urine collection kit that can be used to collect a 3- to 5-mL urine sample, which is then shipped directly to the company.

BLOOD TESTS

Nu.Q Vet

What is it?

The Nu.Q Vet cancer test (Volition Veterinary, veterinary.volition.com) measures the total nucleosome concentration in plasma. Nucleosomes are the structural unit of DNA packaging. They consist of DNA wrapped around proteins called histones. When cancer cells die, nucleosomes are released into the blood. Increased nucleosome concentrations identify patients who may have cancer.¹⁰

The Nu.Q test detects 7 common cancers in dogs with a sensitivity of 50% and a specificity of 97%.¹⁰ The highest sensitivity was seen for hemangiosarcoma (82%) and lymphoma (77%).^{11,12} These values represent the performance of the test in patients with a known cancer diagnosis.

Clinical Applications

The Nu.Q Vet cancer test is marketed as a cancer

In veterinary medicine, liquid biopsy is marketed primarily as a cancer screening tool for senior dogs and high-risk breeds.

screening test for dogs 7 years and older during all routine wellness examinations or for high-risk breeds. In dogs with a moderate or high risk of cancer based on nucleosome concentration, additional diagnostic tests should be performed to confirm a cancer diagnosis.

Results should be interpreted cautiously, as nucleosomes can be elevated with systemic inflammation, immune-mediated diseases, sepsis, or trauma and in dogs that have not been fasted a minimum of 4 hours before obtaining a sample.^{11,12}

What Samples Are Necessary and How Are They Submitted?

A 2- to 5-mL plasma sample obtained from spun whole blood is required. Samples must be shipped overnight on ice. A point-of-care version of this test is expected in the future.

OncoK9

What is it?

OncoK9 (PetDx, petdx.com) analyzes cell-free DNA (cfDNA). cfDNA consists of short fragments of DNA released into the blood during cell death. Sequencing of cfDNA detects gene mutations specific to a variety of cancers. Dogs with malignant tumors have significantly higher levels of cfDNA than those with benign tumors and healthy controls.¹³

The presence of a cancer-associated mutation in a sample is reported as “cancer signal detected.” If no cancer-associated mutation is detected, the result is reported as “cancer signal not detected.”

The detection rate for the most common canine cancers analyzed is 62%, with an overall sensitivity of 55%.¹⁴ When only lymphoma, hemangiosarcoma, and



osteosarcoma are being considered, the sensitivity increases to 85%. The specificity of the test is 99%.¹⁴ Like the Nu.Q test, these values represent the performance of the test in patients with a known diagnosis of cancer.

Clinical Application

OncoK9 may be incorporated as part of the annual screening for all dogs starting at 7 years of age; it can be used for younger dogs of breeds in which cancer is more likely to develop at an earlier age. As a screening test, the positivity rate for the detection of cancer is only 4.5%.¹⁵ It is also marketed as an “aid in diagnosis” for dogs with suspected cancer based on clinical signs or other clinical findings. Finally, it may have the potential to detect minimal residual disease (i.e., the presence of tumor-specific biomarkers and cancer recurrence following cancer treatment).

What Samples Are Necessary and How Are They Submitted?

OncoK9 provides a blood collection kit to collect 14 mL of whole blood from a peripheral vein. The tubes can be kept at ambient temperature for up to 7 days.

INCORPORATING LIQUID BIOPSY INTO VETERINARY PRACTICE

The ability to detect cancer at earlier stages is an important consideration for veterinarians. Approximately 1 in 4 dogs will develop cancer; in dogs older than 10 years, this rate increases to almost half.¹⁶ Cancer is a leading cause of death in pets, and most forms, while treatable, are not curable.

However, while early detection is important, there is no substantial evidence-based information to prove early intervention improves outcomes such as disease-free intervals or survival times. Additionally, veterinary oncologists are not in universal agreement regarding the institution of therapy based on positive liquid biopsy test results alone. Therefore, with any positive result, complete staging should be considered, in addition to obtaining a diagnosis via tissue biopsy, if feasible and applicable, before any therapeutic interventions are performed.

Staging aims to find the cancer, if present, to obtain a definitive diagnosis. This includes complete laboratory

testing, thoracic and abdominal imaging, and tissue sampling, if applicable. When the diagnosis and the extent of the disease are unknown, choosing suitable diagnostic tests may present challenges. Preferred diagnostic modalities vary by tumor type and treatment recommendations. It is appropriate to consult with a veterinary oncologist in cases of positive liquid biopsy to determine the next best course of action.

If staging tests detect cancer, further consultation or referral to a specialist should be considered to further elucidate the grade, additional staging tests, and possible treatment options. Conversely, if complete staging is performed in a dog with a positive liquid biopsy result and no cancer is found, this could represent a false-positive test or a scenario in which the staging tests lack the diagnostic power to detect disease. Additional laboratory tests and advanced imaging may be considered on a case-by-case basis.

False-positive and false-negative results can occur with liquid biopsy. False-positive results may incur additional costs with unnecessary repeat and/or serial diagnostic testing and, in some instances, potentially prompt premature decisions about euthanasia. On the other hand, false-negative results can give a false sense of security and perhaps lead to a missed opportunity to detect cancer earlier in its course.

False-negative results have largely been associated with an assay’s technical detection limit. If the overall disease burden in the patient is low, the test is less likely to pick up on the biomarker in question. In more disseminated cases with higher disease burdens, the test is more apt to return as positive. In this latter scenario, conventional diagnostics (e.g., physical examination, thoracic and abdominal imaging) are likely as sensitive as liquid biopsy, if not more sensitive, for detecting disease. Therefore, liquid biopsy tests do not replace standard-of-care clinical assessment methods and should be performed only as an adjunct diagnostic tool for cancer detection.

The cost of liquid biopsy tests can vary, but it is typically a few hundred dollars. Insurance coverage may vary by provider, plan, and case for each test. Pet owners with insurance should contact their insurance provider to discuss coverage. When facing limited financial resources, it is recommended to prioritize allocating funds toward diagnostic tests that provide a definitive diagnosis and offer guidance for treatment. **TVP**



References

1. Chibuk J, Flory A, Kruglyak KM, et al. Horizons in veterinary precision oncology: fundamentals of cancer genomics and applications of liquid biopsy for the detection, characterization, and management of cancer in dogs. *Front Vet Sci.* 2021;8:664718. doi:10.3389/fvets.2021.664718
2. Tidd-Johnson A, Sebastian SA, Co EL, et al. Prostate cancer screening: continued controversies and novel biomarker advancements. *Curr Urol.* 2022;16(4):197-206. doi:10.1097/CU9.0000000000000145
3. Rossman P, Zabka TS, Ruple A, et al. Phase I/II trial of vemurafenib in dogs with naturally occurring, BRAF-mutated urothelial carcinoma. *Mol Cancer Ther.* 2021;20(11):2177-2188. doi:10.1158/1535-7163.MCT-20-0893
4. Mochizuki H, Shapiro SG, Breen M. Detection of BRAF mutation in urine DNA as a molecular diagnostic for canine urothelial and prostatic carcinoma. *PLoS One.* 2015;10(12):e0144170. doi:10.1371/journal.pone.0144170
5. Dhawan D, Ramos-Vara JA, Utturkar SM, et al. Identification of a naturally-occurring canine model for early detection and intervention research in high-grade urothelial carcinoma. *Front Oncol.* 2022;12:1011969. doi:10.3389/fonc.2022.1011969
6. Gentilini F, Palgrave CJ, Neta M, et al. Validation of a liquid biopsy protocol for canine BRAFV595E variant detection in dog urine and its evaluation as a diagnostic test complementary to cytology. *Front Vet Sci.* 2022;9:909934. doi:10.3389/fvets.2022.909934
7. Wen Q, Boshier P, Myridakis A, Belluomo I, Hanna GB. Urinary volatile organic compound analysis for the diagnosis of cancer: a systematic literature review and quality assessment. *Metabolites.* 2020;11(1):17. doi:10.3390/metabo11010017
8. Namgong C, Kim JH, Lee MH, Midkiff D. Non-invasive cancer detection in canine urine through *Caenorhabditis elegans* chemotaxis. *Front Vet Sci.* 2022;9:932474. doi:10.3389/fvets.2022.932474
9. Hirotsu T, Sonoda H, Uozumi T, et al. A highly accurate inclusive cancer screening test using *Caenorhabditis elegans* scent detection. *PLoS One.* 2015;10(3):e0118699. doi:10.1371/journal.pone.0118699
10. Wilson-Robles HM, Bygott T, Kelly TK, et al. Evaluation of plasma nucleosome concentrations in dogs with a variety of common cancers and in healthy dogs. *BMC Vet Res.* 2022;18(1):329. doi:10.1186/s12917-022-03429-8
11. Wilson-Robles HM, 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
12. Dolan C, Miller T, Jarvis J, et al. Characterizing circulating nucleosomes in the plasma of dogs with lymphoma. *BMC Vet Res.* 2021;17(1):276. doi:10.1186/s12917-021-02991-x
13. Tagawa M, Shimbo G, Inokuma H, Miyahara K. Quantification of plasma cell-free DNA levels in dogs with various tumors. *J Vet Diagn Invest.* 2019;31(6):836-843. doi:10.1177/1040638719880245
14. Flory A, Kruglyak KM, Tynan JA, et al. 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.* 2022;17(4):e0266623. doi:10.1371/journal.pone.0266623
15. O'Kell AL, Lytle KM, Cohen TA, et al. Clinical experience with next-generation sequencing-based liquid biopsy testing for cancer detection in dogs: a review of 1,500 consecutive clinical cases. *JAVMA.* 2023;261(6):827-836. doi:10.2460/javma.22.11.0526
16. Vail DM, MacEwen EG. Spontaneously occurring tumors of companion animals as models for human cancer. *Cancer Invest.* 2000;18(8):781-792. doi:10.3109/07357900009012210



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