target antigen) visible on microscopy. While this technique is well established within histopathology its application to routine prestained cytology slides is relatively new.

Applications include determination of the immunophenotype in lymphoma, differentiation of mesenchymal and epithelial cells in the context neoplasia and identification of amelanotic melanoma.

These molecular techniques now allow the advantages of fine needle aspirate cytology in terms of minimal tissue invasion, speed and convenience to be coupled with a significant enhancement of the diagnostic information available from a single sample.

KEY LEARNING OBJECTIVES

- Develop an understanding of quantitative polymerase chain reaction (qPCR) for infectious agents and the application of this technology to effusions and washing fluids in order to increase their diagnostic yield
- Appreciate the applications of PCR for antigen receptor rearrangement (PARR) in relation to the identification of lymphoma and determination of its immunophenotype
- Understand the potential application of immunocytochemical analysis of cytology slides to assist characterization of cell populations which cannot be definitively identified based on morphological features alone

MULTIPLE CHOICE QUESTIONS

1. Which one of the following statements is FALSE regarding PARR analysis of cytology slides in the context of canine lymph node aspirates
 - (A) PARR analysis cannot be undertaken on cytology slides stained with Diff Quik in practice

- (B) PARR analysis may occasionally identify lymphoma cases as being a dual immunophenotype containing clonal DNA sequences of both T and B cell origin
 - (C) Samples containing a high proportion of lysed or disrupted lymphocytes are unsuitable for PARR analysis
 - (D) PARR analysis generally reliably differentiates between lymphoid hyperplasia and neoplasia
2. Which of the following statements regarding qPCR analysis of BAL samples in cats is UNTRUE?
 - (A) Results for *Mycoplasma felis* can be influenced by oropharyngeal contamination of the sample
 - (B) Rapid analysis of samples for *Bordetella* and *Mycoplasma* spp. is essential for reliable qPCR results
 - (C) Co-infections of respiratory viral and bacterial infections are not uncommonly detected in symptomatic cats
 - (D) qPCR has a much higher sensitivity for the identification of *Mycoplasma* spp. than conventional culture
3. Which one of the following statements is FALSE with regard to immunocytochemistry undertaken on cytological preparations?
 - (A) It is useful for the differentiation of B cell versus T cell lymphoma
 - (B) It is useful for the differentiation of epithelial versus mesenchymally derived cells
 - (C) It is useful for the differentiation of benign versus malignant neoplasia
 - (D) It is useful for the differentiation of amelanotic melanoma versus soft tissue sarcoma

Diagnosis and classification of canine lymphoma from FNA samples with a combination of cytology and flow cytometry

Elizabeth Villiers

Lymphoma is classified according to the World Health Organization system, which identifies several types of

high- and low-grade T and B cell lymphoma. The most common types are diffuse large B cell lymphoma (DLBCL), high-grade T cell lymphoma (HGTCL), T zone lymphoma (TZL) and marginal zone lymphoma. TZL has the longest median survival time, followed by DLBL, whilst HGTCL has the shortest survival times when standard protocols are used. There are different treatment protocols for low- and high-grade lymphoma and recent studies have shown HGTCL may respond better to protocols with L-asparaginase and lomustine, whilst standard CHOP protocols with doxorubicin are less effective. These different types of lymphoma can usually be identified with a combination of flow cytometry and cytology.

Samples for flow cytometry are collected into a cell medium or a mix of saline and plasma. Several fine-needle aspiration samples are injected into one vial, mixing carefully to prevent clotting. Aliquots of cells are labelled with lineage-specific antibodies tagged with a fluorescent dye. The cells pass through a laser beam causing the laser light to scatter at different angles,

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depending on cell size and complexity, which is used to identify cell populations. When the laser light encounters a fluorochrome-tagged antibody, fluorescence at a longer wavelength is emitted which is measured at a detector. Cells may be dual or triple stained with antibodies conjugated to different fluorochromes.

Important features to assess on cytology are the size and shape of the nuclei, chromatin pattern, location and number of nucleoli, colour/appearance of cytoplasm and mitotic rate.

In DLBCL nuclei are 2–2.5 times the diameter of a small lymphocyte with coarsely stippled chromatin and several peripheral nucleoli. Cytoplasm is deeply basophilic, and the mitotic rate is high. These cells stain with CD21, CD79a (B cell markers), CD45 (panleucocyte marker) and MHCII. Reduced expression of the latter is a negative prognostic indicator.

In TZL the nuclei are 1.5 times the diameter of small lymphocytes with finely clumped chromatin, no nucleoli, and pale-staining cytoplasm which extends as a projection on one side (hand mirror cells). The mitotic rate is very low. These cells stain with T cell markers CD3, CD5, MHCII, CD4 or CD8, but do not stain with CD45, and often have aberrant expression of CD21. This unique immunophenotype along with the morphology confirms the diagnosis.

KEY LEARNING OBJECTIVES

- Outline the importance of identifying different types of lymphoma for prognosis and choice of treatment

- Outline how flow cytometry works
- Outline how a combination of morphology and immunophenotype is used to classify lymphoma

MULTIPLE CHOICE QUESTIONS

1. Which type of lymphoma has the longest median survival?
(A) Marginal zone lymphoma
(B) Diffuse large B cell lymphoma
(C) T zone lymphoma
(D) High-grade T cell lymphoma
2. Which of the following would NOT be expected in T zone lymphoma?
(A) Negative staining with CD45
(B) Positive staining with CD21
(C) Intermediate-sized nuclei with two to three round nucleoli
(D) Pale cytoplasm extending as a projection on one side of the nucleus
3. Which of the following is a negative prognostic indicator in B cell lymphoma?
(A) Positive staining with the stem cell marker CD34
(B) Reduced staining with MHCII
(C) Reduced staining with CD45
(D) Reduced staining with CD79a