REVIEW ARTICLE

ARTICLE DE REVUE

Enzyme immunohistochemical staining of formalin-fixed tissues for diagnosis in veterinary pathology

Deborah M. Haines, Edward G. Clark

Abstract

Disease diagnosis often relies on the detection of specific antigens in tissue specimens. Enzyme-based immunohistochemical stains of formalin-fixed, paraffin-embedded tissues may be used to identify antigens associated with viral, bacterial and protozoal microorganisms, autoimmunity, and neoplasia. The detection of antigens in routinely fixed tissues offers several advantages over other diagnostic techniques. Sample submission is convenient and facilitates safe handling of potential human pathogens. Retrospective studies of stored specimens are possible. The technique is relatively rapid and enables detection of nonviable microorganisms. In addition, the ability to detect antigens in fixed specimens allows simultaneous visualization of the antigen and the histological lesion which may enhance the accuracy of diagnosis.

Résumé

Coloration par procédé immunohistochimique enzymatique de tissus fixés à la formaldéhyde comme moyen de diagnostic en pathologie vétérinaire

Le diagnostic d'une maladie est souvent basé sur la découverte d'antigènes spécifiques dans les tissus soumis. La coloration de tissu préalablement fixé à la formaldéhyde et enrobé dans la paraffine par immunohistochimie enzymatique peut être employée pour l'identification antigénique d'infections virales, bactériennes ou par protozoaires et lors de processus auto-immun ou néoplasique. L'identification d'antigènes à partir de tissus préservés par des techniques standard offre plusieurs avantages comparativement à d'autres moyens de diagnostic. Le transport et la manipulation des échantillons se font sans inconvénient et de façon sécuritaire considérant qu'ils sont susceptibles de contenir des agents pathogènes représentant un danger pour l'homme.

Une étude rétrospective peut être effectuée sur les échantillons gardés en réserve. La technique est relativement rapide et permet l'identification de microorganismes non viables. De plus, ce procédé permet de visualiser simultanément l'antigène et les lésions histologiques améliorant ainsi l'exactitude du diagnostic. (*Traduit par Dr Thérèse Lanthier*)

Department of Veterinary Microbiology (Haines) and Department of Pathology (Clark), Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0.

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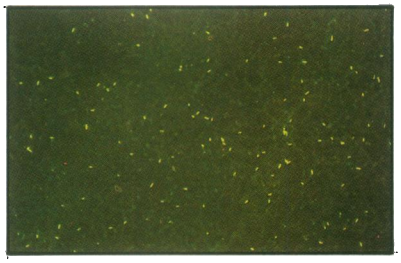
Introduction

Immunohistochemical staining allows detection of antigens in tissue specimens using specific antibodies labelled to be visible microscopically. Immunohistochemical stains are widely used in human and veterinary medicine for diagnosis of diseases associated with autoantibody deposition, infectious disease microorganisms and most recently, for the identification of tumors. In this paper, we summarize recent advances in immunohistochemical staining technology which allow their application to formalin-fixed tissues and will emphasize the applications of these methods in veterinary diagnostic laboratories.

The first immunohistochemical stain described was immunofluorescence (1). When tissues stained with fluorescein-labelled antibodies are viewed under ultraviolet light, sites of antibody binding appear bright yellow-green. Figure 1 shows an immunofluorescence stain for *Clostridium chauvoei*, the causative organism of blackleg in cattle. Immunofluorescence stains are used extensively for disease diagnosis, however there are limitations associated with the characteristics of the fluorescein dye. Fluoresceinated antibodies can only be viewed with expensive, ultraviolet microscopes and the dye fades rapidly, thus permanent records can only be maintained by photomicroscopy. In addition, tissues stained with fluoresceinated antibodies cannot be counterstained with the stains used for routine histological evaluation, so it is not possible to simultaneously view the histological lesions and the distribution of the immunohistochemical stain.

These shortcomings have led to the development of alternative immunostaining methods. One alternative to fluoresceinated antibodies is the use of antibodies labelled with enzymes (2). When antibodies conjugated to enzymes are applied to tissues followed by an



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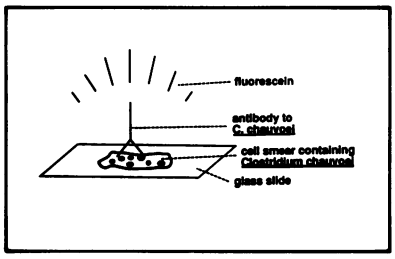

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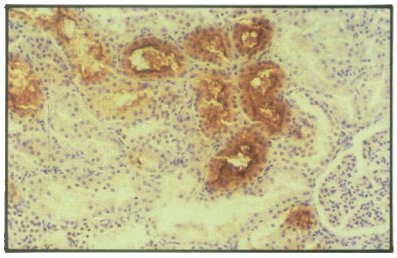

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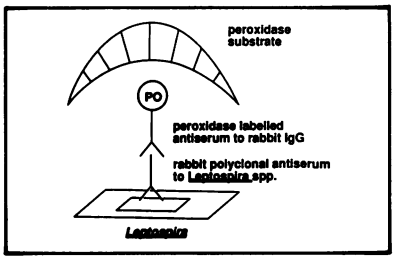

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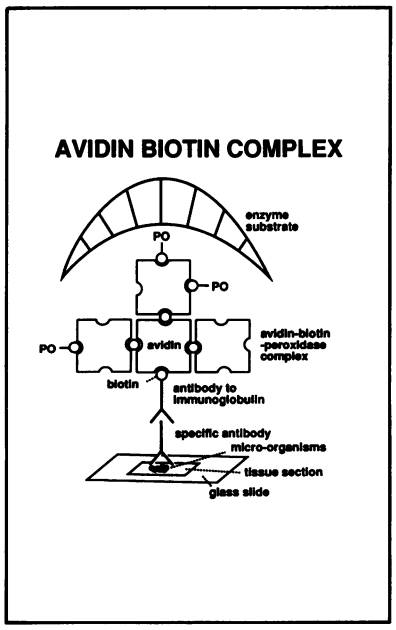

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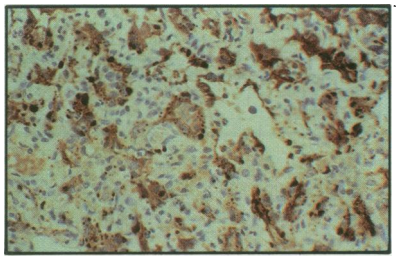

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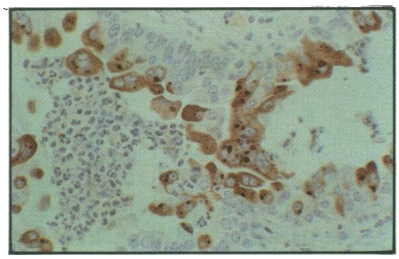

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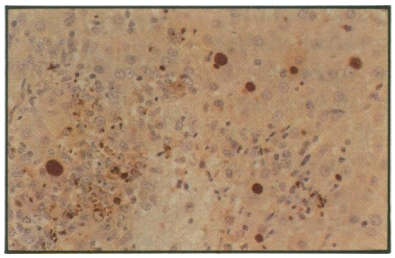

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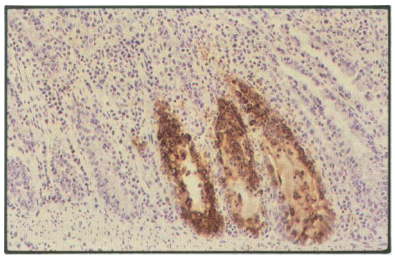

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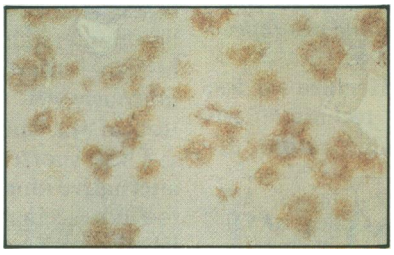

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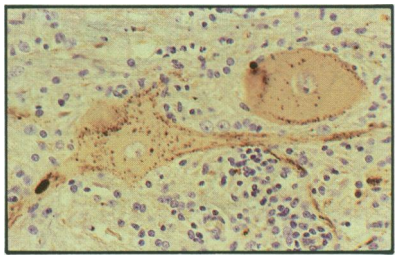

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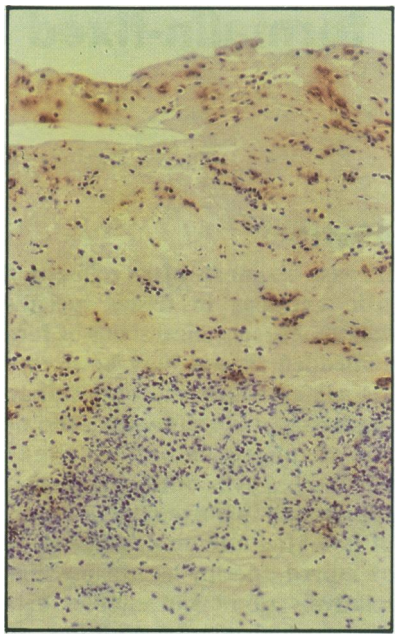

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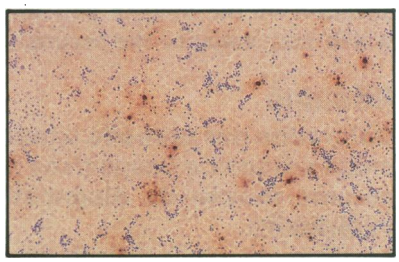

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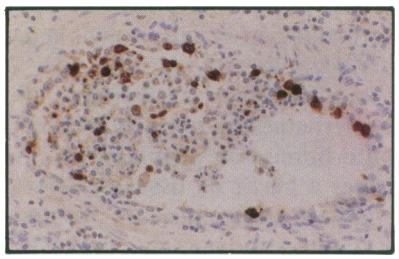

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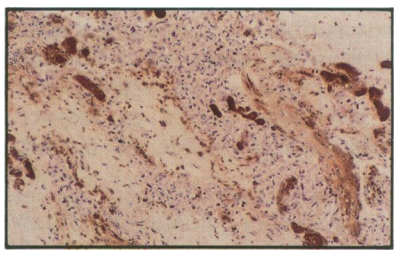

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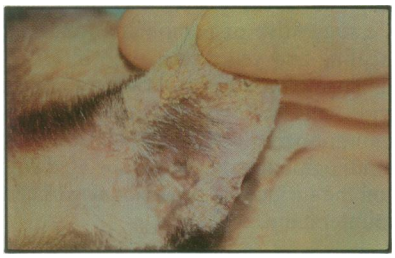


Figure 14a

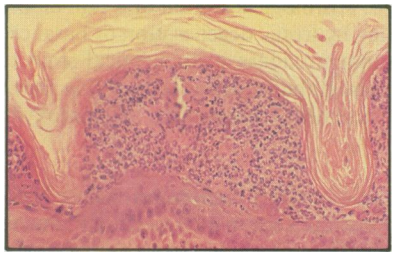


Figure 14b

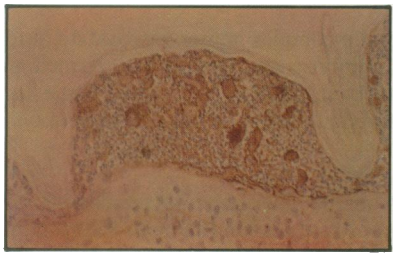


Figure 14c

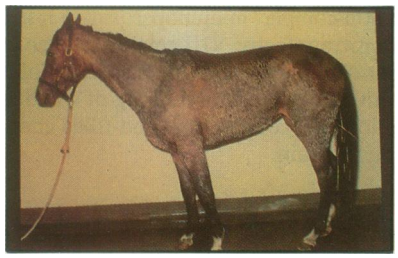


Figure 15a

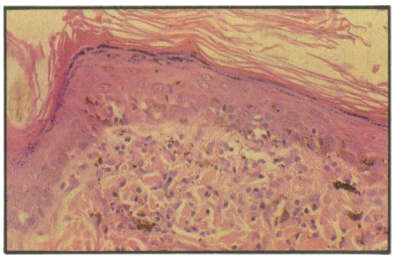
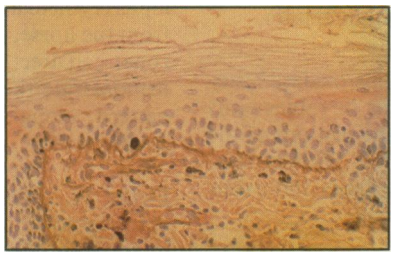


Figure 15b



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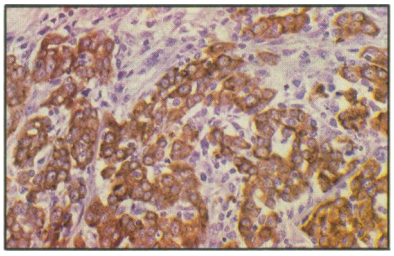

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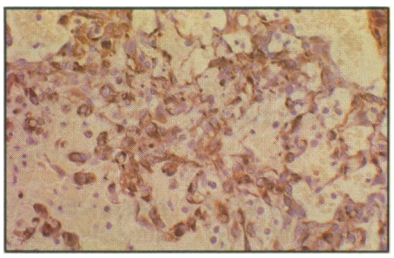

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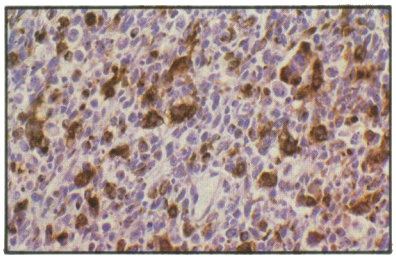

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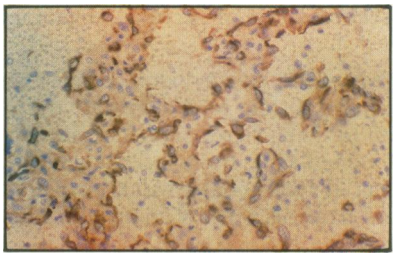

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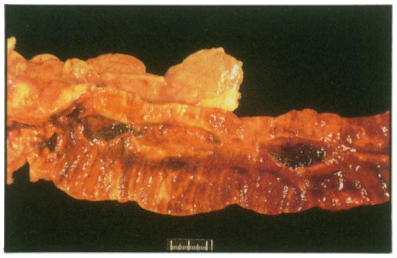

Figure 20a

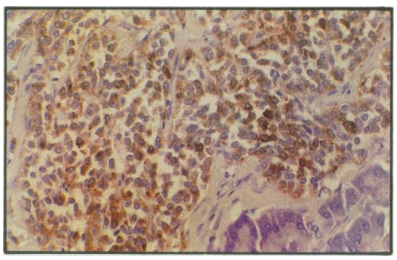

Figure 20b

Figure 1a. Immunohistochemical staining for antigens of *Clostridium chauvoei* in an impression smear of muscle from an ox. Direct immunofluorescence viewed under ultraviolet light. b. Diagrammatic representation of the direct immunofluorescence staining method used to demonstrate *Clostridium chauvoei*.

Figure 2a. Indirect immunoperoxidase immunohistochemical stain for antigens of *Leptospira interrogans* serovar *pomona* in a formalin-fixed, paraffin-embedded kidney section of a pig. The section has been counterstained with hematoxylin to demonstrate nuclei. Note the staining of organisms lining tubular lumens and of antigens within tubular epithelial cells. b. Diagrammatic representation of the indirect immunoperoxidase staining method used to demonstrate leptospiral antigens.

Figure 3. Diagrammatic representation of the avidin-biotin-complex immunohistochemical staining method.

Figure 4. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of canine distemper virus in formalin-fixed, paraffin-embedded tissue sections of the lung of a dog. Positive staining is dark brown. Note the intense staining of cytoplasmic inclusion bodies. Hematoxylin counterstain.

Figure 5. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of bovine respiratory syncytial virus in formalin-fixed, paraffin-embedded tissue sections of the lung of an ox. Positive staining is dark brown. There is diffuse cytoplasmic and intense inclusion body staining in bronchiolar epithelial cells. Hematoxylin counterstain.

Figure 6. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of *Toxoplasma gondii* in formalin-fixed, paraffin-embedded tissue sections of the brain of a dog. Positive staining is dark brown. There is intense staining of scattered tachyzoites in addition to large pseudocysts. Hematoxylin counterstain.

Figure 7. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of bovine coronavirus in formalin-fixed, paraffin-embedded tissue sections of the colon of a calf. Positive staining is dark brown. Staining is apparent in lining epithelial and sloughed cells of the colonic crypts. Hematoxylin counterstain.

Figure 8. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of bovine herpes type 1 (infectious bovine rhinotracheitis virus) in formalin-fixed, paraffin-embedded tissue sections of the liver of a calf. Positive staining is dark brown and is apparent in randomly scattered foci of necrosis. Hematoxylin counterstain.

Figure 9. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of rabies virus in formalin-fixed, paraffin-embedded tissue sections of the brain of a pig. Positive staining is dark brown. There is staining of Negri bodies within perikarya, dendrites and axons of large neurons. Hematoxylin counterstain.

Figure 10. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of *Haemophilus somnus* in formalin-fixed, paraffin-embedded tissue sections of a bovine lung. Positive staining is dark brown. Note stained clumps of organisms in pleural fibrin. Hematoxylin counterstain.

Figure 11. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of *Chlamydia psittaci* in formalin-fixed, paraffin-embedded tissue sections of the liver of a bird. Positive staining is apparent as dark brown multifocal deposits. Hematoxylin counterstain.

Figure 12. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the antigens of influenza A virus in formalin-fixed, paraffin-embedded tissue sections of the lung of a pig. Positively stained sloughed and attached bronchiolar epithelial cells are dark brown. Hematoxylin counterstain.

Figure 13. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate the colonies of *Campylobacter jejuni* in formalin-fixed, paraffin-embedded tissue sections of a bovine placenta. Positive staining is dark brown. There is dense staining of intra- and extravascular clusters of organisms. Hematoxylin counterstain.

Figure 14a. Photograph of the ear of a cat with generalized superficial skin disease suggestive of pemphigus foliaceus. Note the superficial scaling and yellow crusting. b. Histological section of a skin biopsy from the cat showing a pustule containing acantholytic cells (arrows) in the superficial epidermis. c. Direct immunoperoxidase histochemical stain to demonstrate the immunoglobulin deposits associated with acantholytic cells and on the surfaces of perilesional epithelial cells. Hematoxylin counterstain.

Figure 15a. Photograph of a horse with generalized exfoliative and alopecic skin disease suggestive of systemic lupus erythematosus. b. Histological section of a skin biopsy from the horse, showing hydropic degeneration of basal cell zone. c. Direct immunoperoxidase histochemical stain to demonstrate the immunoglobulin deposits in a fine linear pattern along the basement membrane. Hematoxylin counterstain.

Figure 16. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate keratin intermediate filaments in formalin-fixed, paraffin-embedded tissue sections of a squamous cell carcinoma from a dog. Hematoxylin counterstain.

Figure 17. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate vimentin intermediate filaments in formalin-fixed, paraffin-embedded tissue sections of an osteosarcoma from a dog. Hematoxylin counterstain.

Figure 18. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate desmin filaments in formalin-fixed, paraffin-embedded tissue sections of a rhabdomyosarcoma from a dog. Hematoxylin counterstain.

Figure 19. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate factor VIII-related antigen in formalin-fixed and paraffin-embedded tissue sections of a hemangiosarcoma from a dog. There is staining of spindle-shaped to fusiform endothelial cells lining blood-filled channels. Hematoxylin counterstain.

Figure 20a. Photograph of an ulcerative lesion in the duodenum of a dog. b. Avidin-biotin-complex immunoperoxidase histochemical stain to demonstrate gastin in formalin-fixed, paraffin-embedded tissue sections of a focus of small tumor cells in the pancreas of this dog. Hematoxylin counterstain.

enzyme substrate, colored chromogen deposits stain the tissues at the sites of antibody binding. These deposits are visible with an ordinary light microscope and produce permanent stains. In addition, the tissue sections may be concurrently stained with routine histochemical stains so that the morphological details of the tissue can be seen simultaneously with the distribution of the immunostain.

One of the most common enzymes used in immunohistochemical staining is peroxidase. The binding of peroxidase-labelled antibodies to tissue antigens, followed by the peroxidase substrate (3,3) diaminobenzidine (DAB), results in rich chocolate-brown deposits in the tissues. Figure 2 shows a peroxidase-based immunohistochemical stain which demonstrates detection of leptospiral antigens in sections of porcine kidney.

The method shown in Figure 2 is an *indirect* immunostain while the method diagrammed in Figure 1 is a *direct* technique. Direct staining methods are those in which the primary antibody (antibody specific for the antigen of interest) has been directly labelled with the enzyme or fluorescent dye, whereas in indirect techniques the primary antibody is unlabelled. In indirect immunostains, the binding of the primary antibody to the tissues is detected by a second antibody to immunoglobulin which has been labelled with the enzyme or fluorescent dye. In the example shown in Figure 2, the primary antibody was raised in a rabbit, therefore the second antibody is directed against rabbit

immunoglobulin. Indirect methods are a means of increasing the sensitivity of the staining method so that smaller amounts of antigen may be detected. Since approximately five to seven second antibodies will bind to each primary antibody, this results in five to seven fold enhancement of the visible fluorescence or enzyme signal.

There are other immunohistochemical techniques that result in even greater amplification of the visible signal than is provided by indirect methods. One amplification method is the avidin biotin complex (ABC) technique (3) diagrammed in Figure 3. The ABC immunostain relies upon the high affinity of the B-vitamin biotin, for the egg-white glycoprotein, avidin. In ABC stains, the tissue antigen is bound by an unlabelled primary antibody followed by a second antibody to immunoglobulin labelled with biotin. The biotinylated antibody is then detected by application of preformed complexes of avidin and biotin. Each avidin has binding sites for four biotin molecules, and the complexes are generated with free biotin-binding sites, which ensures attachment of the complexes to the biotinylated antibody. The biotin molecules are labelled with an enzyme, usually peroxidase. Binding of the large avidin-biotin complexes, containing many peroxidase molecules, results in an amplification which enables detection of much smaller amounts of antigen in tissues. The ABC stain is estimated to be 1000-fold more sensitive than a direct immunoenzyme staining method (4).

All immunohistochemical stains are dependent upon the ability of the primary antibody to bind to the antigen of interest in the tissue specimen, therefore it is critical that the antigenic integrity of the specimen is conserved. For this reason, immunohistochemical stains have traditionally been performed on fresh or frozen tissue specimens. Immunohistochemistry has not been applied to fixed tissue specimens because the process of fixation, while preserving the morphological details of the specimen, damages the antigenicity so that antibodies are less able to bind. Recently, however, there have been numerous descriptions of successful immunohistochemical stains using routinely fixed and processed tissues (5–9). The ability to accomplish immunohistochemical stains on formalin-fixed and paraffin-embedded tissue is attributable to two improvements in immunohistochemical staining methodology. The first is the enhanced sensitivity afforded by techniques such as the ABC method which enables the detection of binding of small numbers of primary antibody molecules to tissues with diminished antigenicity. The second is the discovery that digestion of tissue sections with proteolytic enzymes improves the reactivity of antibodies with antigens in fixed tissues (10). The explanation for this improvement is unclear. It may be a combination of increasing the permeability of the tissue for antibody binding and of breaking down the formalin-induced cross-linkage of the tissue proteins.

The ability to specifically detect antigens in formalinized tissues and to produce stable immunohistochemical stains visible with ordinary light microscopy adds a new dimension to diagnostic pathology. This paper focuses on the applications and advantages of immunohistochemical stains of routinely fixed tissues for disease diagnosis in veterinary medicine.

Detection of infectious disease-producing microorganisms

In veterinary medicine, most immunohistochemistry on formalin-fixed tissues has been used to detect infectious disease-producing microorganisms. While most routine diagnostic procedures for detection of infectious disease agents will continue to use fresh or frozen tissue specimens, the ability to diagnose infectious disease agents in fixed tissues offers advantages in some situations.

Diagnosis of infectious disease agents in fixed tissues is convenient with respect to submission of specimens. Since it is common practice to fix tissus in formalin for histological examination, the ability to detect microorganisms in these tissues enables submission of a single sample which may be used for both evaluations. This also negates the requirements for special transport media and for rapid transport of the specimens. The ability to detect antigens in fixed tissues may be especially beneficial in instances in which the diseased animals are long distances from the diagnostic laboratories. Figure 4 shows an ABC immunoperoxidase stain to demonstrate canine distemper virus (CDV) antigen in formalin-fixed tissue specimens. This method was used to diagnose CDV infection in animals dying in Canada's eastern Arctic, several hundred miles from any diagnostic laboratory (11). The ability to detect the virus in formalin-fixed tissues enabled convenient collection and transport of the specimens.

Detection of microorganisms in processed tissue specimens facilitates retrospective studies. Most diagnostic laboratories store, as paraffin-embedded blocks, samples of all submitted specimens, and most antigens are stable indefinitely in tissues within paraffin blocks. Immunohistochemical staining of these paraffin-embedded specimens permits large scale and/or long-term retrospective studies. Figure 5 demonstrates a section of bovine lung stained for bovine respiratory syncytial virus (BRSV) antigens. We have demonstrated this virus in paraffin-embedded specimens of bovine lung stored for over 15 years. The ability to detect antigens in stored specimens means that retrospective surveys for changes in the incidence and prevalence of diseases may be readily achieved. Immunohistochemical stains on formalin-fixed tissues also permits short-term retrospective diagnoses. This need most often arises in cases in which the correct diagnosis is suspected only following histological examination and fresh tissue specimens are no longer available. Figure 6 demonstrates an example of the application of immunohistochemical staining of fixed tissues to achieve short-term retrospective diagnosis. The tissues are from a dog which died with acute neurological disease suspected to be granulomatous meningoencephalitis, however postmortem histological examination revealed large protozoan cysts in the brain. These could be further identified by immunohistochemical staining of serial sections of the fixed tissues with antibodies directed against *Toxoplasma gondii*.

Another advantage of immunoenzyme stains on formalin-fixed tissues is that these methods produce permanent preparations which can be viewed by light microscopy. This enables the pathologist to associate the immunohistochemical stain, demonstrating the infectious disease agent, with the lesions present in the tissues. This association may be desirable when diagnosing disease due to organisms which are ubiquitous and might be isolated from an unaffected animal. The detection of the organism in the lesion helps to confirm that it is involved in the pathogenesis of the disease and not simply an irrelevant isolate. Figure 7 shows the demonstration of bovine coronavirus antigen in tissues from a calf with diarrhea. The detection of coronavirus antigens in the tissues in association with histological lesions helps to confirm this organism as a cause of disease in the animal.

Demonstration of the organism in association with the lesion is also useful in diagnosis of diseases due to organisms for which live virus vaccines have been used. In these cases the demonstration of the organism in the lesion is necessary to implicate the organism in the disease, since isolation of the organism alone is not unexpected following vaccination with live microorganisms. Figure 8 shows tissue stained for bovine herpes type 1 virus (BHV-1) the causative agent of infectious bovine rhinotracheitis. This calf died following vaccination with a modified live virus vaccine for BHV-1. Simple isolation of the organism independent of the lesions is insufficient to link the organism with the disease since the vaccine virus might be expected to be present without causing disease. This immunohistochemical stain demonstrating the microorganism in the site of the histological lesion strengthens the evidence that the virus was involved in the pathogenesis of the disease.

Immunohistochemical staining on formalin-fixed tissues may improve the sensitivity of diagnosis of infectious diseases. This improved sensitivity may be attributed to the ability to examine routinely stained sections to identify the histological lesions, then to immunohistochemically stain serial sections of these lesions for detection of the suspected microorganisms. Figure 9 shows an immunohistochemical stain for rabies viral antigens in a pig. When sections with histological lesions were specifically selected, and stained, the virus was readily demonstrated in the affected areas. The direct immunofluorescence test for rabies virus on fresh tissue was negative in this case, probably due to the inability to preselect the affected areas of the tissues for testing.

Improved sensitivity of diagnosis by immunohistochemical stains may also be attributable to the fact that the organism does not have to be alive or to proliferate in order to be detected. This may be advantageous in cases of bacterial infections in which the likelihood of culture is reduced due to systemic treatment of the animal with antibiotics. Figure 10 shows staining of a section of bovine lung for antigens of *Haemophilus somnus*. It is not uncommon to demonstrate *Haemophilus* antigens in lesions of antibiotic-treated animals in which it has not been possible to culture the organism.

Immunohistochemical stains may also be an efficient means of detecting organisms which are inherently slow or difficult to diagnose by viral isolation or bacteriological culture. Figure 11 shows immunohistochemical staining of the liver of a budgie for antigens of *Chlamydia psittaci*. Isolation of *Chlamydia psittaci* can take up to three weeks; in contrast, this organism may be detected in formalin-fixed tissue sections in a single-day using an immunohistochemical stain. Immunohistochemical stains may also be used to detect viruses which are difficult to isolate. Figure 12 shows immunostaining of sections from a porcine lung demonstrating the antigens of influenza A virus. The isolation of this virus is tedious and insensitive, however it is readily demonstrated in formalin-fixed tissues with immunohistochemical stains.

An additional advantage of using formalin-fixed tissues for diagnosis of infectious diseases in that this minimizes the risks of handling microorganisms which are potential human pathogens. Testing tissues for pathogens such as rabies virus, *Chlamydia psittaci*, leptospiral serovars, *Toxoplasma gondii* and *Campylobacter* may be a threat to human health if handling fresh tissue specimens, however these organisms do not pose a risk if tested as formalin-fixed sections. Figure 13 shows formalin-fixed sections of a placenta stained to demonstrate *Campylobacter* antigens.

Autoimmune skin diseases

Immunohistochemical stains are also used to assist in the diagnosis of autoimmune skin diseases (AISD). The diagnosis of AISD relies upon the demonstration of autoantibody deposits in the skin. Traditionally, immunofluorescence staining of snap-frozen cryostat sections of fresh tissues are used for this test. As well as the inconvenience involved in transporting fresh tissues from the veterinary hospital to the laboratory, there are additional disadvantages associated with this test method. For accurate diagnosis of AISD, three criteria must be fulfilled: a clinical syndrome suggestive of AISD, histological lesions consistent with the suspected syndrome, and the demonstration of autoantibodies in a skin biopsy in a distribution consistent with the suspected AISD. It can be difficult to obtain skin biopsies which demonstrate histological lesions or immunoglobulin deposits typical of AISD. Often the lesions become secondarily infected with bacteria or are mutilated by self trauma. When several biopsies are submitted from an affected animal only one or two may show histological lesions or immunoglobulin deposits characteristic of AISD. When using frozen sections for immunohistochemical staining, the histological and immunohistochemical examinations are carried out on different biopsies. Typical histological lesions or immunoglobulin deposits may be seen in one set of biopsies which were not present in the other and, as a consequence, conflicting results are reported. In contrast, immunohistochemical demonstration of immunoglobulin deposits in formalin-fixed skin biopsies enables precise correlation of immunohistochemical and histological findings. Once the histological sections of the biopsies have been

evaluated, the paraffin blocks identified to have lesions consistent with AISD are resectioned and submitted for immunohistochemical staining for antibody deposits. The immunoglobulin deposits are thus viewed in serial sections of tissues which were examined histologically, and the findings of the immunology and pathology laboratories can be accurately correlated. Figures 14 and 15 demonstrate examples of the clinical findings, histological lesions, and immunohistochemical stains used to diagnosis pemphigus foliaceus in a cat and systemic lupus erythematosus in a horse.

Tumor diagnosis

In veterinary medicine, immunohistochemical stains on formalin-fixed tissues have been used primarily for the identification of infectious disease microorganisms. This is in contrast to the situation in human medicine where these techniques have chiefly been used for the identification of tumors. Recently, however there have been several reports of applications of immunohistochemical stains for improved diagnosis of tumors in animals (12–16).

Identification of a tumor begins with the determination of the tissue of origin of the tumor population. In normal tissue, intermediate filament expression is tissue-type specific; epithelial cells express only keratin intermediate filaments, mesenchymal cells express vimentin intermediate filaments, and in addition muscle cells express desmin proteins. This restricted expression is generally maintained in tumor cells and may be used to identify the origin of the tumor population. Figures 16, 17 and 18 demonstrate immunohistochemical staining of formalin-fixed tumors to identify keratin, vimentin and desmin intermediate filaments, respectively.

Further identification of the origin of the tumor may also be achieved by demonstration of organ or cell-specific antigens. Figure 19 shows the staining of a hemangiosarcoma with antibodies to factor VIII related antigen (F VIII Ra) substantiating the endothelial cell origin of the tumor cells. Figure 20a shows a photograph depicting mucosal ulceration of the duodenum of a dog. One cause of duodenal ulceration may be the presence of a tumor producing the hormone gastrin. Figure 20b shows an immunohistochemical stain for gastrin in a small focus of cells infiltrating this animal's pancreas confirming the presence of a gastrin-producing tumor.

Immunohistochemistry may also be used for the diagnosis of plasma cell tumors. In order to demonstrate the monoclonal nature of a plasma cell tumor cell population, it is desirable to show that the cells express a single immunoglobulin light chain and single class of immunoglobulin. This can be achieved by staining a series of sections of the tumor with antisera specific for IgG, IgM, and IgA classes and for kappa and lambda light chains.

Conclusions

Enzyme-based immunohistochemical staining of formalin-fixed tissues has advantages over other diagnostic methods for detection of microorganisms in convenience of sample submission and in enabling retrospective diagnoses. In addition, these tests can improve diagnostic accuracy by permitting visualization of the distribution of the infectious disease agent simultaneously with the histological lesions. The sensitivity of diagnosis may also be increased by the ability to preselect the histological lesions to be immunohistochemically examined for the organism. Immunohistochemical stains of fixed tissues have the additional advantage of safe handling of human and animal pathogens. The ability to detect immunoglobulins in formalin-fixed tissues enables precise correlation between histological and immunohistochemical findings for improved diagnosis of autoimmune skin diseases. Finally, immunohistochemical stains for cell and tissue specific antigens may be used to identify tumor cell populations to assist in more accurate tumor diagnosis.

Avidin-biotin complex immunohistochemical stains on formalin-fixed tissues are labor intensive and consequently expensive to perform compared with methods such as direct immunofluorescence on fresh tissues. In addition the necessity for fixation and processing to produce paraffin-embedded blocks means that the test is not accomplished as rapidly as tests which can be performed on fresh tissues. Currently these techniques are used largely as ancillary aids for the diagnosis of infectious disease agents. For the diagnosis of AISD and tumors, where case numbers are low and rapidity of diagnosis is not as critical, immunohistochemical stains on formalin-fixed tissues may replace other routine techniques. The recent development of automated immunohistochemical staining systems may in the near future increase the efficiency of these techniques and result in their increasingly routine application (17).

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Correction

Harland RJ, Jim GK, Guichon T, Townsend HGG, Janzen ED.
Efficacy of parenteral antibiotics for disease prophylaxis in feedlot calves.
Can Vet J 1991; 32: 163–168

Table 1. Formulas for the calculation of various percentage risks and relative risk

BRD[a]	= *#Calves first treated for BRD during time period* / #Calves untreated for BRD prior to time period	× 100%
Fatal fibrinous pneumonia	= *#Fatal fibrinous pneumonia cases first treated during time period* / #Calves untreated for BRD prior to time period	× 100%
All fatalities	= *#Fatal cases first treated during time period* / #Calves untreated prior to time period	× 100%
Case fatality	= *#Fatal cases first treated during time period* / #All cases treated during time period	× 100%
Relative risk	= *Risk for prophylactic medication group* / Risk for control group (no prophylactic medication)	

[a]Bovine respiratory disease

The rule lines which indicate the equations in Table 1 were inadvertently omitted; a corrected version of Table 1 appears above. The *CVJ* regrets this error and extends apologies to the authors.