IMMUNOCYTOCHEMISTRY LECTURE
(Also Called Immunochemistry or Immunoperoxidase)

1. Introduction
2. Antigens and Antibodies
3. Staining Methods
4. Staining Procedures
5. Interpretation of Antibodies

INTRODUCTION

With the introduction of immunochemical techniques into the routine histology laboratory, a new era of tissue staining evolved. These very sensitive and specific methods; utilizing antigen-antibody complexes, allow visualization of previously undetectable cell components. This will guide you through scientific knowledge necessary to perform immunoperoxidase staining techniques.

We will start at the beginning by explaining exactly what immunochemicals are, and what they can do. Once we have an understanding of the various methods that are used, we can then outline step-by-step procedures; discuss controls; fixation and processing of specimens; and provide special hints to achieve successful staining.

3

ANTIGENS AND ANTIBODIES

It is necessary to have a basic knowledge of the building blocks of immunology (antigens and antibodies) to more fully understand immunoperoxidase methods.

Antigens

Antigens have two main properties. The first is immunogenicity, which is the ability to induce antibody formation. The second property is specific reactivity, which means that the antigen can react with the antibody it caused to be produced. The reaction between an antigen and its antibody is one of the most specific in biology, and is the reason that immunohistochemical reactions are more precise than ordinary histochemical techniques.

An antigen then, is a substance foreign to the host which stimulates formation of a specific antibody and which will react with the antibody produced. This reaction involves the formation of immune complexes comprised of several antigen and antibody molecules. These complexes may become very large and form precipitates which can be measured by various techniques.

Antibodies

An antibody is a serum protein that is formed in response to exposure to an antigen, and reacts specifically with that antigen to form immune complexes either in the body or in the laboratory. Antibody production is a response by the body to foreign material (an antigen), and is designed to rid the body of this invader.

Antibodies are contained in the gamma globulin fraction of serum, and are often called immunoglobulins (Ig). They can be divided into five classes based on their size, weight, structure, function, and other criteria. The classes are IgA (immunoglobulin A), IgD, IgE, IgG, and IgM. Antibody solutions utilized in immunohistochemical staining contain mostly IgG type antibodies, with lesser amounts of the other classes.

Antibody Structure

Structurally, an antibody is made up of two kinds of protein chains - heavy and light chains. Immunoglobulins are named for their heavy chains, so the IgG molecule in Figure 1 will have heavy chains of the gamma type. An IgA antibody has alpha heavy chains; IgD, delta heavy chains, IgE, epsilon heavy chains; and IgM has mu heavy chains. A primary antibody for immunoperoxidase staining that is "specific for gamma chains" will localize the heavy chain of an IgG molecule.

There are only two types of light chains common to all five groups: kappa and lambda. An IgG molecule has two identical light chains, either two kappa chains or two lambda chains (Figure 2). A single antibody can never have both kappa and lambda chains. This is important when discussing the interpretation of light chain staining in lymphoma cases.

The orientation of an IgG antibody is shown in Figure 3. The Fc portion stands for fragment crystalline, and will crystallize out upon purification. This region is involved in complement fixation and transfer of antibody across the placenta. The remaining portions are called the fragment antigen binding or Fab regions. These are the parts of the antibody molecule capable of specifically binding to the antigen. This IgG molecule has the ability to bind two antigen molecules, one at each Fab site.

Antibody Production

In order to produce an antibody for laboratory use, it is first necessary to purify an antigen. A source for the antigen such as serum, urine or tissue is subjected to a combination of procedures including precipitation, centrifugation, dialysis, chromatography and electrophoresis to obtain a highly purified antigen. The antigen is then injected into an animal of different species than that of antigen source. The animal will identify the antigen as foreign matter, and produce an antibody directed specifically against it. Antibody production begins within twenty minutes after injection, although a measurable quantity of antibody cannot be detected for 5-10 days. Small

blood samples are usually obtained and pooled at two week intervals. Booster injections of antigen are often administered every month to promote consistent antibody production.

The choice of an animal for injection depends upon the antigen used, housing facilities available, amount of antibody needed, and personal preference. Usually several animals of the species chosen will be injected with an antigen, as each animal will vary in how it responds to the antigen, and in the amount of antibody it produces. After several bleedings are pooled, contaminants present must be removed. This is usually accomplished by either liquid or solid phase antigen absorption techniques.

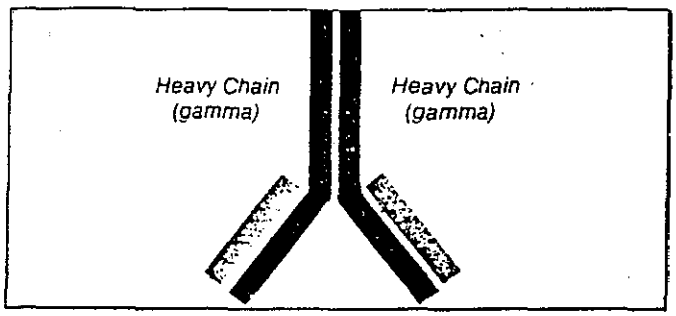


Figure 1. *IgG molecule showing paired heavy chains of gamma type.*

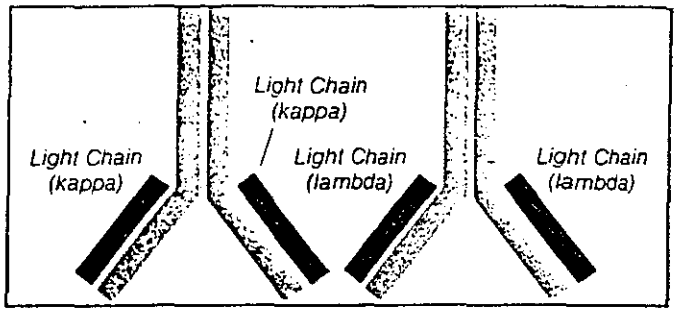


Figure 2. *IgG molecules showing only possible light chain configurations.*

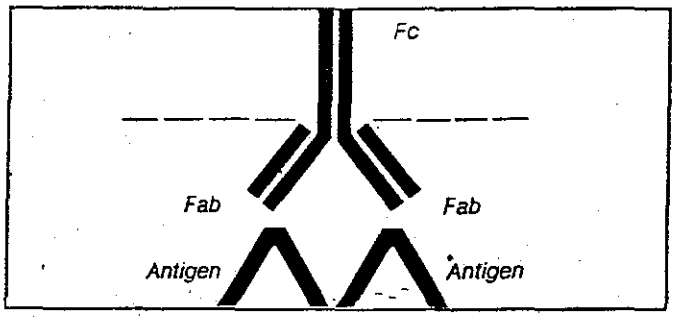


Figure 3. *Division of IgG molecule into Fc and FAb fragments. Antigen binding occurs at the two FAb sites.*

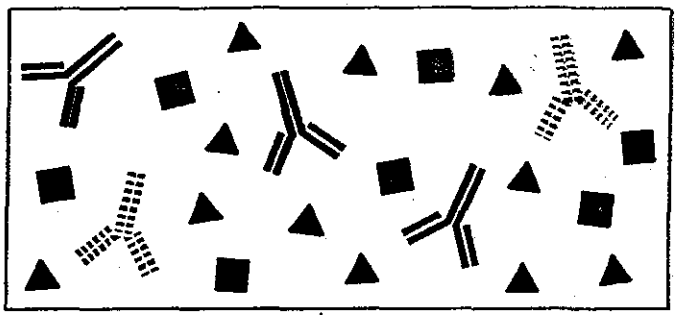


Figure 4. *Whole serum antibody solution containing all normal animal serum components in addition to specific antibody.*

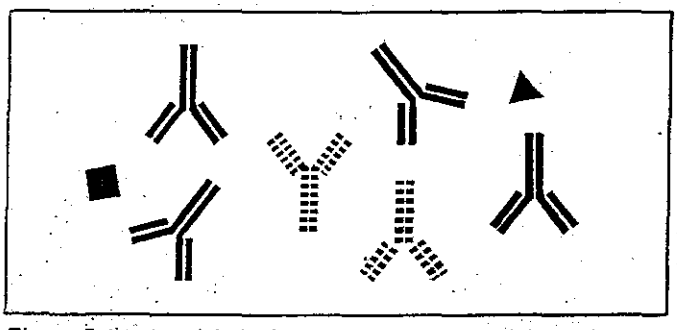


Figure 5. *Immunoglobulin fraction of serum containing only antibodies.*

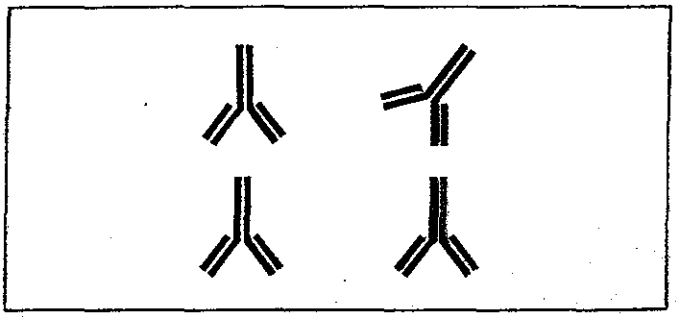


Figure 6. *Antigen specific antibody is more specific than usually necessary.*

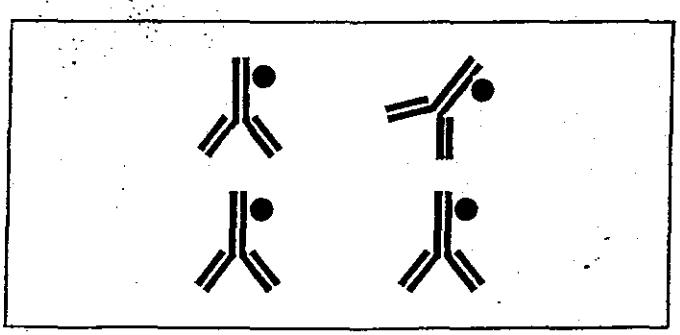


Figure 7. *A conjugate combines an antibody and some type of visual marker.*

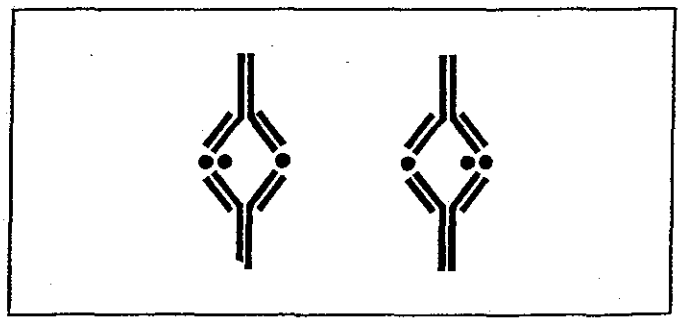
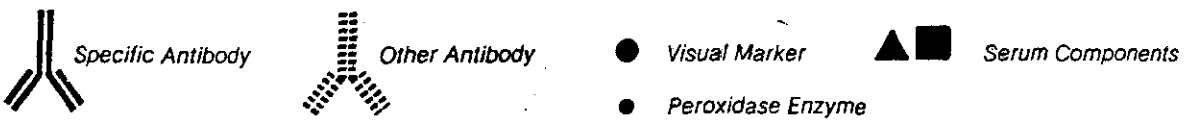


Figure 8. *A peroxidase-antiperoxidase immune complex formed by natural affinities between antigens and antibodies.*

***Key** to Figures 4 through 8.*



1

TYPES OF ANTIBODY SOLUTIONS

There are several antibody preparations available for use in immunoperoxidase procedures. The easiest to produce, and therefore the most common and least expensive, is whole serum. Animal blood containing the antibody is centrifuged to separate the cells from the serum, and any contaminating antibodies are absorbed out.

A whole serum solution is pictured schematically in Figure 4. It will contain antibodies specific for the antigen the animal was immunized with. Other antibodies which are products of the animal's normally functioning immune system will also be present. These should not interfere with staining procedures. The bulk of the whole serum fraction is made up of ordinary serum components such as enzymes, electrolytes, and serum proteins. Occasionally, these other serum elements can cause unwanted background staining in some techniques. This is due to the affinity of serum proteins, most notably albumin, alpha and beta globulins, for certain tissue components.

Since the only element necessary for immunoperoxidase methods is antibody, all other serum components can be eliminated. This type of preparation, called an immunoglobulin or Ig fraction, is depicted in Figure 5. This solution contains mostly antibodies, both specific and naturally occurring, plus a very small amount of residual serum protein. The removal of the majority of proteins will reduce the chances of nonspecific reactions in various techniques.

It is possible, as shown in Figure 6, to prepare a solution containing only antibodies directed against a specific antigen. This is called antigen specific antibody. It is not commonly available, and has greater specificity than is necessary for most procedures.

A fourth type of preparation, and one which is readily available, is conjugated antibody (Figure 7). Conjugation is the process of chemically linking some type of marker onto an antibody molecule. This can be a fluorescent label such as

fluorescein and rhodamine, or an enzyme such as alkaline phosphatase or horseradish peroxidase. A wide variety of conjugates are available for use in various direct and indirect immunohistological stains.

Unfortunately, in the chemical process of conjugation, small amounts of antibody and label can be destroyed. This can decrease the sensitivity and specificity of these reagents. An alternate to artificially combining a marker to an antibody is an immune complex - the combination of an antigen and its specific antibody utilizing the natural affinity they have for one another. These complexes are specially prepared to remain soluble and not to form precipitates in solution. An example of this is the peroxidase antiperoxidase (PAP) complex which consists of the enzyme peroxidase (the antigen) and an antibody specific for peroxidase (Figure 8). The use of these naturally formed immune complexes instead of chemical conjugates, makes PAP staining procedures as much as 1,000 times more sensitive than immunofluorescence.

MONOCLONAL ANTIBODIES

A single antigen molecule contains several characteristic antigenic determinants or epitopes. When an antigen is injected into an animal as described previously, the B-lymphocytes will make antibodies against the antigen. One B-cell can form antibodies against only one antigenic epitope. Since there are many B-cells producing antibodies against each epitope, this is called a polyclonal (many cells) antibody.

In some techniques it is desirable to have an antibody specific for a single epitope. Since this is produced by a single B-cell line (called a clone) it is termed a monoclonal (single cell) antibody.

The selection of the B-cell clone producing the desired antibody must be performed outside the animal. However, these cells cannot grow and divide once removed from their host, and will die in approximately one week. In order to preserve the antibody production capabilities of the clone, the B-cell is fused with a myeloma cell (a cancerous B-cell) that can live almost indefinitely outside the host animal. The hybrid myeloma cell (hybridoma) that is formed by this fusion can be grown in cell culture as can the myeloma cell, and will produce the antibody that was being made by the B-cell.

To obtain a consistent supply of monoclonal antibody, the hybridoma formed must be stable. First, the B-cell and the myeloma cell must come from the same animal species. The myeloma cell must be suitable for hybridizing and be easily propagated in culture. A large number of B-lymphocytes must be available for fusion. The animal that most readily fulfills these criteria is the mouse.

The first step in producing a monoclonal antibody is identical to that of making a polyclonal antibody (Figure 9). A mouse is injected with a purified antigen, and will begin making antibody against it. When large amounts of antibody are being produced, the mouse is sacrificed and the spleen, containing large quantities of B-lymphocytes, is removed. A cell suspension

is made and is mixed with the myeloma cells in a medium that will cause the cells to fuse. Unfused and improperly fused cells will die, while the desired hybridomas will live and grow in culture. The hybridomas are tested to determine which clone is producing antibody against the desired epitope. This is the most difficult and time-consuming part of the procedure.

Once the appropriate cell line is identified, it can be injected back into a mouse where it will produce a tumor. The ascitic fluid from the tumor will contain high concentrations of the antibody, as well as other mouse immunoglobulins and proteins which can cause increased background staining in immunoperoxidase techniques.

Another method of producing monoclonal antibodies is to grow the hybridoma in tissue culture. the supernatant fluid will contain the antibody produced by the hybridoma. Culture supernatants contain lower concentrations of antibody than ascitic fluid, but nonspecific background staining due to undesired proteins is eliminated.

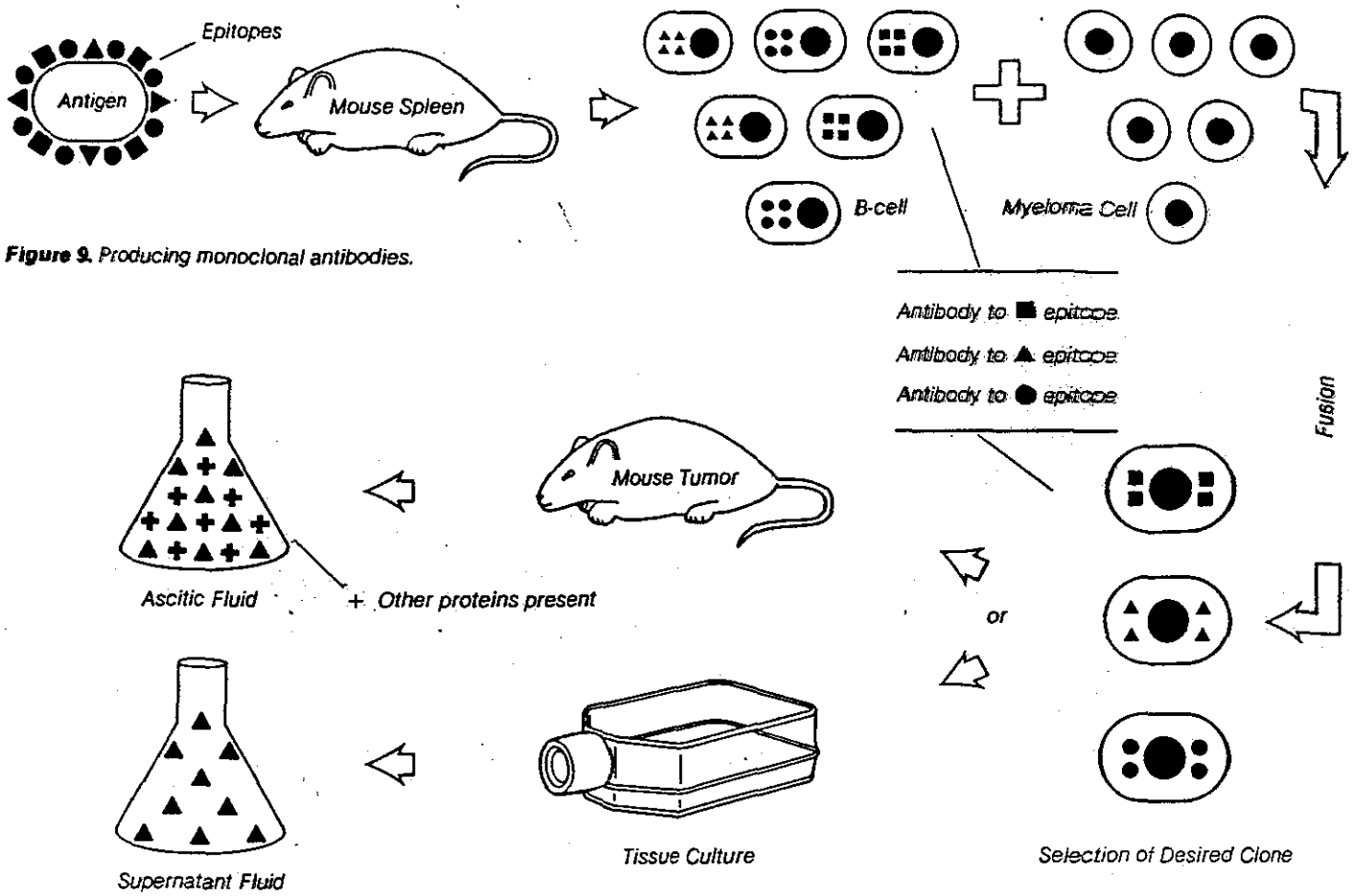
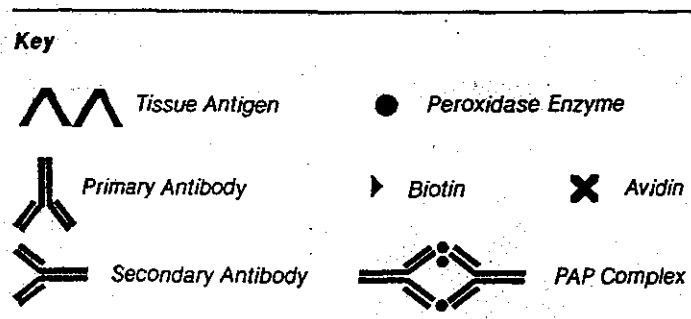


Figure 9. *Producing monoclonal antibodies.*



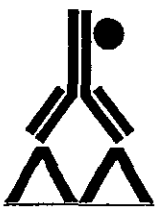


Figure 10. *Direct Method.*

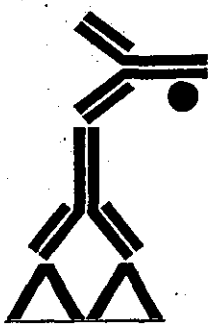


Figure 11. *Indirect Method.*

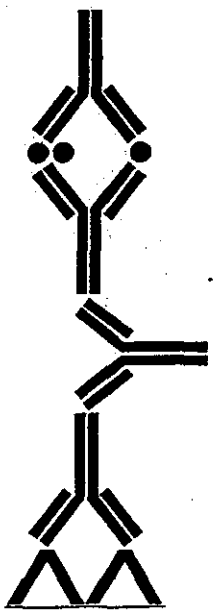


Figure 12. *PAP Method.*

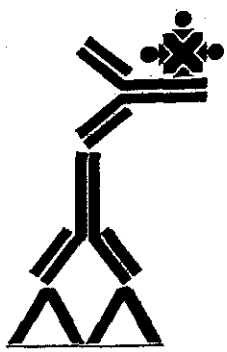


Figure 13. *Avidin-Biotin Method.*

STAINING METHODS

There are four main methods of immunoperoxidase staining that can be used to localize cellular antigens. The direct, indirect, PAP, and avidin-biotin methods each have certain advantages and disadvantages which must be evaluated prior to selection of the most effective procedure for the work to be performed.

Immunoperoxidase procedures allow visualization of cell components in a variety of specimens including paraffin sections, cryostat sections, smears, imprints and cytospins. For a specific antigen it can be determined what type of cells produce this substance in normal and neoplastic tissue, levels of the substance produced, the identification of cells of unknown origin, and the determination of tumor cell differentiation.

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PEROXIDASE

Immunoperoxidase staining involves the use of antibodies and the enzyme peroxidase. Peroxidase is commonly used for several reasons.

- Its small size will not hinder the binding of antibodies to adjacent sites.
- It is easily obtainable in highly purified form so that the chance of contamination is minimized.
- It is very stable, and therefore will remain unchanged during manufacture, storage and application.
- Only small amounts are present in tissue specimens, and this endogenous peroxidase activity is easily quenched.
- There is a wide availability of chromagens which can be acted upon by peroxidase to form a colored end product that will precipitate at the site of the antigen to be localized
- It is inexpensive.

DIRECT METHOD

The simplest way to localize a certain antigen is by using an antibody directed specifically against it. In the direct immunoperoxidase method this specific antibody is chemically linked to peroxidase. The conjugated reagent is applied to the specimen and will react with the antigen (Figure 10). A substrate is then applied which will produce a colored end product precipitating at the site, and thus mark the localized antigen.

Direct technique can be performed very quickly, with low probability of nonspecific reactions. The main drawback is that for every antigen to be localized, a different conjugated antibody is needed. If the antibody cannot be obtained in conjugated form, then the user must either perform the conjugation himself, or choose another procedure.

The most common application of the direct immunoperoxidase method is for the detection of immunoglobulin, complement and immune complex deposits in kidney biopsies from patients with various types of renal disease. These same antigens can also be localized in skin biopsies from cases of systemic lupus erythematosus (SLE) and other connective tissue disorders. The most common antigens identified in these cases are IgG, IgA, IgM, C3 and C4.

INDIRECT METHOD

In the indirect application, an unconjugated antibody will bind to the antigen in the specimen. To localize this attachment, a peroxidase conjugated antibody is needed to bind to the first antibody. For example, if the primary antibody was made in a rabbit, then the conjugated secondary antibody must be specific for rabbit antibody. a substrate is added to localize the reaction (Figure 11).

This method is more versatile than the direct method because a variety of primary antibodies made in the same animal species can be used with one conjugated secondary antibody. Therefore, this procedure can be used to advantage with any primary antibody when a peroxidase conjugated second antibody is available. However, the procedure takes approximately twice as long to complete as the direct method, and there is greater chance of nonspecific reactions occurring.

The primary use for the indirect immunoperoxidase technique is to identify antibodies in the serum of patients with various autoimmune, bacterial and parasitic diseases. In this procedure, the patient's serum is applied to the antigen containing specimen in place of the primary antibody. The peroxidase conjugated secondary antibody is specific for human immunoglobulin. If the patient has an antibody that can react with the antigen in the specimen, the peroxidase conjugated anti-human immunoglobulin will bind to the patient antibody, and show positive staining for the antigen. If the patient's serum contains no antibody to that antigen, the secondary antibody cannot bind, and no staining of the antigen will be seen. Some of the more common patient antibodies identified are against nuclear, thyroidal, mitochondrial, and smooth muscle antigens; treponema pallidum; herpes simplex virus; and cytomegalovirus.

PAP METHOD

This method utilizes three reagents: Primary and secondary antibodies, and PAP Complex - comprised of the enzyme peroxidase and an antibody against peroxidase. The primary antibody is specific for the antigen. The secondary or "link" antibody is capable of binding to both the primary and to the PAP Complex, because both are produced in the same animal species.

Functionally, the link antibody is added in excess so that only one of its Fab sites will bind to the primary, leaving the other Fab site free to bind to the antibody in the PAP Complex. The peroxidase enzyme is visualized via a substrate-chromagen reaction (Figure 12).

Absence of conjugated antibodies in this method means greater sensitivity than that attributed to the direct and indirect techniques. This is especially evident in formalin fixed, paraffin embedded tissue where strong staining can be observed even though much of the antigen has been destroyed by fixation and processing. Due to this loss of antigen during fixation, the direct and indirect techniques must be performed on frozen sections to achieve consistent results. The greater flexibility of the PAP method in specimen processing seems to compensate for the increased time required by this method.

One of the most important applications of the PAP method is in determining the origin of tumors by identifying specific antigens the cells produce. This allows for more accurate classification - especially of poorly differentiated and metastatic tumors - than can be achieved on the basis of morphology alone. The fact that the PAP method is applicable to routinely fixed, paraffin embedded material circumvents the need for frozen tissue and also permits retrospective studies.

Some of the tumor markers commonly used are prostate specific antigen (PSA) to identify tumors of prostatic origin; immunoglobulins such as kappa and lambda light chains, IgG, IgA and IgM to distinguish B-cell lymphomas from undifferentiated carcinomas; glial fibrillary acidic protein (GFA) which stains

all tumors of glial origin, both primary and metastatic; and human chorionic gonadotrophin (HCG) which can be localized in normal trophoblastic cells and the trophoblastic element of germ cell tumors of the ovary, testis and extragonadal sites.

ENDOGENOUS PEROXIDASE ACTIVITY

The substrate-chromagen reaction used to visualize peroxidase cannot distinguish between the enzyme immunologically localizing the cellular antigen, and similar enzymatic activity present in the specimen before staining. The endogenous peroxidase activity is confined mostly to red and white blood cells. If it is not removed before adding the marking enzyme, positive staining will be observed that is due not to the specific antigen alone, but also due to peroxidase activity already present in the specimen.

There are several ways to irreversibly inhibit endogenous peroxidase, and one of these techniques should be performed at the beginning of the staining procedure.

Most common method of blocking endogenous peroxidase is the use of 0.3% H_2O_2 in absolute methanol. This method insures that little or none of the antigen sites are lost.

ENZYME DIGESTION

Overfixation of tissue specimens in formalin causes formation of an excess of aldehyde linkages which will mask the tissue antigen and prevent its localization by the primary antibody. This is a particular problem when staining lymph nodes for immunoglobulin. To unmask the antigen sites, the aldehyde bonds can be digested with proteolytic enzymes.

The most common enzyme used is trypsin. It is the least destructive to the tissue, and its reaction can be easily controlled. Trypsin, like many other enzymes, requires certain temperature and pH values for optimal activity. The incubations are carried out at 37°C using prewarmed buffer on prewarmed slides. Temperatures below 37°C will reduce the activity of the enzyme.

Masking Antigenic sites

- fixation so well preserves the tissue integrity that the large molecules used in these techniques are prevented from penetrating the tissue to the antigenic sites.
- fixation works by producing cross linkages with proteins thereby "masking" the antigenic sites.
- use of trypsin @ 37°C proteolytic (enzyme) breaks these cross linkages and releases antigenic sites.
- it is the least destruction to the tissue.

NONSPECIFIC BACKGROUND STAINING

Positive staining of a specimen that is not a result of antigen-antibody binding is termed nonspecific background staining. The most common cause is attachment of protein to highly charged collagen and connective tissue elements of the specimen. Antibodies are proteins. If the first protein solution applied to the tissue is the primary antibody, it can be nonspecifically absorbed to these charged sites. The secondary antibody can still bind to the primary and the peroxidase color reaction will occur. Positive staining of these sites is due not to localization of the tissue antigen, but to nonspecific antibody attachment to collagen and connective tissue (Figure 15).

The most effective way to prevent this nonspecific staining is to add an innocuous protein solution to the specimen before applying the primary antibody. This protein will fill the charged sites, leaving no room for absorption of the primary antibody. The most common source of the protein solution is nonimmune serum from the same animal species that produced the secondary antibody. This avoids positive staining due to binding of the secondary antibody to components in the protein solution.

Poor fixation - fix properly overnight.

Drying of section - only deal with a few slides at a time when adding antisera, when sections are allowed to dry, false positive staining occurs.

Improper dewaxing of sections - allow to heat in 56° incubator overnight and dewax with 4 changes of xylene - 10 minutes each.

P.A.P.

Day I

Prepare Slides:

- Cut sections @ 3 microns on clear glass slide.
- Place in 58-60 degree C. incubator overnight

Day II

- Place 200 ml 1/10 PBS in a 37 degree C. incubator.
- Weigh out 0.2 gm trypsin.
- Make up albuminized 1/10 PBS:

  - 1 ml bovine albumin per 100 ml 1/10 PBS (This solution is used to prepare all antibody dilutions and for washing slides).

Procedure

(1) Transfer slides directly from 58-60 degree C. incubator to:

1. xylene ..... 10 min.
2. xylene ..... 10 min.
3. xylene ..... 10 min.
4. xylene ..... 10 min.
5. 95% alcohol ..... 5 min.
6. Cold Tap H_2O ..... 5-10 min.
7. 37 degree Tap H_2O ..... 3-5 min.

(2) Add 0.2 gm trypsin to 200 ml 1/10 PBS in 37 degree C. incubator.

(3) Transfer slides to trypsin solution for 6 minutes at 37 degrees C. (Discard after use).

(4) Rinse in cold tap H_2O for 5 min.

Inhibition of Endogenous Peroxidase

(5) Transfer slides to freshly prepared 200 ml of methanol + 2 ml 30% H_2O_2 for 30 minutes

(6) Rinse in cold tap water for 5 minutes

(7) 95% alcohol ..... 5 min.

(8) Abs. alcohol ..... 5 min.

(9) Chloroform ..... 5 min.

(10) Acetone ..... 5 min.

(11) Cold Tap H_2O ..... 5 min.

(12) Albuminized PBS ..... 5 min. minimum

Primary Antibody

(13) Prepare dilutions of primary antibody using albuminized PBS 140 - 200 ul per slide.

(14) Prepare slides one by one by drying outside and leaving a film of the albuminized PBS around the section (NO AIR BUBBLES). (DO NOT LET THE SECTIONS DRY!).

(15) Cover section with diluted primary antibody (one slide at a time) for 30 minutes in a humidification chamber.

(16) Wash each slide carefully with 1/10 PBS (albuminized) using a squeeze bottle.

(17) Transfer to a staining dish of the albuminized PBS.

Secondary Antibody

(18) Prepare dilution as follows:
for 20 slides
PBS 1/10 - 2700 ul
Normal Human - 300 ul
Serum

Shake well and discard 200 ul of this solution.
Add 200 ul peroxidase conjugated rabbit immunoglobulines to mouse immunoglobulins. Mix well.

(19) Prepare slides as for primary antibodies and add secondary antibodies for 30 minutes.

(20) Wash with albuminized PBS and return to staining dish of PBS.

Teritary Antibody

(21) Prepare dilutions as for secondary antibody using peroxidase conjugated swine immunoglobulins to rabbit immunoglobulins.

(22) Prepare slides as before and add antibody for 30 minutes.

(23) Wash well with PBS and return to staining dish of PBS.

Revelation of Peroxidase Staining:

(24) Prepare DAB:

Dissolve 6 mg of DAB (diaminobenzidine) for 6 slides in 10 ml Tris-HCL (0.05M). Add 3 ul of 30% H_2O_2 just before use. Keep in dark. (If larger quantities are needed, use several tubes ready for H_2O_2.

10 ml 10 ml 10 ml

(25) Arrange slides in humidification chamber. Put DAB on each slide for 3 to 5 minutes. (Do not attempt to reveal too many slides at one time - maximum 15 slides).

(26) Rinse with old tap H_2O to stop reaction.

(27) Return to a staining jar of water.

Counterstain

(28) Haematoxylin for 10 to 20 seconds

(29) Rinse in cold tap H_2O.

(30) 95% alcohol

(31) Absolute alcohol

(32) Acetone

(33) Xylene

Mount with Permount

24

INTERPRETATION OF RESULTS

EMA

- is of value in confirming the epithelial origin of an anaplastic tumor.
- should be used in conjunction with LCA
- distinction can be drawn between carcinomas (+EMA -LCA) and lymphomas (-EMA +LCA).
- plasma cells stain +VE
- also of value for detecting metastatic CA cells in sections of lymphoid tissue.

LCA

- also of value in confirming origin of anaplastic tumors
- plasma cells - VE
- lymphoid cells +
- + staining with LCA indicates the tumor is of white cell origin and likely to be a lymphoma.

UCHL -1

- the great majority of T-cell lymphomas is labelled by UCHL-1.
- + VE thymocytes (subpopulation of T-cells) and mature T-cells.
- useful for subclassing lymphomas.
- normal B-cells are negative.

L-26

- B-cell lymphomas are labelled by L-26.
- useful for subclassing lymphomas.
- reacts with B-cells.

Monoclonal & Polyclonal antibodies.

a) Paraffin section immunohistochemistry

MONOCLONAL (3-step immunoperoxidase)

Intermediate & Microfilaments

anti-cytokeratin	- KL1 (55 kD) -Immunotech
	- MNF116 - private source (Chittal)
anti-vimentin	- Dako
anti-desmin	- Dako
anti-neurofilament	- Dako
anti-GFAP	- Dako
HMB 45	- Dako (melanoma cells)

Other

anti-CEA	- private source (Ford)
anti-EMA	- Dako

Hormones

anti-thyroglobulin	- Monosan
anti-calcitonin	- Dako

Kit - Dako
Insulin
Glucagon
Somatostatin

Lymphocytes

LCA (CD45)	- leukocyte common - Dako
UCHL1 (CD45R0)	- T-cell - "
MT1 (CD43)	- T-cell monocytes, macrophages, B-lymphoblast
L26 (=CD20)	- B-cell
LN1 (CDw75)	- B-cell (GCC) - Clonlab
	- rbc, epithelium
DNA7 LN1-like	- - pr.source
DBB42	- B-cell differentiation - pr.source
DBA44	- mantle zone B-cells monocytoid B-cells, hairy cells - pr.source
LN2 = MB3 (CD74)	- HLA-DR (cyto)
DND53 (CD74 like)	- " - pr. source

MB2 - B-cell (cyto) - Clonlab
endo/epi weak

Lymphocyte activation

BerH2 (CD30) - RS cells, ALC, - Dako
embryonal ca

anti-EMA - see above

Other

Leu M1 (CD15) - x-hapten
granulocytes, RS cells,
epithelium - Becton-Dickinson

Leu-7 - NK cells,
myelin basic protein - "

Factor VIII - endothelial cells

BNH9 - BG-related
endothelial cells - private source

anti IgM - Dako
anti-kappa - "
anti-lambda - "

POLYCLONAL (PAP) all from Dako

anti-IgGh
anti-IgMh
anti-IgAh
anti-kappa
anti-lambda

anti-fibrinogen
anti-lysozyme
anti-alpha-1-anti trypsin
anti-alpha-1-fetoprotein
anti-B2 microglobulin
anti-myoglobin

anti-TSH
anti-ACTH
anti-prolactin
anti-HCG
anti-GH

S-100
anti-NSE
anti-chromogranin A
anti-GFAP

anti-cytokeratin

anti-prostate specific antigen
anti-prostatic acid phosphatase