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INTRODUCTION OF NU.Q® VET CANCER TEST INTO A CLINICAL LABORATORY

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Introduction

Nucleosomes are DNA strands which are wrapped around a histone protein core. Multiple repeated nucleosomes form the final chromatin [1] (figure 1). During apoptotic and necrotic cell death [2-3], chromatin breaks up into oligo- or mono- nucleosomes which are released into the blood stream. As a liquid biopsy test nucleosomes can easily be accessed using a simple blood test. Multiple studies evaluating nucleosomes in dogs have been performed [4-7]. Increased nucleosome concentrations are associated with several neoplastic diseases in dogs, like lymphoma, hemangiosarcoma and others [4-6].

The Nu.Q® Vet Cancer Test is a new ELISA assay to evaluate nucleosomes in dogs. The method in the studies carried out so far showed a specificity of 97% and a sensitivity of 49.8%, with an area under the curve of 68.74% for the overall detection of a variety of different cancer types [6] Interference values not altering the test result are summarised in table 1 [8].

Table 1: Nu.Q® Vet Cancer Test. Information about thresholds of interference substances not influencing the assay.

Interfering substance	Threshold concentration
Triglycerides	3000 mg/dL
Haemoglobin	500 mg/dL
Bilirubin, unconjugated	20 mg/dL
Bilirubin, conjugated	20 mg/dL
Protein (total)	8 g/dL
Cholesterol	300 mg/dL

Objective

To describe our experience with the Nu.Q® Vet Cancer Test protocol, after three months from the introduction in our laboratory.

Material and Methods

The manufacturer's instructions (Belgian Volition SRL, Isnes, Belgium)e Nu.Q® Vet Cancer Test was carried out according to. The assay is an indirect quantitative sandwich ELISA with a capture antibody directed to histone H3.1. Briefly, wells come coated with the anti-H3.1 antibody and are washed prior to adding buffer and samples, standards and controls into the wells. After a first incubaton followed by a washing step, the detection antibody coupled to horseradish peroxidase (HRP) is added. After a second incubation and washing step, the TMB (3,3′,5,5′-Tetramethylbenzidine) substrate is added. Following the addition of the stop solution, the absorbance is read at OD 450 nm using a standard plate reader. Using the positive control stock provided, a standard curve was generated with Graphpad Prism 9 software (figure 2). Unknown data sets were interpolated using a sigmoidal 4PL, X concentration model. The signal obtained is proportional to the concentration of H3.1 nucleosome concentration present in the sample. Descriptive statistics were performed using Microsoft Excel (table 2). A normal and a high control are run simultaneously with every sample batch. Quantitative results were allocated into three levels of suspicion for the presence of neoplastic disease: low suspicion <50 ng/mL, medium suspicion 51-80 ng/mL, and high suspicion >81 ng/mL.

Owner consent was achieved before participation. Dogs were apparently healthy and fasted for a minimum of four hours. Up to 5 mL of whole blood was collected into EDTA tubes from jugular or a peripheral vein. Samples were centrifuged at room temperature for 10 min at 3000 g within 2 hour of collection. The plasma was separated, transferred into a clean tube, shipped cooled to the laboratory and analysed within 72 hours.

Patients with a Nu.Q® Vet Cancer Test result in the high suspicion category (>80 ng/mL) underwent further diagnostics by the referring veterinarian to characterize the disease.

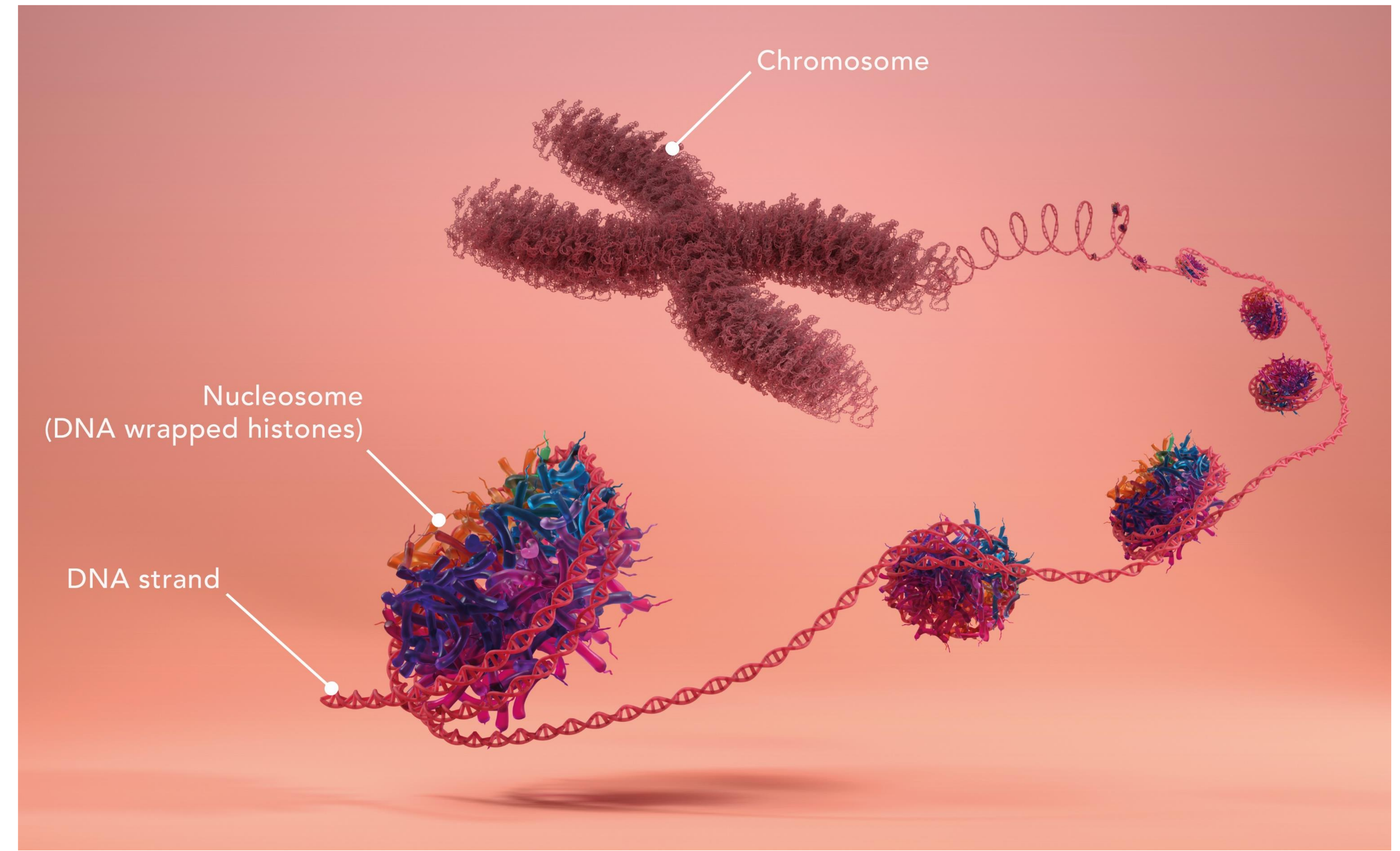

Figure 1: Scheme of nucleosomes.

Results

Within the evaluated time frame, 273 samples were examined. Execution of the Nu.Q® Vet Cancer assay was easy and kit controls were in the target range (table 2).

Table 2. Raw data presentation to generate the standard curve and calculate patient results. Std. A-F = standards, KC1+2 = controls. Result shown as Mean, SD, and CV%. Result of sample 1 gained a nucleosome concentration in the medium risk range with a value between 51-80 ng/ml.

	H3.1 (ng/mL)	OD-450nm		Mean	SD	CV %					
std A	0	0,0519	0,0515	0,052	0,000	0,5					
Std B	35,2	0,2269	0,2296	0,228	0,002	0,8					
Std C	72	0,3466	0,3592	0,353	0,009	2,5					
Std D	178,3	0,6548	0,692	0,673	0,026	3,9					
Std E	358,1	1,3249	1,3431	1,334	0,013	1,0					
Std F	779,8	2,0717	2,1129	2,092	0,029	1,4	H3.1 (ng/mL)		Mean	SD	CV %
KC1	(42,7-79,3)	0,3358	0,3462	0,341	0,007	2,2	64,51849008	67,28909013	65,9	2,0	3,0
KC2	(222,5-413,2)	1,2437	1,2833	1,264	0,028	2,2	365,6252966	381,2656347	373,4	11,1	3,0
Sample 1	1706	0,3076	0,3084	0,308	0,001	0,2	57,1064295	57,31462784	57,2	0,1	0,3

Results of 18 patients (18/273, 6.6%) were rejected. 12 samples (12/273, 4.4%) due to the presence of interfering substances (lipemia, see table 1) or inadequate storage temperature during transportation (e.g. freezing instead of cooling) while in a batch of 6 samples (6/273, 2.2%) failure to generate the correct standard curve was present. Upon owner consultation it revealed that patients with lipemic samples were not fasted adequately. All 18 patients were re-sampled and re-tested: 17 samples showed a result of <50 ng/mL and 1 sample gave a value between 51-80 ng/mL. All results are summarised in table 3.

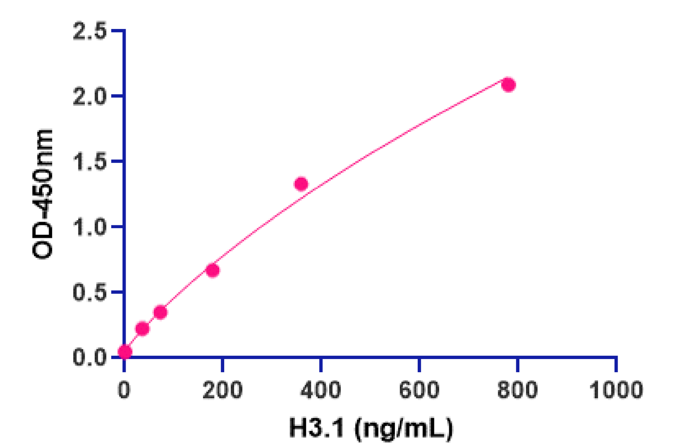

Figure 2: Nu.Q® Vet Cancer Test, standard curve.

Table 3: Nu.Q® Vet Cancer Test result distribution based on suspicion level of the 273 cases examined.

Level of suspicion for neoplasia	N° of cases
Low suspicion < 50 ng/mL	260 (including 17 re-tested)
Medium suspicion 50-80 ng/Ml	9 (including 1 re-tested)
High suspicion > 80 ng/mL	4

Patients in the high suspicion category underwent further diagnostics. Table 4 summarises the data for the 4 samples (4/273, 1.5%) with a value > 81 ng/mL. In all 4 cases, a neoplastic condition was detected through preliminary clinical pathological exam, i.e. PCR clonality testing, cytologic evaluation, cytofluorimetry, and other.

Table 4: Data for the 4 cases with H3.1 values above 81 ng/mL.

SIGNALMENT	H3.1 [ng/mL]	Associated disease
Small Italian Greyhound, Male, 12 yrs old	117	Myeloid leukemia
Lagotto, Male, 14 yrs old	463,3	Lymphoid leukemia
Irish Setter, Female, 2 yrs old	204,8	Intestinal Lymphoma
Mongrel, Female, 9 yrs old	334,3	Hepatic Lymphoma

Conclusions

Nu.Q® Vet Cancer Test can be run easily in a commercial laboratory. Customer communication is important to minimize preanalytical error due to inadequate fasting and failure to respect transport conditions. Increased nucleosome concentrations in the high suspicion for neoplasia range were observed in 1.5% of patients evaluated. Follow-up exams diagnosed neoplasia in all 4 cases. Nucleosome elevations may allow earlier detection of neoplasia and can be a useful blood-based marker to screen patients for neoplastic disease. If preanalytical error cannot be excluded, follow-up examination is recommended to confirm high results.

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