Is it cancer? Use of blood-based test as an aid-in-diagnosis for cancer detection in dogs

This session, sponsored by PetDX will review the basic principles of liquid biopsy testing and how this technology may be useful as an aid-in-diagnosis.

Speaker Bios:

Dr. O'Kell is a board-certified internal medicine specialist with experience in both academia and private practice. At PetDx, she serves as a vet-to-vet resource for OncoK9 clinical use consultations as well as supporting clinical research studies. After graduating from the Western College of Veterinary Medicine at the University of Saskatchewan, she completed a rotating Small Animal Medicine and Surgery internship at the Virginia Maryland Regional College of Veterinary Medicine, followed by a combined Small Animal Internal Medicine Residency and Master's degree at the same institution. Dr. O'Kell enjoys all aspects of internal medicine and clinical research, the latter of which has focused on novel disease biomarkers and canine diabetes pathogenesis. She was awarded a prestigious National Institutes of Health Career Development Award in 2018 to support this research. Dr. O'Kell has published numerous articles in peer reviewed journals, has presented at a variety of veterinary conferences, and has a wealth of experience providing education for veterinary students and CE for the veterinary community.

Dr. Cohen is a board-certified internal medicine specialist with more than a dozen years of experience in specialty private practice. At PetDx, he serves as a vet-to-vet resource for OncoK9 clinical use consultations as well as supporting clinical research studies. After earning a Doctor of Veterinary Medicine from UC Davis School of Veterinary Medicine, Dr. Cohen completed his internship at the University of Pennsylvania,

followed by his internal medicine residency back at UC Davis. Before PetDx, he practiced at VCA Animal Specialty & Emergency Center in Los Angeles. both private practice and academic settings. I have been studying diabetes in dogs at the University of Florida since 2016 and am currently supported by an NIH K08 Career Development Award. My research focus is canine diabetes pathogenesis and early disease biomarker discovery.

Learning Objectives:

1. Describe basic principles of cancer development and understand how cell-free DNA can be used to detect cancer in dogs
2. Identify clinical scenarios in which liquid biopsy may be useful as an aid-in-diagnosis for cancer in dogs
3. Review appropriate interpretation of liquid biopsy results in the aid-in-diagnosis scenario

Is it cancer- Use of a blood-based test as an aid-in-diagnosis for cancer detection in dogs

[00:00:00.18] Dr. Cohen and I are really excited to be here tonight to discuss this topic, the use of a blood-based test as an aid and diagnosis for cancer detection in dogs. So just a disclosure, as both Dr. Cohen and I are full-time employees and shareholders of PetDx.

[00:00:18.86] So we are all here today because cancer is a big health concern in dogs, and is actually the leading cause of death in dogs in North America. So even if you don't diagnose cancer every day, it's certainly a differentiable on your list every day for your sick patients. And overall, 4 to 6 million dogs per year are diagnosed with cancer. So the scope of the problem is huge.

[00:00:45.40] And so if we think of the cancer detection paradigm as it exists today, most cases of cancer in dogs are actually detected due to the presence of clinical signs. Or you might detect cancer incidentally when that pet presents to you for another problem. And so we know we have a number of traditional diagnostic tools in the workup to diagnose cancer, or other diseases, that we are all likely quite familiar with.

[00:01:10.85] So our elements of a typical clinical workup in your sick patient would include a physical exam, some laboratory testing, probably some diagnostic imaging, and maybe some tissue sampling. But what happens if you hit a roadblock in your diagnostic workup?

[00:01:27.73] So roadblocks might include you might find a mass in a patient that's in a difficult to access location for a fine needle aspirate or a biopsy. You might actually get to do cytology or histopathology, but those results are inconclusive or unrewarding. You may have lack of timely access to specialist care, which I think we all know has been a major problem in the recent past, and currently. Or you may have an owner that's hesitant to pursue recommended testing, even if you do have a path moving forward.

[00:01:59.88] So thinking about what tools do you have to use in these scenarios, Today, we're actually going to discuss a novel noninvasive test called liquid biopsy that may help in some of these situations, whether that be to help you focus and prioritize your diagnostic plan, or maybe help convince a hesitant owner to proceed with additional diagnostics.

[00:02:23.02] So we're going to start by talking about the science behind liquid biopsy, which is cancer genomics. So genomics is the field of science that studies an individual's entire genome, or the set of instructions in their DNA. And if we think back to the science classes that we've all taken at some point, the genome is present in every cell except for mature red blood cells. And at its core, it's made up of base pairs of nucleotides that form a DNA strand. And then that DNA strand is packaged up into chromosomes that fit into the nucleus of each cell.

[00:02:58.63] Advances in molecular medicine over the past two decades have revealed that cancer is truly a disease of the genome, and that normal cells will accumulate random genomic alterations over time. And that is really the root cause of cancer.

[00:03:13.73] So as clinicians, we typically will define cancer by its organ or tissue of origin, or by its cellular characteristics. The cause of cancer really is when one or more alterations in the genome confer an uncontrolled growth advantage to a population of cells. So this can be a small alteration, such as a single letter spelling mistake like a substitution, or it can be an insertion or deletion of several nucleotides.

[00:03:40.42] We also can have larger genomic events that affect thousands to millions of nucleotides. And these are known as structural alterations, which can include copy number variants, or CNVs, which involve gains or losses of large structures of DNA. Or we can have

translocations, in which DNA strands from unrelated parts of the genome are joined together and result in the fusion of genes.
[00:04:05.86] So these genomic alterations lead to a single cell developing an uncontrolled growth advantage. But over time, we have successive genomic alterations so that the first microscopic clone will acquire a second alteration. And then that clone may also acquire another alteration. And you can see in this diagram, these colors represent different subclones.
[00:04:29.70] And so when the cancer grows larger, we have continued clonal expansion and novel mutations. So that by the time we actually recognize that patient has cancer, it actually has cancers and are made up of a heterogeneous group of cells. When considering how genomics fits into liquid biopsy, we need to discuss cell-free DNA.
[00:04:52.81] So when cells die, either by apoptosis or necrosis, the contents are released into the circulation. And this includes cell-free DNA. And so cell-free DNA can be released from both normal and cancer cells. And the fraction that originates in cancer cells is called circulating tumor DNA, or ctDNA.
[00:05:15.18] CfDNA in plasma can be collected, and then sequenced and analyzed with next generation sequencing. And the unique genomic structure of cancer can be identified because the cancer cells have genomic alterations that do not exist in the healthy cells.
[00:05:32.07] And it's important to note that cell-free DNA only lasts minutes to hours. And so that's key for a couple of reasons. One is that the measurement or the analysis of cell-free DNA represents, a snapshot in time of what is happening right now in the body. And the second is that special blood collection tubes must be used in order to stabilize and preserve that DNA.
[00:05:57.27] So we're going to talk about a schematic of next generation sequencing and how that can analyze cell-free DNA to detect these genomic abnormalities. So we just talked about having cell-free DNA shed into the bloodstream from both normal and cancer cells. We collect a blood sample. And that blood sample is separated into its components, including the plasma, and then the buffy coat which contains white blood cells.
[00:06:23.76] DNA from the plasma and the White blood cells are extracted, amplified, and sequenced. And then a sophisticated bioinformatics pipeline is used to identify those cancer-associated genomic alterations. And these are not found in normal cells. So when we find these, that indicates the presence of cancer in the body.
[00:06:45.00] So if we think back to our typical cancer management paradigm, there are several areas in which liquid biopsy might actually fit in. So we can consider using liquid biopsy for cancer screening. And this might be in a dog that's at high risk for cancer, whether due to advanced age, or it may be a breed that is predisposed to cancer.
[00:07:07.45] We can also use liquid biopsy in a clinical workup as what we call an aid in diagnosis. So these are cases in which liquid biopsy can help us further understand the case, in which cancer is on the differential list. And these are considered pre-diagnosis use cases. This means that we don't have a diagnosis of cancer, but the liquid biopsy may help us get there.
[00:07:34.05] There are also some post-diagnosis use cases. And we're not going to talk about those today, but I just want to mention what they are so you can get an idea of the full spectrum of liquid biopsy. So it can be used for targeted treatment selection, after you already have a diagnosis of cancer. It can also be used for minimal residual disease detection and recurrence monitoring, as well as treatment response monitoring.
[00:08:01.56] We're going to now move into talking about the clinical validation of liquid biopsy in canine cancer. The CANcer Detection in Dogs, or CANDiD study, was published in PLOS One in April of 2022. And this was a validation study looking at OncoK9 in over 1,000 dogs. And it is available in PLOS One, which is a peer-reviewed open access journal. So it is available for you to download and read for free.

[00:08:30.38] The study population in the CANDiD study included dogs both with and without cancer. And so there were several different protocols. And in protocol 101, these were dogs that were presumably cancer-free So all of these dogs had a thorough history and physical exam by their enrolling veterinarian. And at the time of enrollment, they had no history of cancer and no current suspicion of cancer.
[00:08:53.06] Dogs in this cohort were allowed to have common benign skin and subcu tumors, such as lymphomas, skin tags, and sebaceous adenomas. And they were also allowed to have other acute and chronic medical conditions. They just couldn't have a current suspicion of cancer. So importantly, this represented a real world population of dogs in the presumably cancer-free group.
[00:09:16.33] The dogs with cancer were included in the 201 protocol, which consisted of dogs that had confirmed cancer but were not having surgery as part of their treatment plan, or dogs in the 301 protocol that had suspected or confirmed cancer, and were planning to have surgery as part of their treatment plan. And overall, this included 1,100 dogs.
[00:09:41.75] So these 1,100 dogs represented 85 breeds, more than 40 cancer types, in 41 clinical sites around the world. The veterinarians in this study included DVMs, that were both general practitioners and specialists. And so this study was used to validate OncoK9, and it is the largest clinical validation study ever performed in veterinary cancer diagnostics.
[00:10:08.92] We're going to talk about the results. And first, we're going to break these up into cohorts. And because this is a multi-cancer detection study that has a wide variety of cancer types, we wanted to look to see how the detection rate was in various groups, starting with the most aggressive cancers, then moving on to the most common cancers, and then looking at all of the cancers that were detected in the study.
[00:10:32.72] So of the three most aggressive canine cancers, which were lymphoma, hemangiosarcoma, and osteosarcoma, the detection rate was 85%. When we look at eight of the most common cancers, and these cancers were defined this way based on a 2016 AHA paper, they included both the three most aggressive cancers as well as the additional ones seen in the metal box here. The detection rate was 62%.
[00:11:01.66] And then of all cancer-diagnosed subjects in the study, the detection rate was 55%. Importantly, the specificity for the study was 98.5%, which corresponds to a false positive rate of 1.5%. And it's really important to note that OncoK9 was optimized for specificity, as a low rate of false positives is really important for a test such as this one.
[00:11:30.23] So we also looked at detection rate of cancer by extent of disease and tumor size. And so on the right side of this chart, we have disseminated or metastatic disease grouped by size. So either less than 5 centimeters or greater than 5 centimeters. And you can see here that the detection rate was greater than 80%. And we have localized or regional disease on the left side of this chart. So localized being defined as confined to the organ of interest, or involving regional lymph nodes only. The detection rate ranged from around 20% to 50%.
[00:12:07.55] So and this makes sense if you think about how the test works because the cell-free DNA is released in circulation. And the larger or more disseminated the tumor is, the more cell-free DNA that it may release. And so when you think about how we typically diagnose, or detect cancer, we actually did a recent study that looked at cancer detection on a wellness exam. And in wellness, it was detected about 4% of the time.
[00:12:36.08] And so if we have a test that could even detect 20% of cancers at their earliest stages, this could represent a big improvement over our standard of care right now. And it's really exciting to think about how this might improve our ability to detect cancer earlier.
[00:12:54.01] This is a full list of cancer types detected in the CANDiD study. And so there were 30 different cancer types, and they're listed here in this alphabetical list that's divided up by a

combination of both cancer type and anatomic location. And those in bold represent the most common cancer types, that we mentioned on the slide earlier.
[00:13:17.07] So we've just talked about the performance metrics of OncoK9. And so we wanted to compare that to some tests that you use regularly in practice, and that you're likely pretty comfortable with. So this might help you put into perspective how the performance of a newer test like OncoK9 compares to performance of some other tests.
[00:13:38.76] And so this table includes information that's already in the published literature. And so you can see that, for example, an FNAC cytology of a lymph node looking for a neoplastic disease has the detection rate of about 67%, and a false positive rate of 8.5%. When we look at diagnosing pancreatitis, we often use a SNAP cPL or a Spec cPL as part of the workup.
[00:14:03.75] And depending on the study, you can see the detection rate varies between 59% and 100%, and the false positive rate between 2.5% to 79%. And that's just based on the study in the population that they looked at. And so when you look at OncoK9's detection rates, they compare similarly to some of these tests that we're already using and feel comfortable with, and a false positive rate of 1.5% is quite low.
[00:14:33.35] OncoK9 is currently recommended as an annual screening test for dogs that are at high risk of cancer, whether that be too advanced age, so over seven, or certain breeds that have a higher risk of cancer potentially at younger ages. It's also recommended as an aid-in-diagnosis test. And that might be for patients in which cancer is suspected, but the site is challenging to access. Or you do suspect cancer, but the site is actually not clinically evident. and the aid-in-diagnosis use is what we're going to focus on today.
[00:15:06.85] So we're going to move on to how to interpret test results. The results of OncoK9 in general are binary. So either a cancer signal not detected or a cancer signal detected. We will discuss a subset of cases that may receive a cancer signal of origin prediction of hematologic malignancy a little bit later on. But for right now, we're going to focus on the cancer signal not detected or cancer signal detected.
[00:15:33.69] So when you get a positive or a negative result, how do you actually interpret that with respect to the patient you have in front of you? So sensitivity and specificity, that we just talked about, are characteristics of the test itself. And when you apply that test to a real world population, you actually need to take into account the disease prevalence. And the positive and negative predictive values take this into account, and actually become more relevant when you're interpreting a test in light of the patient that you have sitting in front of you.
[00:16:04.93] So the positive predictive value is the probability that a patient has cancer, given a positive OncoK9 result and that patient's unique clinical presentation. The negative predictive value is the probability that a patient does not have cancer, given a negative OncoK9 result. And the formulas are listed here for those that like math. I'm not going to go through them in detail, but they're there for you if you need. But just know that this takes into account sensitivity, specificity, and prevalence.
[00:16:37.18] And so, as I mentioned prevalence, the PPV and NPV depend on this. And so we need to come up with an estimate of prevalence in the population that we're using the test for. So to estimate prevalence for aid-in-diagnosis cases, we actually polled veterinarians.
[00:16:53.88] And they estimated that when cancer was on their differential diagnosis list in the top two to three differentials, they ended up diagnosing cancer about half of the time. So that led us to estimate a 30% to 50% prevalence of cancer in that patient that presents to you with clinical signs in which cancer is suspected.
[00:17:14.61] And so when you plug those numbers in, we get an estimated positive predictive value of 94% to 97% for a positive test, meaning that your patient with suspected cancer and a

positive OncoK9 has a 94% to 97% probability of having cancer.
[00:17:33.00] Now, if that patient gets a negative OncoK9 result, the negative predictive value is 68% to 84%. Meaning that there's a 68% to 84% chance that that patient with a negative OncoK9 result does not have cancer. And we're going to walk through some scenarios in a little bit more detail to just give a better picture of how this works.
[00:17:55.51] But I did just want to mention that since OncoK9 is also validated for screening, we did the calculation for this screening use as well. And so at an estimated prior probability of cancer of 8% to 10% in our high risk population, so whether that be due to advanced age or breed, the estimated positive predictive value of a positive test result is 76% to 80%. So 76% to 80% chance that patient has cancer. And a negative result in a screening case has a 95% to 96% chance that patient does not have cancer.
[00:18:34.55] All right. So as I mentioned, we're going to walk through a hypothetical scenario in order to illustrate in more detail how to apply and think about the PPV and NPV and test interpretation. And I just want to mention. This doesn't just apply to OncoK9 specifically. With any test, thinking about PPV and NPV are really important.
[00:18:56.18] So here we have a population of 100 dogs. And we're going to assume, for this example, that there's a 30% prevalence of cancer here. And so those dogs are represented in black. So with a sensitivity overall of 55%, this means that 17 out of these 30 dogs will receive a true positive test result. And so these dogs are in the pink circles. With a specificity of 98.5%, only 1 of 70 dogs without cancer will receive a false positive test result.
[00:19:30.81] So when we do the numbers and add them up, we have 18 positive results in this population of 100 dogs. 17 are true positive, and 1 is false positive. And that gives us a positive predictive value of 94%. So again, there's a 94% chance that your patient with clinical signs of cancer does have cancer when it has a positive OncoK9.
[00:19:54.38] It's really important to note, though, that while a positive OncoK9 in this situation increases the likelihood the patient has cancer, it is not a diagnostic test for cancer. So it's really one piece of the puzzle in your diagnostic workup, and it may help you focus your workup or other diagnostic testing. We recommend that a positive OncoK9 test result should not be used alone to make important decisions, such as treatment or euthanasia.
[00:20:21.59] So it's somewhat similar to if you're doing a workup on an older golden retriever with lethargy. And you might do an abdominal ultrasound as part of that workup, and you find a splenic mass. Now, you have a very high suspicion that splenic masses cancer in this dog, but you need additional testing to confirm that cancer is definitively present. And the ultrasound result is one piece of the puzzle. And so similarly, OncoK9 is one piece of the puzzle in your workup.
[00:20:51.96] All right. So what about negative results? So how much reassurance can we have if you get a negative OncoK9 result? And again, that's related to the apriory risk of cancer, or the prevalence of cancer in that population.
[00:21:05.83] So going back to our 100-dog scenario with a 30% prevalence. So 30% of these dogs do have cancer. At a sensitivity of 55%. That means 14 out of 30 dogs, or 45%, will receive a false negative result. So those are in the blue rectangles. With the specificity of 98.5%, 69 out of 70 dogs with cancer will receive a true negative result. So underscoring the high specificity here.
[00:21:38.57] When we add up the numbers and do the calculations, this comes out to 83 negative results, 69 are true negative, and 14 are false negative, with a negative predictive value here of 83%. So there's an 83% probability that your patient with a negative OncoK9 does not have cancer. And so this means, while a negative OncoK9 makes cancer less likely, and you could probably move that cancer lower on your differential list a negative result does not rule out

cancer.
[00:22:12.27] And so back to our ultrasound example, think of a workup that you might do for a patient with acute azotemia. You may do an ultrasound as part of that workup, and the kidneys might actually look completely normal. So does that mean that the patient does not have acute kidney injury as a cause for that is azotemia. So no, it doesn't because we do know that the kidneys are not always abnormal on ultrasound in acute kidney injury.
[00:22:38.96] And so if other pieces of information you have for that patient do support an AKI, you would continue to work up that patient for AKI and its underlying causes. So this is the same situation. If you have other pieces of information that support cancer in your patient, you do still need to consider it as a differential diagnosis.
[00:23:01.69] And so that was a lot of information and a lot of numbers. So I'm just going to quickly summarize the key numbers that we talked about. So for an aid-in-diagnosis scenario, meaning a patient with clinical signs that you suspect might have cancer, a positive result, or cancer signal detected, means there's a 94% to 97% chance that the dog has cancer. With a negative result in this scenario, there's a 68% to 84% chance the dog does not have cancer.
[00:23:33.15] All right. So now, I'm going to hand it over to Dr. Cohen, who's going to discuss some interesting cases that use OncoK9 as an aid-in-diagnosis, and hopefully help you get an idea of how to use this in practice.
[00:23:49.94] Thanks Dr. O'Kell,. And thank you everyone for being here tonight. So what I'm going to do is walk you through a few cases in which OncoK9 was used as an aid and diagnosis tool for a variety of reasons.
[00:24:03.22] So the first case that I'm going to talk to you about is Molly. So Molly's a nine-year-old female spayed Labrador who presented to her local veterinarian for left thoracic limb lameness in April of 2022. She had some radiographs taken of both her elbows and her shoulders, which showed just some very mild osteoarthritis of both of her elbows, as well as some mild changes to her left shoulder. But given the degree of lameness that Molly had, these radiographic changes were deemed to be relatively underwhelming and unlikely to be the cause of her clinical signs.
[00:24:39.22] So given the lack of significant radiographic changes, an OncoK9 was performed to investigate Molly's lameness further and reveal the cancer signal detected. As a result of that cancer signal detected result and Molly's signalmen clinical signs, a more thorough physical exam was performed focusing on that left thoracic limb, and revealed a soft tissue mass in her axillary region associated with her proximal humerus. A fine needle aspirate was performed. However, it was inconclusive, revealing mostly laced cells. Therefore, an incisional biopsy of the mass was performed with histopath revealing a poorly differentiated soft tissue sarcoma.
[00:25:26.16] To further evaluate the mass, a CT scan was performed for surgical planning and revealed a rim-enhancing mass effect, medial to the proximal humerus, that was extending through the musculature. And there, you can see a blown up version of that rim-enhancing mass. Following the CT, an amputation was performed, and the histopathology of the mass did indeed confirm a poorly differentiated soft tissue sarcoma.
[00:25:55.17] So to summarize Molly, she's a nine-year-old female spayed Labrador, with a left thoracic limb lameness and radiographic changes deemed not sufficient enough to account for her clinical signs. As a result, and given her signalmen, and OncoK9 was performed to investigate her lameness further and show a cancer signal detected. Fine needle aspirate, incisional biopsy, and CT led to a definitive diagnosis and staging of her poorly differentiated soft tissue sarcoma, an amputation was performed, and her diagnosis was confirmed.
[00:26:29.27] So the takeaways from Molly's case are that OncoK9 led to closer examination of that left thoracic limb and/or axillary region, and also led to discovery of a mass in a challenging

location to detect. This discovery allowed for a diagnosis to be made and a treatment plan to be developed for Molly.
[00:26:50.81] So moving on to the next case. I'm going to talk to you about a dog named Arthur. So Arthur is a 13-year-old male, castrated plot hound mix who had about a one week history of diarrhea, with some intermittent vomiting mixed in as well. He had had a past history of a malignant melanoma, but had no current evidence of disease.
[00:27:10.92] Given his signalmen clinical signs and previous history, an OncoK9 was performed to look for evidence of underlying cancer as a cause of his GI signs, and the OncoK9 revealed a Cancer Signal Detected. In addition, we were able to issue a cancer signal origin prediction, as Dr. O'Kell mentioned earlier, for hematologic malignancy, namely lymphoma or leukemia.
[00:27:35.04] A cancer signal origin basically means that the signal seen is typical of a specific cancer type. We're able to issue that cancer signal origin prediction for about 50% of lymphoma, and are hopeful that in the coming months, we'll be able to issue additional cancer signal origin predictions.
[00:27:55.71] So given the positive OncoK9, her clinical signs, and hematologic cancer signal origin prediction, an immediate referral was recommended by her veterinarian. Arthur was referred to an internist, where ultrasound revealed multiple enlarged abdominal lymph nodes, and her heterogeneous appearance to the liver, all of which were concerning for neoplasia, according to the ultra sonographer.
[00:28:20.52] So based upon the ultrasound findings and the hematologic cancer signal origin prediction, ultrasound-guided fine needle aspirate of the enlarged abdominal lymph nodes were performed. And cytology yielded a diagnosis of lymphoma.
[00:28:37.21] So to summarize Arthur's case, he's a 13-year-old male castrated plot hound mix with GI signs, whose OncoK9 showed a Cancer Signal Detected, but also is able to issue a hematologic cancer signal origin prediction. A confirmatory cancer evaluation was recommended. And given the clinical signs and cancer signal origin prediction, GI lymphoma was suspected. So Arthur was quickly referred to an internist, where enlarged lymph nodes and liver changes were noted on ultrasound. Ultrasound-guided aspirates and cytology of the enlarged lymph nodes led to a diagnosis of lymphoma.
[00:29:13.03] So in Arthur's case, his OncoK9 results helped guide his workup, and help expedite referral to an internist. The takeaway is that OncoK9 can help guide workup for patients with clinical signs potentially suggestive of cancer, and cancer signal origin predictions can help prioritize referrals, as well as help prioritize the most pertinent diagnostics for these patients.
[00:29:37.27] Moving on to the next patient. Let's talk about Whiskey. So Whiskey is a 12-year-old spayed female Jack Russell terrier, who in late 2021 had a screening ultrasound performed that found both splenic and hepatic masses. As a result, Whiskey was taken to surgery for a splenectomy and an incisional liver biopsy. The splenic mass came back benign, however showing nodular hyperplasia. However, the liver mask was a lot less straightforward, with the pathologist waffling between this representing hepatocellular carcinoma versus just a benign adenoma. And even stating, quote, "It's difficult to make a definitive determination regarding malignancy," which this is not an uncommon scenario with hepatic masses.
[00:30:24.44] So fast forward a few months, Whiskey is doing great. Recheck blood work reveals an elevated ALP of about 1,500. So a repeat ultrasound was performed and revealed a relatively static liver mass, but another ill-defined mass noted within the liver as well. As a result of this new mass within the liver, an OncoK9 was performed to further evaluate the changes within the liver and reveal the Cancer Signal Detected result.

[00:30:52.73] Based on this result, whiskey was staged with bloodwork, thoracic radiographs, and a repeat ultrasound. His CBC showed mild anemia and a thrombosis ptosis, while his chem panel revealed an ALP of 1,254. His thoracic radiographs were within normal limits, and his liver was relatively stable on ultrasound. So in early August, whiskey had a lobectomy performed. And his towpath revealed hepatocellular carcinoma that was completely excised.
[00:31:21.65] So to summarize Whiskey's case, she's a 12-year-old female spade Jack Russell, with a known liver mass. However, the previous incisional biopsy was not definitive in differentiating between an adenoma and hepatocellular carcinoma. An OncoK9 was performed to evaluate this mass further and reveal the Cancer Signal Detected. Whiskey was stage and a repeat ultrasound performed revealed possible progressive disease.
[00:31:47.99] At that time, the options were either to continue monitoring her or consider pursuing surgical excision. Excisional biopsy was elected. And histopath confirmed a hepatocellular carcinoma, which is potentially very exciting news for Whisky as this is a tumor type that can be cured with complete excision, as was the case with Whisky's mass. Whisky's OncoK9 canine encouraged the owners to pursue further diagnostics, resulting in a definitive diagnosis and therapy. Whisky is doing great post-op, and both his owners-- sorry, her owners and veterinarian are very happy with their outcome.
[00:32:24.60] The takeaways from whiskies case are that OncoK9 helped the owners decide to proceed with obtaining a definitive diagnosis and therapy for her hepatic mass. And the OncoK9 proved to be a useful tool when histopath pass or cytology, in some cases, is inconclusive in tumor types that are notoriously difficult to diagnose such as those pesky hepatic masses.
[00:32:48.30] Moving on, let's talk about a dog named Kerry. So Kerry is a nine-year-old female spayed Akita who presented to her local veterinarian for what the owner described as neck swelling. On physical exam, Kerry had enlarged mandibular lymph nodes with some mild associated edema, as well as a 6-centimeter right caudal memory mass, excuse me. She had some blood work performed, which was relatively unremarkable. And then the mandibular lymph nodes were aspirated. However, cytology revealed reactive lymphoid hyperplasia.
[00:33:21.00] As a result, Kerry was treated with a course of antibiotics. However, there was no response in terms of her lymphadenopathy. To evaluate that lymphadenopathy further, an OncoK9 was submitted and revealed a cancer signal detected, as well as a cancer signal origin prediction of likely lymphoma. Because of her OncoK9 result and her hematologic cancer signal origin prediction, additional lymph nodes were aspirated by Kerry's veterinarian, revealing a diagnosis of lymphoma. As a result, Kerry was referred to an oncologist immediately. And the owners pursued that consultation in order to discuss Carrie's lymphoma, as well as her mammary tumor.
[00:34:03.22] So to recap, Kerry is a nine-year-old female spayed Akita with enlarged mandibular lymph nodes. Cytology of the nodes didn't fit with what the clinician was suspecting. So an OncoK9 was performed as an additional evidence-based test. OncoK9 came back with both a cancer signal detected, as well as a cancer signal origin prediction of likely lymphoma. This OncoK9 result prompted Kerry's vet to aspirate additional lymph nodes, shortening the diagnostic path to a diagnosis of lymphoma.
[00:34:36.81] This diagnosis allowed for an onco consultation, giving the owners all of the options necessary to make the best possible decisions for Kerry going forward in terms of therapy. Kerry's takeaways or that one cytology and clinical impression don't match up, OncoK9 may help as an aid and diagnosis tool in those cases. In addition, cancer signal origin prediction can help provide additional information and guidance in challenging to diagnose cases.
[00:35:10.68] Moving on, let's chat about a little dog named Daisy. So Daisy is a seven-year-old female intact Corgi who became acutely blind and unfortunately, as a result of her blindness, fell

into a swimming pool. She was taken to her local veterinarian who diagnosed her with uveitis and started a workup for systemic disease. Her uveitis panel for infectious diseases came back negative, as you can see on the right, while her basic blood work revealed a mildly elevated ALT of 416, whereas it had been in the mid to high 100s previously.
[00:35:45.62] Daisy was then seen by an ophthalmologist who noted hyphema and diagnosed her with pan uveitis, prompting further workup. She had an ultrasound that revealed a heterogeneous liver, as well as a plump right adrenal gland. Her coags at that time were normal. And so her liver was aspirated for cytology.
[00:36:04.14] Cytology revealed macular degeneration, which is a benign non-worrisome change within the liver. Daisy also had an ocular ultrasound performed, which revealed a detached right retina with an dissociated poor prognosis for regaining vision in that eye. The ophthalmologist was very suspicious based upon the changes in the eyes, that Daisy had underlying systemic disease that had led to these changes saying, quote, "Primary concern at this time is ruling out systemic, life-threatening disease in Daisy."
[00:36:36.52] So to help look for underlying neoplasia as a potential underlying disease, Daisy had an OncoK9, results of which showed a cancer signal detected. So this is a portion of Daisy's genomic sequencing data. Each vertical section represents a chromosome, which you'll see labeled from 1 to 38 on the x-axis. In a normal sample, all of the dots should be blue and center around the baseline, indicating no gains or losses of genomic material. The lower tracing, which represents Daisy's gDNA or DNA from her white blood cells is a good example of a normal sample.
[00:37:16.43] In Daisy's cell-free DNA above however, you'll see gains involving chromosomes 11, 13, 19, and 30, indicated by red dots elevated above baseline, and losses involving chromosomes 3, 11, 16, 19, 22, 29, 30, and 37, indicated by green dots depressed below baseline. The identification of these genomic alterations indicates the likely presence of cancer in the body.
[00:37:49.04] So given these OncoK9 results, as well as her ocular changes, additional diagnostics were discussed, including sampling of her anterior chamber, lymph node aspirates, thoracic radiographs, and even possibly a CT scan. Rather than pursuing further diagnostics, Daisy's owner opted for humane euthanasia due to rapidly worsening quality of life for Daisy. But she did want to pursue a necropsy in an effort to obtain a definitive diagnosis for Daisy's underlying systemic illness.
[00:38:20.28] So these are some of her necropsy images. And on necropsy, you can see the presence of numerous pigmented masses throughout Daisy's body, including her GI tract with that jejunal mass in the upper left, her body wall, her brain, her lungs, her liver, and her right eye. Based upon the gross appearance of these lesions, hemangiosarcoma was suspected. Microscopic evaluation of these lesions confirmed the presence of diffuse metastatic hemangiosarcoma in Daisy's liver, eyes, jejunum, peritoneum, lungs, heart, brain, adrenal gland, and popliteal lymph node. In addition, she had a mild hemoabdomen at the time of necropsy, presumed to be secondary to ruptured hemangiosarcoma.
[00:39:11.59] So here we have the tracing from Daisy's cell-free DNA on the top, showing gains and losses in several chromosomes, as we discussed a few slides ago. We were interested in this case for research purposes. And so we looked at a few of her post-mortem tissues with OncoK9 in a research setting. If you look at the two traces below, these are tracings from her liver and popliteal lymph nodes respectively, showing very similar genomic alterations to the cell-free DNA trace. These help confirm that her hemangiosarcoma was the source of the cancer signal detected within her plasma.
[00:39:50.17] So again, Daisy was a seven-year-old female intact Corgi with acute onset of

blindness that the ophthalmologist felt was very likely secondary to severe underlying systemic disease. Her original workup did not reveal an obvious diagnosis. So an OncoK9 was performed to look for evidence of underlying neoplasia. And a cancer signal was detected.
[00:40:10.67] Further diagnostics were recommended as part of her confirmatory cancer evaluation. However, the owner elected euthanasia given Daisy's declining quality of life. A necropsy was performed in hopes of obtaining a definitive diagnosis and revealed diffuse metastatic hemangiosarcoma.
[00:40:29.27] In Daisy's case, OncoK9 helped assure the owner that humane euthanasia was a reasonable decision, given her clinical picture and rapid decline in quality of life. Takeaways from her case are that onco canine helped guide the clinical decisions of both the owner and the veterinarian. And necropsy and research samples helped confirm that her hemangiosarcoma was the source of her original plasma cancer signal.
[00:40:57.95] Let's go through one more case. And we're going to talk briefly about Hank. So Hank is a nine-year-old male castrated Boxer, who presented to his veterinarian for mild hyporexia, mild PUPD, and mild lethargy. He had some blood work to investigate these clinical signs that showed hypercalcemia.
[00:41:17.19] Thoracic radiographs were performed as part of his evaluation for hypercalcemia. And a cranial mediastinal mass was found. The veterinarian strongly suspected cancer and advised oncology referral. However, because his clinical signs were quite mild in nature, his family was reluctant to pursue referral right away. They elected to submit an OncoK9 while they were considering their options.
[00:41:41.13] When the OncoK9 result came back, it showed a cancer signal was detected and also revealed a cancer signal origin, as we've seen a few times now, suggesting either lymphoma or leukemia as the source of his cancer signal. Given these results, Hank's veterinarian now had further support for her suspicion of cancer. And she was able to counsel the family on the importance of immediate referral, which they did elect to pursue.
[00:42:08.41] When considering Hank's entire clinical picture, including his OncoK9 results, the veterinarian had a really strong suspicion of cancer and was able to convince Hank's owner to pursue referral to an oncologist. As a result, Hank had additional workup done in the form of ultrasound-guided fine needle aspirates of his cranial mediastinal mass, the cytology of which revealed large cell lymphoma.
[00:42:32.71] So in summary, Hank had OncoK9 performed as an aide in diagnosis test to gain further information about his vague clinical signs, hypercalcemia, and cranial mediastinal mass. Prior to OncoK9 testing, his owner was hesitant to pursue oncology referral, and instead chose to start with OncoK9 as a noninvasive option. When a cancer signal was detected, a cancer signal origin prediction for hematologic malignancy was also provided.
[00:42:59.49] All together, this information helped convince the client to go for immediate referral for workup of suspected lymphoma, as recommended by the managing veterinarian. During the onco consultation, an ultrasound-guided fine needle aspirate of the cranial mediastinal mass was performed and confirmed the diagnosis of lymphoma, allowing the initiation of chemo, which immediately palliated his clinical signs.
[00:43:24.07] So overall in Hank's clinical journey, the benefits of liquid biopsy testing included helping the owner decide to pursue referral and prompting the ultrasound-guided fine needle aspirate, which achieved a diagnosis, which ultimately allowed for initiation of chemo and helped Hank feel better. Takeaways from Hank's case are that OncoK9 can be used as another tool in the toolbox, in cases where it can be difficult to access the lesion in question, and that again, a cancer signal origin prediction is available for about half of hematologic malignancies with more cancer types likely coming soon.

[00:44:03.05] So in closing, I just want to summarize some of the potential uses of OncoK9 in an aide in diagnosis setting. For those dogs with cancer on the list of differentials, but that mass is difficult to access, such as Hank's mediastinal mass, or those cases in which you suspect cancer, but it's not readily obvious where it's hiding, for those clients that need a stepping stone going forward with additional diagnostics, OncoK9 can be a great non-invasive next step diagnostic while conservative management is being attempted. With the turnaround time of approximately 9 to 10 calendar days, results are usually available within that typical recheck window.
[00:44:44.21] Along the same lines, OncoK9 can be helpful for those clients that want to try to get more information about what's going on with their pet while they're waiting that couple of weeks for an ultrasound or a referral appointment, which we can be hard to come by these days. And that pretty much wraps things up on our end.
[00:45:03.90] So Dr. O'Kell, I just want to thank you guys for coming this evening and point you to a couple of useful items here. On our website, we have a Resources page that has some peer reviewed literature. We've got some case studies, some past webinars, some conference posters, and more. And then we also just want to make sure that you guys are aware that Dr. O'Kell and I, as the directors of clinical support, we're here to support you guys. I mean, that's our job description.
[00:45:31.69] So if you need to discuss a case, you have questions, you want to talk about OncoK9, its uses, the results, how to interpret them, what the next steps are, please feel free to reach out to us at any time. And with that, we will gladly answer any questions that you guys might have.
[00:45:49.34] Thank you guys so much. That was great. I especially like the case studies. It's so nice to see the real world examples of how to use your test.
[00:45:57.55] And I wish this was out when I was in clinical practice. I definitely would have used it. I had quite a few clients who would have appreciated this extra bit of information. So I'm a bit jealous for everyone who gets to use it now.
[00:46:09.94] We do have a few questions for you. The first one-- are these tests being developed for other species? And is this test available for cats?
[00:46:19.14] That's typically the first question we get. So the answer is yes. It's coming, is what it comes down to. We don't yet have this test for cats. And you cannot run OncoK9 on a feline.
[00:46:35.10] But Onco Feline is hopefully in our future. It's a little bit trickier with cats. We know a whole lot more about the genome of dogs than we do about the genome of cats. But we're working on it.
[00:46:48.54] And it's not that we don't like cats. They're just a little bit trickier when it comes to their genomics. So we are definitely working on it.
[00:46:57.21] That's great news. And hopefully, you guys get there. I would love to see that. Does OncoK9 classify what kinds of cancers are detected?
[00:47:07.50] So I can take that one. So in general, our result is a cancer signal detected or cancer signal not detected. And so the result really will give you whether cancer is likely present in the body or less likely to be present.
[00:47:24.27] So currently, our cancer signal of origin prediction, as we discussed in a couple of cases, is available for lymphoma or leukemia. And in about 50% of lymphoma and leukemia cases, we can provide that prediction. And so we are certainly working on other cancer signals origin predictions. It's a really high priority, and we know it will be very useful in practice. So please just stay tuned as more of those should be coming in the future.
[00:47:54.56] Awesome. Next question we have is, in order to obtain the cfDNA, do the blood samples need to be collected at a specific time, handled in a special way, or is it just a special

tube?
[00:48:06.39] So that's a great question. And Dr. O'Kell, I don't know if you want to pull up the slide. So we have very specialized tubes made by Roche that help stabilize the cell-free DNA within the blood. Cell-free DNA tends to break down very quickly, within minutes to hours. These tubes can actually stabilize the cell-free DNA at room temperature for up to seven days.
[00:48:30.42] They don't need to be refrigerated. They don't need to be spun. In fact, we recommend not doing either one of those things. But these specific tubes, which you can either get directly from us, you can order these kits from Antech or IDEXX. But these are tubes that you will not have on your shelf in your clinic. You need to get them from one of those different channels so that you can stabilize the cell-free DNA in your patient's sample and then get that sample to us.
[00:49:00.09] Great. That's even better news when you don't have to refrigerate or freeze something.
[00:49:04.14] Super easy.
[00:49:04.72] Actually.
[00:49:05.20] Just put the blood in there, agitate it a couple of times, and put it on the counter, and forget about it.
[00:49:11.61] I always appreciate that. Our next question is, can we do this diagnostic test in house? Or do we always need to send it to a diagnostic laboratory?
[00:49:21.04] Yeah, that's a great question. And so yes, you do have to send it to the diagnostic lab So the PetDx lab, as it is very highly specialized testing. And it's been clinically validated by our lab. So this is something that you do have to send to PetDx.
[00:49:38.31] But as Dr. Cohen mentioned, it is available directly through PetDx, but also through IDEXX and Antech. So it's actually fairly easy to have this test performed, as there are multiple ways that you can order kits and then submit your sample.
[00:49:54.80] Excellent. Next question is, can you remind us what the turnaround time is for the OncoK9 test?
[00:50:00.92] Yeah, absolutely. So turnaround time is about 9 to 10 calendar days. So not business days, calendar days, so that's better. And we are steadily improving upon that turnaround time as well. We're starting to do more runs per week, which is gradually, but progressively cutting down on that turnaround time.
[00:50:23.35] That's great news. What is the cost of the test? And is it available for Canadian clinics?
[00:50:30.14] Let the Canadian take that question.
[00:50:32.08] Yes, I actually am Canadian. So yes, the cost of the test to veterinarians, it is cost in the high 300s. Most veterinarians are retailing it or selling it to their clients around the $500 mark. But the markup, of course, is up to each clinic. And yes, it is available in Canada through IDEXX and Antech.
[00:50:58.66] That's great. I see more and more Canadians joining us. And so I really appreciate having a fellow Canadian and be able to speak to them. Our next question-- so if I was using this for a liver mass seen on ultrasound, would a hepatic adenoma have a cancer signal?
[00:51:15.14] So hepatic adenoma will not have a cancer signal. And that's why it's so useful. Like in the case that I presented of the hepatic mass, it can help differentiate between an adenoma and a carcinoma, which can be very challenging in general, whether it's via incisional biopsy, cytology. I mean, these can be really a headache for pathologists, clinical pathologists, and clinicians. So the good news is that this is a test that can help differentiate between a benign adenoma and a hepatocellular carcinoma.
[00:51:54.44] That's great news too. I particularly remember quite a few really challenging liver

cases that just drove me up a wall, drove me batty and the owners too. So that's really, really great news and definitely a good reason to use this test for sure.
[00:52:11.63] We have someone from London. Is this test available in the UK?
[00:52:18.59] So currently, it is not available in the UK. We are hoping to be able to open it up in the future to international customers other than Canada. But currently, unfortunately, we're not able to offer it to other countries.
[00:52:35.87] Understood. Someone else wanted to know, I see that the kit includes Vacutainer and butterfly. Is it required to use that? Or can I use a regular needle and syringe?
[00:52:46.07] So it's not required to use that. It's recommended to use that. These tubes take about 7 and 1/2 to 8 mLs of blood each. And we do ask that you fill both of these tubes. So the easiest way and the fastest way to get that blood into those tubes is with the Vacutainer.
[00:53:06.86] That being said, if you would prefer to use just a more conventional needle and syringe, that's fine. We do recommend using at least a 21, if not larger gauge needle, to try to prevent hemolysis from occurring. But we do recommend using the supplies that we send to you in the kit, because that gives us the best sample quality at the end of the day.
[00:53:33.08] That makes sense. All right, we have another couple of questions. I assume this type of test is available for people. And if so, what's its name?
[00:53:43.01] Yeah, so it certainly is. And the test is called Galleri, and it's made by Grail. And it's a multi-cancer early detection test. And there's actually several different tests out there. But the one that's most similar is that one. And that is available and can detect multiple types of cancer, and certainly is kind of at the forefront of technology in the human field.
[00:54:09.29] Great. If a pet is already on chemotherapy, will that affect the results of the canine test? So if there's still circulating tumor DNA in that dog's blood, we will still detect it. But if that chemotherapy is doing a really good job of wiping out that cancer and wiping out, as a result, the circulating tumor DNA in the bloodstream, we may get a false negative as a result of that therapy. So it's very case-dependent.
[00:54:40.98] But simply because a dog is on chemo doesn't mean that you shouldn't consider running OncoK9 on that dog. If there is still tumors, significant amount of tumor in that dog, that's then producing a significant amount of cell-free and circulating tumor DNA, we should be able to still detect a signal in that dog.
[00:55:00.91] That's actually kind of good news. I'm really glad that's the case, because it seems like that would be another tool in the tool belt if you've got someone with--
[00:55:09.34] Stay tuned. Stay tuned. That's one of the post-diagnosis use cases that Dr. O'Kell is referring to. And we're working on getting that stuff out to the world very soon.
[00:55:20.56] That's awesome. That's great news. That was our last question Thank you guys so much for joining us.
[00:55:25.55] We'll go ahead and wrap up. Thank you so much. Have a good evening. Bye, everyone.
[00:55:30.56] Thank you guys for coming.
[00:55:31.75] Bye.