Characterization of the T-cell receptor gamma chain gene rearrangements as an adjunct tool in the diagnosis of T-cell lymphomas in the gastrointestinal tract of cats

Verena Greß[1], Birgitt Wolfesberger[2], Andrea Fuchs-Baumgartinger[3], Nora Nedorost[3], Armin Saalmüller[1], Ilse Schwendenwein[4], Barbara C. Rütgen[4], Sabine E. Hammer[1]

contact:
vena.gress@gmail.com
sabine.hammer@vetmeduni.ac.at

[1]Institute of Immunology, Department of Pathobiology; [2]Clinic for Internal Medicine, Department for Small Animals and Horses; [3]Institute of Pathology and Forensic Medicine and [4]Clinical Pathology Unit, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria

Background & Objectives

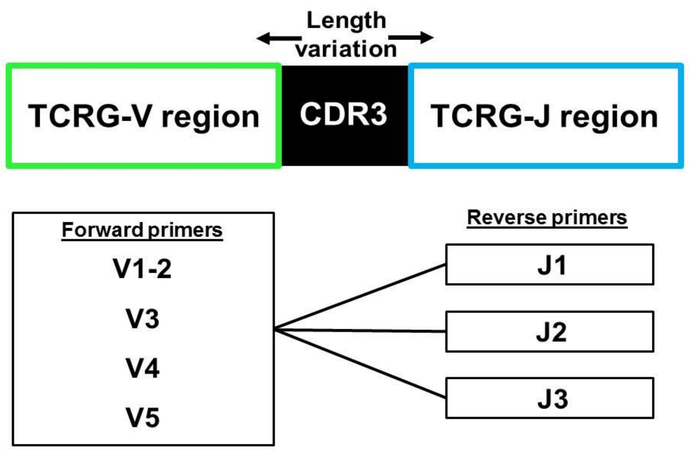
Feline lymphomas represent one-third of all neoplasms descending from haematopoietic tissues. Localizations in the small intestine are most frequently of T-cell origin. Morphologic differentiation between reactive and neoplastic lesions is sometimes difficult so that confirmatory evidence for the presence of lymphoma is necessary. These cases call for complementary tools like PCR-based T-cell receptor (TCR) clonality testing for confirmation. The aim of the study was to create a standardized protocol for DNA extraction, quality control and interpretation of electropherograms for archived sample material.

Genomic DNAs from formalin-fixed, paraffin embedded (FFPE) tissue samples were subjected to triplicate PCR, followed by size separation of the amplified products by capillary electrophoresis. The use of FFPE samples made it necessary to optimize the DNA extraction and clonality protocols. The improved protocols resulted in enhanced quantity and quality of the extracted DNA allowing the establishment of rules for interpretation of the different clonality patterns.

Material & Methods

Formalin-fixed, paraffin-embedded (FFPE) specimens of feline intestinal T-cell lymphoma cases from 43 cats were used as study material, collected at the Institute of Pathology at the University of Veterinary Medicine Vienna from 2002 to 2014. Samples were immunophenotyped with monoclonal antibodies against CD3 to identify T cells and CD79a to determine B cells.
Paraffin slices were deparaffinized and subjected to DNA extraction followed by quality testing. DNA with concentrations lower than 50ng/µl or failing amplification of the test PCR for the feline androgen receptor gene (fAR) were excluded from further analyses, reducing the sample size to 36.
For clonality testing, three primer mix combinations were used, each containing all four forward primers and one of three J reverse primers.

Location of primers at the TCRG locus



TCRG, T-cell receptor gamma chain; V, variable; J, joining; CDR, complementarity-determining region.

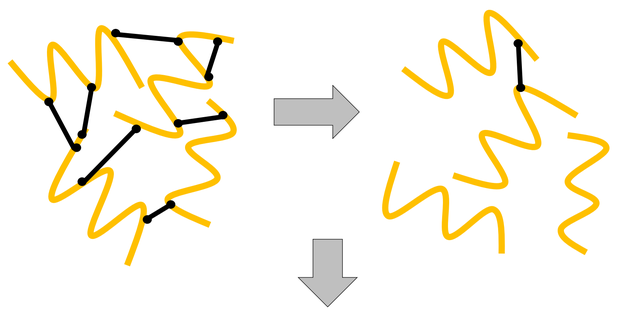
Optimized Workflow

FFPE material

Tissue lysis

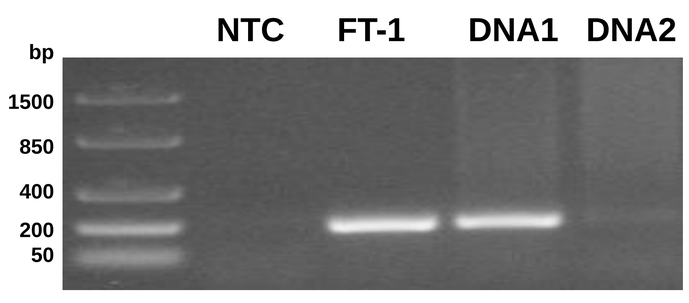
Resolving of formalin crosslinks

90°C 1h



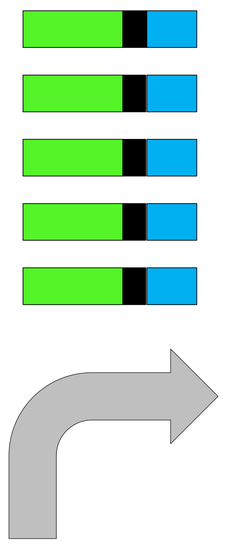
DNA extraction and quality control

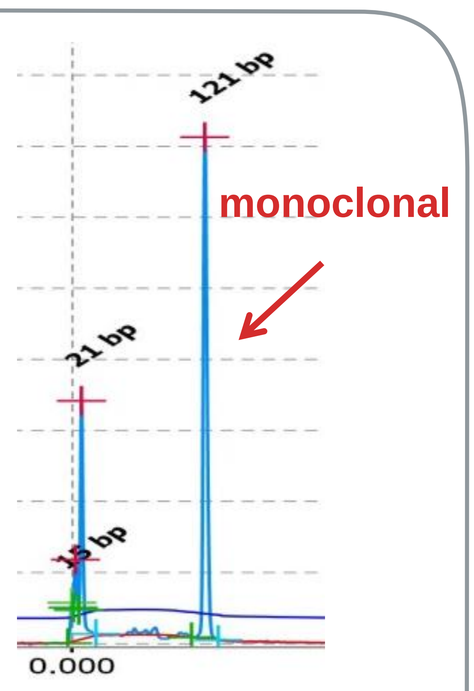
PCR for fAR

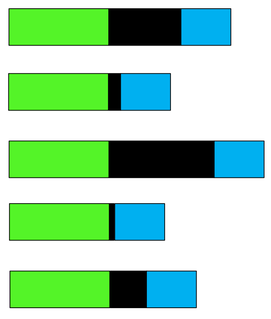


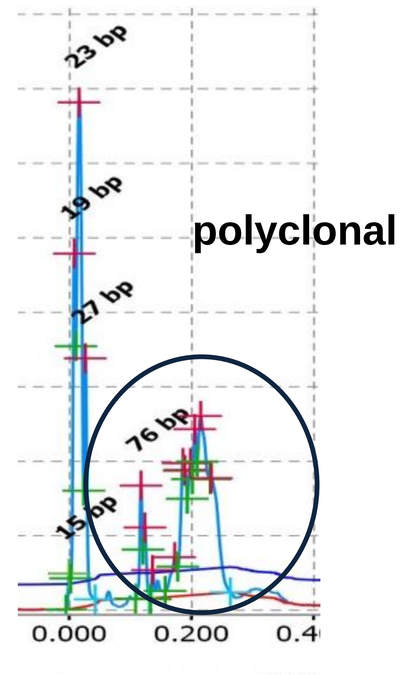
fAR, feline androgen receptor gene; bp, basepairs; NTC, no template control; FT-1, feline T-cell lymphoma cell line.

Multiplex PCR



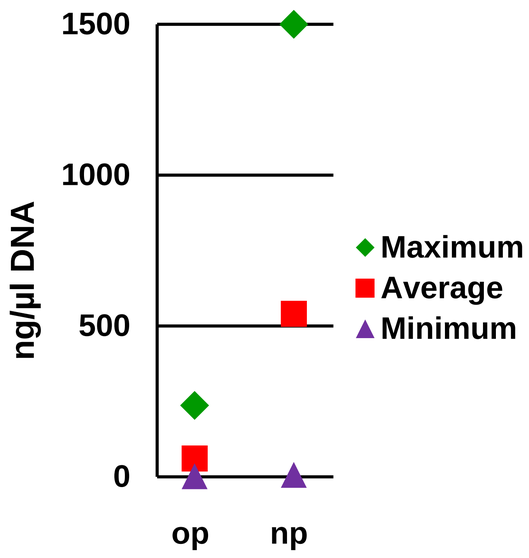




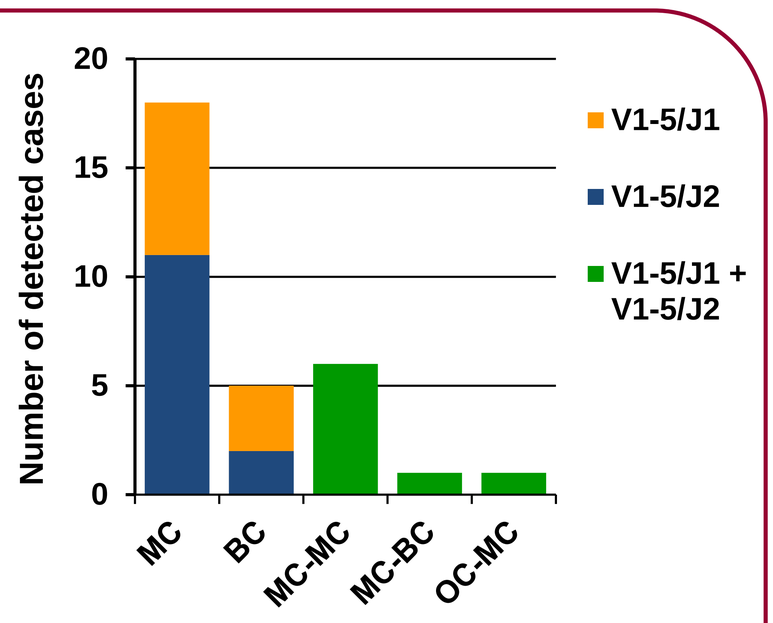


Results

DNA extractions without the additional heating step (“old protocol”, op) showed overall lower DNA yields and high co-purification of organic solvents being indicated by low 260/230 ratios.
In contrast, the “new protocol” (np) resolved most of the formalin crosslinks, resulting in higher DNA concentrations and less contaminations. Maximum and minimum values of both extraction protocols as well as the corresponding averages are displayed in the graph.



op, old protocol without additional heating step; np, new protocol with heating step.



V, variable; J, joining; MC, monoclonal; BC, biclonal; OC, oligoclonal.

In 31 out of 36 T-cell lymphoma cases, clonality was detected with at least one primer setup.

Conclusions

- We optimized the PCR-based clonality testing for archived material.
- 86% of alimentary T-cell lymphomas in cats were detected with the assay.
- A best practice work-flow is recommended for clonality testing of feline FFPE-tissue samples.

References | Mochizuki et al. (2012) Vet. Immunol. Immunopathol. 145:402-409; Greß et al. (2016) Res Vet Sci. 107:261-266. FFPE block image: https://twitter.com/exiqon_science/status/535445899066216449.