Overview

OncoK9 is a multi-cancer early detection (MCED) test for the detection and characterization of cancer-associated genomic alterations in DNA isolated from canine whole blood samples, using next-generation sequencing (NGS) technology. OncoK9 is intended for use in dogs who are at higher risk of cancer. It is recommended as an annual screening test for all dogs starting at 7 years of age[1], and potentially at younger ages for dogs belonging to breeds that are predisposed to cancer. It is also recommended as an aid-in-diagnosis for dogs in which cancer is suspected based on clinical signs or other clinical findings. As with any laboratory test, OncoK9 results should be interpreted by a veterinarian in the context of each patient's medical history and clinical presentation. The test is available by prescription only.

OncoK9 analyzes the genomic profiles of cell-free DNA (cfDNA) extracted from plasma and of genomic DNA (gDNA) extracted from white blood cells. Since the test is designed to detect genomic alterations believed to be associated with cancer, the test report provides a result indicating if cancer-associated genomic alterations were detected or not.

OncoK9 reports can provide three types of results: *Cancer Signal Not Detected*, *Cancer Signal Detected*, and *Not Reportable - Sample Failed* (for samples that fail to meet quality criteria). Results are issued electronically after a technical and clinical review by a team of highly trained veterinarians and cancer genomics specialists at PetDx.

Cancer Signal Not Detected

A *Cancer Signal Not Detected* result indicates that no cancer-associated genomic alterations were detected in the DNA from the patient's blood sample. This significantly reduces the likelihood that cancer is present, but does not rule out the presence of cancer or the possibility of cancer developing in the future. If cancer is clinically suspected, a full diagnostic evaluation should be performed. Consider re-testing with OncoK9 if cancer remains high on the differential diagnosis list, as advancing disease generally provides a higher cancer signal.

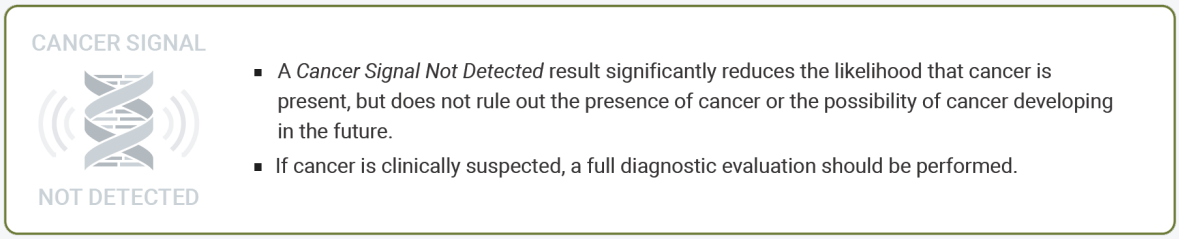

FIGURE 1. A patient with a *Cancer Signal Not Detected* result will not have the Cancer Signal badge highlighted because no cancer-associated alterations were detected.

Cancer Signal Detected

A ***Cancer Signal Detected*** result indicates that cancer-associated genomic alterations were detected in the DNA from the patient's blood sample. This significantly increases the probability that cancer is present, but does not establish a definitive diagnosis of cancer. A full clinical evaluation must be performed to establish a definitive diagnosis in this patient. **This result should not be used as the sole basis for making important decisions such as treatment or euthanasia.**

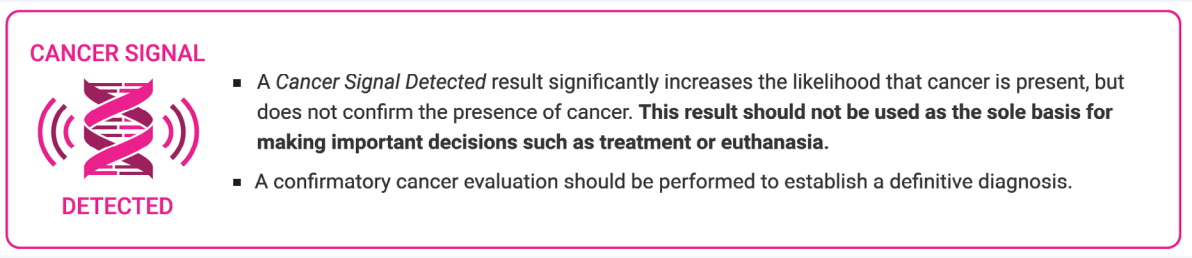

FIGURE 2. A patient with a *Cancer Signal Detected* result will have the Cancer Signal badge highlighted because cancer-associated alterations were detected.

Comments

Cancer Signal Origin Prediction: Hematological malignancy.

Recommended Next Steps

A *Cancer Signal Detected* result does not establish a diagnosis of cancer. A confirmatory cancer evaluation is required to establish a definitive diagnosis. The following confirmatory measures should be considered: thorough clinical history and physical exam (including an oral and rectal exam), complete bloodwork and urinalysis, routine imaging (such as thoracic radiographs and abdominal ultrasound), tissue sampling of any detected masses for pathological analysis, and, if indicated, advanced imaging such as CT and/or MRI. If the confirmatory cancer evaluation does not result in a cancer diagnosis, this could mean that the selected confirmatory measures did not identify the cancer, or that the test result was a false positive. Further evaluation and/or monitoring of this patient should be considered.

Positive (Cancer Signal Detected) reports will provide a set of recommendations for next steps in the evaluation of the patient. These are general recommendations, and the treating veterinarian can decide on their usefulness for managing individual patients.

In some cases, a Cancer Signal Origin (CSO) Prediction will be provided in the Comments field. OncoK9 is currently validated to provide CSO Prediction for a subset of hematological malignancies, such as lymphoma; other cancer types may be added in the future. Please see the CANDiD study for more details.

Not Reportable - Sample Failed

A ***Not Reportable - Sample Failed*** result indicates that the sample sent to PetDx did not meet internal quality criteria for a reportable result, and submission of a new sample is typically advised. The report will provide instructions about submitting a new sample.

Understanding OncoK9 Test Performance and Predictive Values

As discussed above, the two main types of results from the OncoK9 test are ***Cancer Signal Detected*** and ***Cancer Signal Not Detected***. The test performance of OncoK9 has been established in a clinical validation study of over 1,000 canine patients with and without cancer at an overall sensitivity of 55% and a specificity of 98.5%.[2] In a subgroup analysis comprising three of the most aggressive cancer types in dogs (lymphoma, hemangiosarcoma, osteosarcoma), the sensitivity was 85%.[2] In a subgroup analysis comprising eight of the most common cancer types in dogs (lymphoma, hemangiosarcoma, osteosarcoma, soft tissue sarcoma, mast cell tumor, mammary gland carcinoma, anal sac adenocarcinoma, and malignant melanoma),[3] the sensitivity was 62%.[2] As with any laboratory test, false positive and false negative results do occur. To properly contextualize a result for an individual patient based on their unique characteristics and their test result, positive and negative predictive values are helpful.

Once an individual test result has been issued, the clinical question shifts from test sensitivity ('how does the test perform at detecting cases of cancer in a 'cancer-only' population?') and test specificity ('what proportion of individuals without cancer get a negative result?') to questions specific to the individual patient that now has a positive or a negative test result.

After a positive result (***Cancer Signal Detected***), the clinical question becomes 'what is the chance that *this* patient has cancer in light of this positive result *and their own unique clinical presentation*'? The PPV (Positive Predictive Value) answers this important question and guides the discussion around how much clinical concern there should be regarding the presence of cancer.

Conversely, after a negative result (***Cancer Signal Not Detected***), the clinical question becomes 'what is the chance that *this* patient does NOT have cancer in light of this negative result *and their own unique clinical presentation*'? The NPV (Negative Predictive Value) answers this question and guides the level of clinical reassurance that can be provided regarding the absence of cancer. At the individual patient level, the interpretation of a test result needs to be contextualized within the pre-existing clinical scenario unique to that individual patient.

Interpreting Results

- **Positive Predictive Value (PPV)** estimates the probability that the individual with a ***Cancer Signal Detected*** (positive) test result actually has cancer.
- **Negative Predictive Value (NPV)** estimates the probability that the individual with a ***Cancer Signal Not Detected*** (negative) test result actually does not have cancer.

Calculation of **PPV** and **NPV** incorporates the following three elements:

- Test sensitivity (does not change from patient to patient)
- Test specificity (does not change from patient to patient)
- The ***prior probability*** (*prior risk*) of cancer ***before*** the test was conducted; this is related to the prevalence of cancer in the population to which the patient belongs, and it *does* change from patient to patient, based on age, weight, breed, clinical history, clinical signs, etc.

The patient's veterinarian is best suited to estimate the ***prior probability*** of cancer (*prior risk*) in the context of each patient's unique clinical situation.

The table below is intended to assist in the interpretation of OncoK9 test results. To use the table, the veterinarian should determine which clinical use case below is applicable for each individual patient; the corresponding PPV and NPV ranges are listed in the adjacent cells.

Clinical use case	Intended use population	Prior probability of cancer*	PPV (Given a positive test result)	NPV (Given a negative test result)
Screening	≥7 years old and/or predisposed breed	8 - 10%	76 - 80%	95 - 96%
Aid-in-diagnosis	Cancer suspected based on clinical presentation	30 - 50%	94 - 97%	68 - 84%

$$PPV = \frac{\text{sensitivity} \times \textit{prior probability}}{\text{sensitivity} \times \textit{prior probability} + (1 - \text{specificity}) \times (1 - \textit{prior probability})}$$

$$NPV = \frac{\text{sensitivity} \times (1 - \textit{prior probability})}{(1 - \text{sensitivity}) \times \textit{prior probability} + \text{specificity} \times (1 - \textit{prior probability})}$$

**Estimated prior probabilities are based on a review of the literature and a survey of over 300 US-based veterinarians (PetDx data on file).*

Estimated ranges for positive predictive value (PPV) and negative predictive value (NPV) calculated using an overall test sensitivity of 54.7% and specificity of 98.5% (range calculated using the lower and higher ends of prior probability)

REFERENCES

1. Rafalko J, Kruglyak K, McCleary-Wheeler A, 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. bioRxiv doi: 10.1101/2022.03.30.486448
2. Flory A, Kruglyak KM, Tynan JA, McLennan LM, Rafalko JM, Fiaux PC, 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
3. Biller B, Berg J, Garrett L, Ruslander D, Wearing R, Abbott B, et al. 2016 AAHA Oncology Guidelines for Dogs and Cats. J Am Anim Hosp Assoc. 2016;52(4):181–204.