**Frequently-Asked Questions: Immunohistochemistry**

**What does the immunohistochemistry service do?**

At DCPAH, IHC is primarily used for identification of specific infectious organisms, diagnosis of tumor entities, and prognostic evaluation of tumors. In addition, we use it occasionally to determine the primary site of a tumor, whether or not it is malignant, and the therapeutic implications of a diagnosis. Many times a paraffin block is the only sample available for routine diagnostics in veterinary medicine. However, numerous infectious agents and cell types can be demonstrated with IHC in formalin-fixed tissues in a wide variety of animal species. At MSU we have developed diagnostic tumor panels for a number of neoplastic conditions in dogs and cats. In addition, specific markers have been used to increase the accuracy of our prognostic predictions. In comparison with other diagnostic tests for infectious diseases, IHC allows co-localization of an antigen within a lesion, thereby increasing diagnostic accuracy and understanding of pathogenesis.

**What is Immunohistochemistry?**

Immunohistochemistry (IHC) has been proven as a highly specific and sensitive diagnostic method and is especially advantageous as a diagnostic tool for neoplastic and infectious diseases. It is based on the detection of the antigen in question using specific mono- or polyclonal antibodies in tissue sections. After binding to the antigen, the antibodies are detected by secondary antibodies that bind a cascade of streptavidin-biotin or polymer molecules and are labeled with peroxidase or alkaline phosphatase. Both enzymes cause a color reaction that will allow the pathologist to detect the antibody-bound antigen within tissue sections.

**What is Immunocytochemistry?**

Immunocytochemistry (ICC) employs the same techniques as described above on cytological samples. We are pleased to offer a number of antibodies that can be used in a more rapid fashion on cytological preparation to further help determine different cell types. It is extremely important to prepare the cytology slides according to a protocol that was specifically developed in our laboratory to guarantee highest quality results. For more questions in regard to ICC please contact the DCPAH Clinical Pathology laboratory and for a detailed description on how to prepare a cytologic sample for ICC, go to the document, “Preparation of Cytologic Samples for Immunocytochemistry” on the DCPAH CD or website.

**Which antibodies are available?**

For a detailed list of antibodies for both infectious diseases and to differentiate various cell types go to the document “IHC Request Form” on the DCPAH CD or website. In addition to antibodies used on domestic species, we also have a wide range of antibodies that can be used in rodents, especially mice and rats, and we are performing GLP IHC studies and can validate antibodies for research projects.
How much does Immunohistochemistry cost?
The cost for a single IHC test is listed in the current Fee Schedule on the DCPAH CD or website.

How can I request IHC?
You can request specific antibodies using the form below or list the antibodies on the general submittal form. You may also list the infectious diseases or neoplastic entities you want to include in your diagnosis, and we will design a panel that corresponds best with your needs. IHC can be requested as an additional test following a biopsy report. This can be done at the same time the sample is submitted for biopsy or as a retrospective test on archival material (years later).

What are prognostic tumor panels?
In contrast to diagnostic antibody panels that are designed to provide a more accurate diagnosis, these panels are designed to provide a more accurate evaluation of the biological behavior of a specific tumor entity. At DCPAH, we have developed a prognostic panel for canine cutaneous mast cell tumors and canine as well as feline lymphomas. For more detailed description and examples of panels for other neoplastic entities, go to the document “Tumor Panels for Formalin-Fixed Tissues” on the DCPAH CD or website.

What is included in a canine cutaneous mast cell tumor prognostic panel?
The panel includes ki67, PCNA, AgNORs, c-kit (KIT protein as well as c-kit PCR). The cost is listed on the current Fee Schedule on the DCPAH CD or website. For a detailed description of the prognostic canine cutaneous mast cell tumor panel and how each of the components in the panel is interpreted, go to the document “New Canine Cutaneous Mast Cell Tumor Panel Adds Certainty to Prognosis” on the DCPAH CD or website. To see the impact of the prognostic panel for canine cutaneous mast cell tumors on the clinical decision making, go to the document “Mast Cell Tumor Flowchart” on the DCPAH CD or website.

What is included in a diagnostic and prognostic algorithm for the feline enteric panel?
The panel includes immunophenotyping (cd3 and cd79a) of the lymphoid infiltrate followed by PCR for B- or T-cell clonality based on the IHC results. PCR will be done in duplicate and gels of the natured and denatured products will be run. The cost of this panel is listed on the current Fee Schedule on the DCPAH CD or website. There is an additional charge for a biopsy or consultation. For a detailed description on the diagnostic algorithm for differentiating intestinal lymphoma from lymphoplasmacytic inflammation of the small intestine, go to the document “Feline Intestinal Lymphoma” on the DCPAH CD or website.

Is Immunohistochemistry cost effective?
The use of IHC in the diagnosis of various tumor entities is not only useful for an accurate diagnosis and prognosis, but is also a highly cost-effective method. The following examples should illustrate the cost effectiveness of IHC and its benefits for veterinary practitioners and their clients.

Example 1: Canine cutaneous histiocytomas are benign tumors that primarily occur in young dogs and regress spontaneously. In contrast, canine cutaneous T-cell lymphomas are slowly progressing, ultimately fatal neoplasms. Epitheliotropism is one of the major features of cutaneous lymphomas. However, approximately 30% of cutaneous histiocytomas may exhibit epitheliotropism. Especially if
such tumors are found in older dogs, the differentiation between these 2 tumor entities can be difficult. IHC can correctly establish a benign diagnosis, and thereby would increase the life expectancy of the dog. The testing in this case would cost $100 (see current Fee Schedule on the DCPAH website) for a panel of 4 antibodies. If we consider that a puppy can easily cost $500 and additional costs for routine veterinary care, food etc. can quickly amount to an additional $500, the use of IHC would be justified even if only one of 16 dogs with a questionable cutaneous round cell tumor would be diagnosed with a malignant neoplasm (threshold: 0.06 or 1 in 16 cases if the dog lives for at least a year).

**Example 2:** The biopsy of a liver mass results in 2 differential diagnoses: a primary hepatic carcinoid or a metastasizing neuroendocrine tumor. IHC will increase the diagnostic certainty in 9 out of 10 cases. The testing in this case would cost $100 (see current Fee Schedule on the DCPAH website) for a panel of 4 antibodies. An accurate diagnosis would support surgical removal of a hepatic carcinoid and aid in a much better prognosis than for an already metastasizing neuroendocrine tumor. Considering the costs of the surgical removal as well as the cost to purchase and maintain a dog, the use of IHC would be justified even if only one of 20 dogs were diagnosed with a metastasizing neuroendocrine neoplasm (threshold: 0.05, or 1 in 20 cases if the dog lives at least for a year).

**Example 3:** A malignant lymphoma is diagnosed in the lymph node of a dog. Using immunophenotyping, the lymphoma can be identified as a B- or T-cell lymphoma. In this case IHC not only increases the prognostic accuracy, but also helps to determine the therapeutic protocol. Whereas B-cell lymphomas respond well to aggressive chemotherapy, T-cell lymphomas are slower progressing, ultimately fatal neoplasms. The testing in this case would cost $50 (see current Fee Schedule on the DCPAH website) for a panel of 2 antibodies. Considering the cost of chemotherapy as well as the cost to purchase and maintain a dog, the use of IHC would be justified even if only one of 25 dogs were diagnosed with a T-cell neoplasm (threshold: 0.04 or 1 in 25 cases if the dog lives at least for a year).

**How do I contact the Immunohistochemistry service?**

During regular business hours, Monday through Friday 7:30 a.m. to 5:30 p.m., please call (517) 353-1683. For animal health emergencies outside of normal operating hours, please call 517-353-5275 for an automated answering system.