Sensitivity and specificity of the PCR-based lymphocyte clonality assay for the diagnosis of B- and T-cell lymphoma in cats

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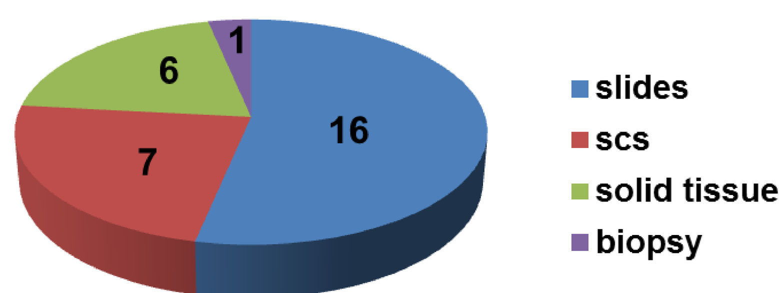
Background & Objectives

With an incidence of 2 per 1.000 individuals, lymphoma is the most common haematopoietic neoplasia in cats. Evaluation of a fine needle aspirate is often the first step in the diagnostic work-up. Diagnostic classification of infiltrates consisting of well differentiated small lymphoid cells is often challenging and the differentiation between a resident mature lymphocyte population and small cell lymphoma cannot be made by cytology alone.

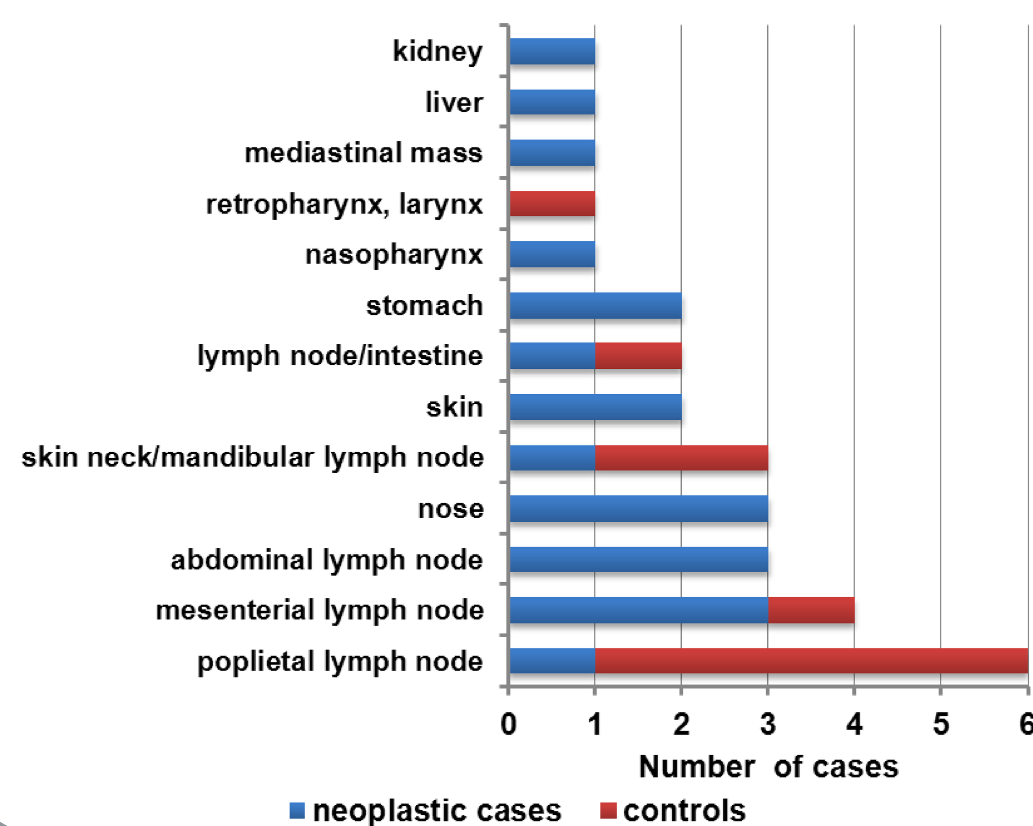
These cases warrant the application of complementary tools like PCR-based immunoglobulin (IG) and T-cell receptor (TCR) clonality testing (PARR) for confirmation. In this study, we evaluated diagnostic sensitivity and specificity of the PARR assay with specified primer sets for routine diagnosis of feline IG heavy chain (IGH2 and IGH3) and TCR gamma (TCRG) gene rearrangements.

Sample Material

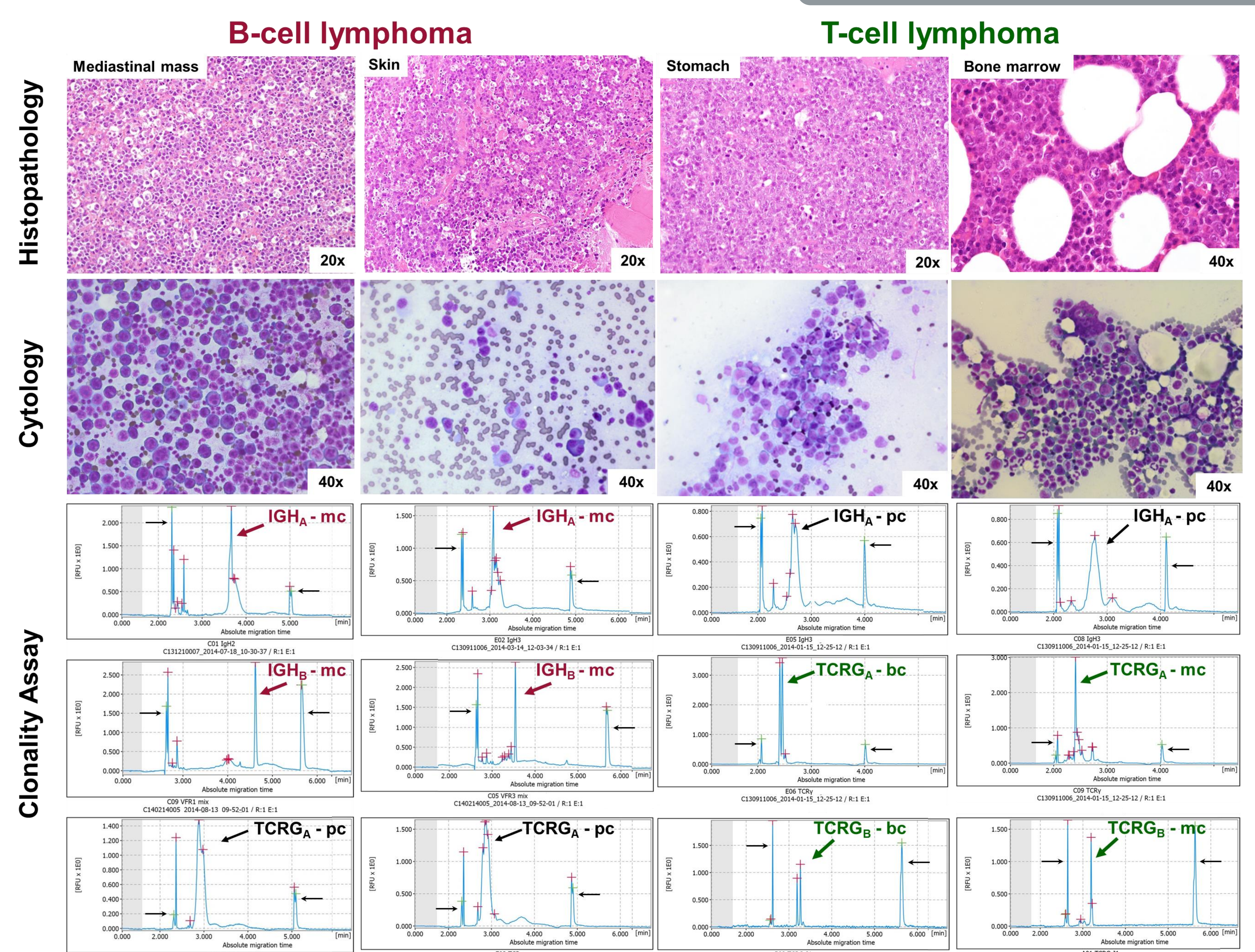
Material from 20 cats with lymphoma confirmed by histopathology and lymphoid tissue from 10 cats without lymphoma collected from patients at the Vetmeduni Vienna from April 2013 to February 2016 were evaluated by clonality testing.



The samples consisted of stained cytology slides, single cell suspensions (scs), fresh solid tissue samples and one biopsy. The anatomic sites of the samples was highly variable.

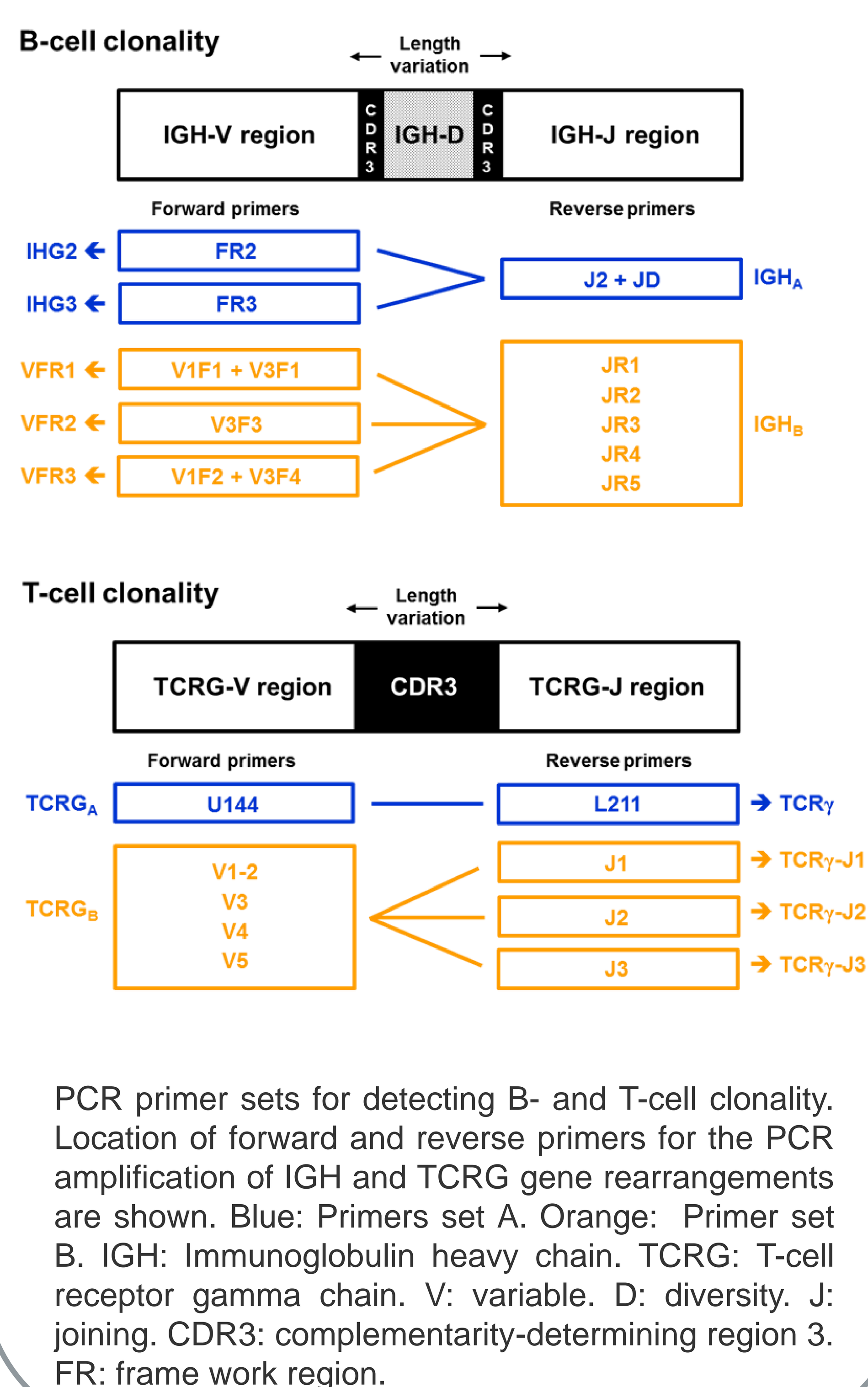


Results of illustrative examples



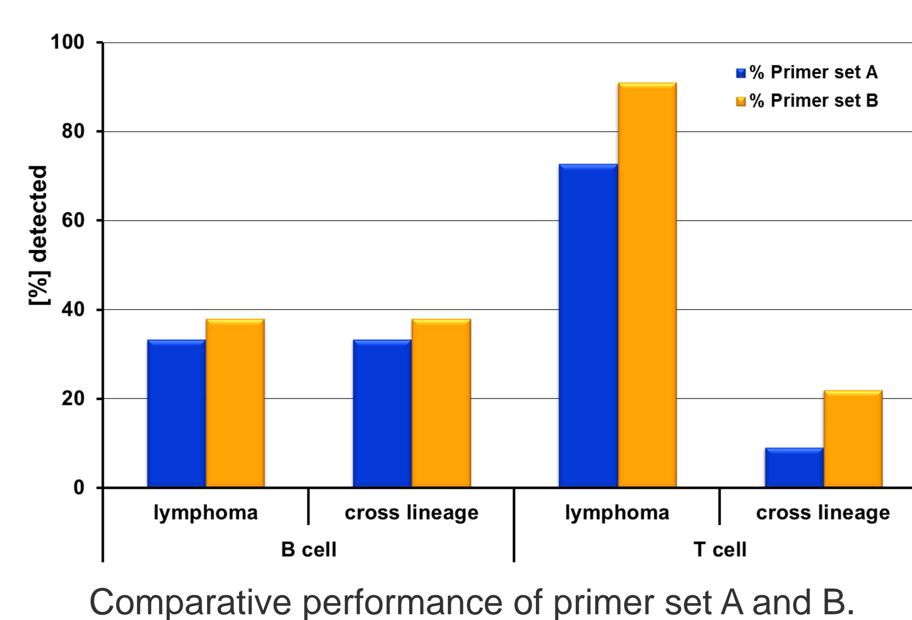
Images of H&E-stained histopathologic and DiffQuick®-stained cytologic samples are compared with the obtained electropherograms of the clonality assays. In the clonality assays, **IGH$_A$ (IGH2 and IGH3)** or **IGH$_B$ (VFR1 and VFR3 mix)** indicate **B-cell clonality**, and **TCRG$_A$ (TCRγ)** or **TCRG$_B$ (TCRG-J1) T-cell clonality**. Black arrows indicate the peaks of the alignment marker at 15 bp and 1000 bp.

PCR strategy of the clonality assay

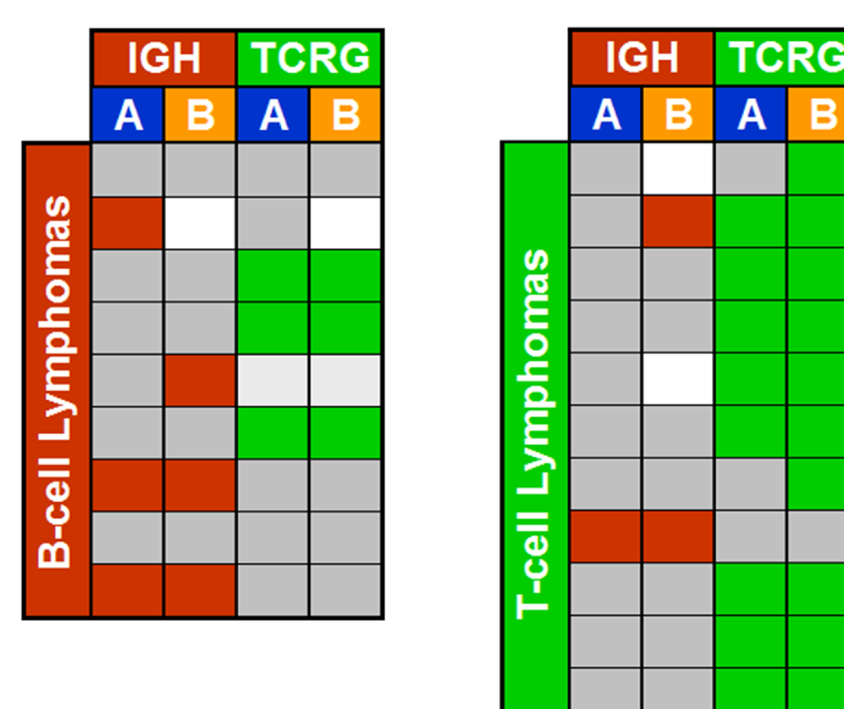


PCR primer sets for detecting B- and T-cell clonality. Location of forward and reverse primers for the PCR amplification of IGH and TCRG gene rearrangements are shown. Blue: Primers set A. Orange: Primer set B. IGH: Immunoglobulin heavy chain. TCRG: T-cell receptor gamma chain. V: variable. D: diversity. J: joining. CDR3: complementarity-determining region 3. FR: frame work region.

Clonality testing outcome



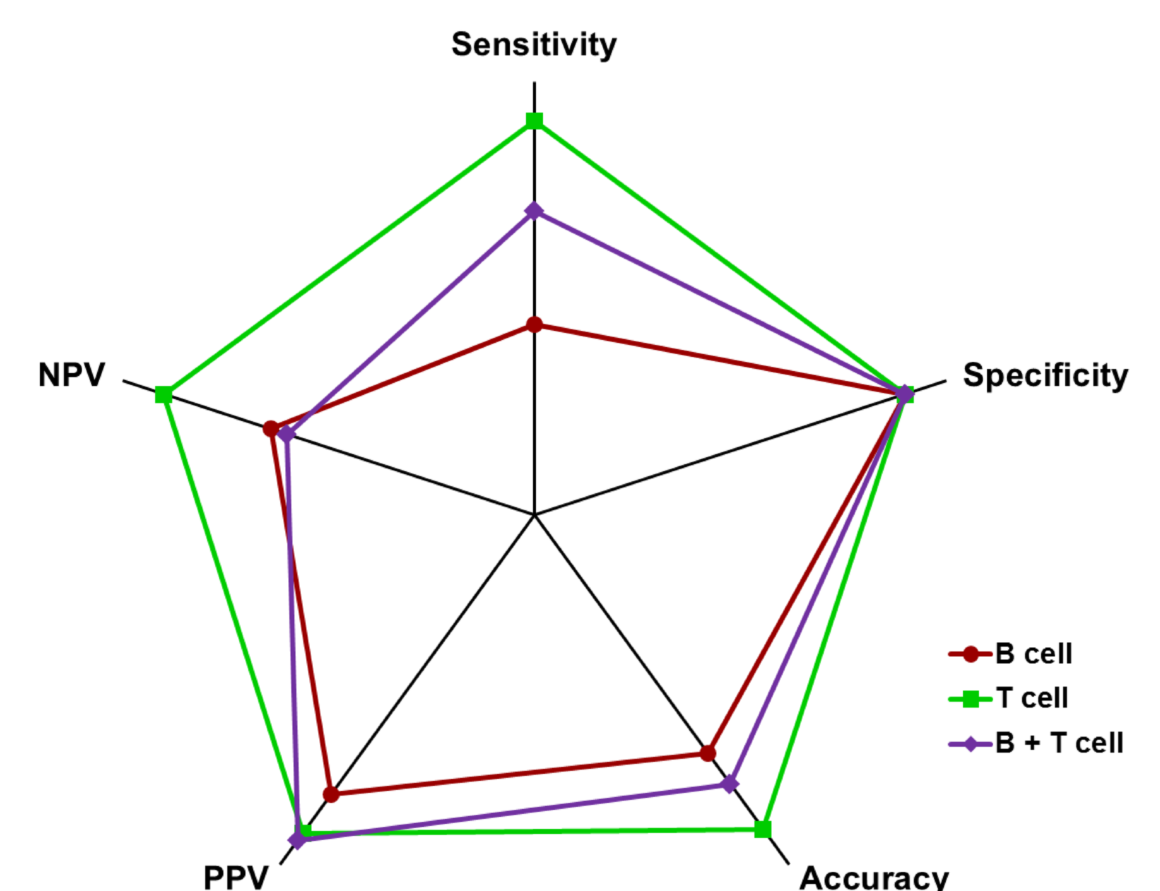
Comparative performance of primer set A and B.



Summarizing heat map for the detection of clonality and cross-lineage rearrangements in the analyzed B- and T-cell lymphomas. Blue: Primer set A. Orange: Primer set B. Grey boxes: No clonality detected. White boxes: Missing PCR reactions.

Summary & Conclusions

- **We tested two PCR primer sets for clonality testing on 30 matched cytological and histopathological reviewed specimens.**
- **Primer set B exhibited an overall better performance, tending to be more prone for cross-lineage detection.**
- **Diagnostic sensitivity and specificity of the clonality assay were 70% and 90%. Overall diagnostic accuracy was 77%.**



NPV: Negative predictive value. PPV: Positive predictive value.
Data points are given in percentages (axis scale: 0-100%).

References | IGH-specific primers: J.A. Werner et al. Vet. Pathol. (2005) 42:596-607. H. Mochizuki et al. Vet. Immunol. Immunopathol. (2011) 140:307-311. TCRG-specific primers: P.F. Moore et al. (Vet. Immunol. Immunopathol. (2005) 106:167-178, H. Mochizuki et al. Vet. Immunol. Immunopathol. (2012) 145:402-409. Financial support : ESVCP - Small Animal Research Award 2013, granted to BCR.