This Week's Citation Classic®

Hsu S-M, Raine L & Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* **29**:577-80. 1981. [Dept. Pathol.. Rhode Island Hosp.. and Div. Biol. and Med. Sci.. Brown Univ.. Providence. RI]

This paper describes the first practical immunohistochemical or histochemical detection technique taking advantage of a unique high affinity between avidin and biotin. [The *SCI*® indicates that this paper has been cited in more than 5.680 publications, making it the most-cited paper published in this journal.]

Avidin-Biotin Detection Methods

Su-Ming Hsu McClellan

Veterans Hospital and
University of Arkansas for Medical Science
Little Rock, AR 72212

As a young medical student in Taiwan, I was always fascinated by the pathologist's ability to make critical diagnoses based on the cytologic and histologic appearance of tumors. Whenever there was a problem diagnosis, the young pathologists would always seek consultations from their more experienced colleagues. No one would ever challenge a diagnosis from these experts. Later, I learned that this was also quite true in Japan, Germany, and even the US.

In 1977, I had an opportunity to enter the residency training program at Rhode Island Hospital and soon learned that no one is immune from making a mistake, it was at this time that I began to be troubled by the inability to apply the principles of immunology, biochemistry, and cell biology to traditional morphologic pathology. I have since dedicated my professional life to finding ways of bridging the gap between basic science and morphologic pathology.

Back in 1942, A.H. Coons[1] developed a technique of using fluorescein-labeled antibodies to detect immunoglobulin synthesis in plasma ceils. This technique was quickly adapted to show Ig or complement deposition in renal disease or autoimmune diseases. Later, enzymes were used in place of the fluorescent substance. Several enzymes and staining methods were proposed. Among them, the peroxidase-anti-peroxidase (PAP) method of L.A. Sternberger[2] gained popularity because of its simplicity and sensitivity and because it could be applied to formalin-fixed paraffin embedded tissue sections.

The PAP method is a powerful technique, but it is not without limitations. For example, the PAP method is not practical for lectin or monoclonal antibody (MAb) staining or for in situ hybridization with cONA or oligonucleotide probes. My experience in doing biochemical research under the supervision of Jen-Kun Lin prompted me to search for an alternative method for immunostaining. The extraordinary affinity between avidin and biotin was established in theory. From this theory, the idea of using a biotin-labeled antibody and an avidin-biotin-peroxidase complex for detection was developed. During my third-year residency training I submitted several papers describing various immunohistochemical or histochemical methods which made use of the avidin-biotin interaction.

The technique soon became popular because of its easy adaptation to immunodetection with MAbs. We now know that a biotin label can also be applied to nucleic acid and that avidin can be conjugated with several types of enzymes or fluorescent substances. Hence, during the last five years, the avidin-biotin techniques have been used not only in immunocytochemistry, but also in in situ hybridization for mRNA, cellular or viral DNAs in tissue sections, Northern or Southern hybridization, and Western blotting. The recent introduction of the chemoluminescent detection reaction has made the avidin-biotin technique even more powerful, eliminating the need for radioactive isotopes.

New scientific discoveries depend on previous knowledge and experience. Several investigators have had significant influence on the design of the avidin-biotin-peroxidase complex method.[3] My greatest satisfaction from this technique is not that it made me famous. Rather, from the bottom of my heart, I know that, somewhere in the US, China, India, or South America, the lives of thousands of patients are improved every day because this method has been employed. Pathologists no longer depend on the naked eye to make a diagnosis. The avidin-biotin method with different antibodies is the first to come to the rescue in case of a problem diagnosis. We all have a much better understanding of the disease process because of the application of this technique to many areas of research in cell and molecular biology. The basic sciences are no longer an in vitro phenomenon; they have found their way to the bedside of patients and, as a result, the quality of human life has been improved.

1. **Coons A H. Creech H J. Jones R N & Berliner** E. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J. Immunol.* 45:159-70. 1942. (Cited 510 times.)
2. **Sternberger L A. Hardy P H, Jr., Cuculis J J & Meyer H G.** The unlabeled antibody enzyme method of immunohistochemislry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-anti-horseradish peroxidase) and its use in the identification of spirochetes. *J. Histochem. Cytochem.* 18:315-33. 1970. (Cited 4.865 times.) [See also: Sternberger L A. Citation Classic. (Barrett J T. ed.) *Contemporary classics in the life sciences. Volume I: cell biology.* Philadelphia: ISI Press. 1986. p. 109.1
3. **Bayer E A & Wilcheck M.** The use of the avidin-biotin complex as a tool in molecular biology. *Methods Biochem. Anal.* 26:1-15. 1980. (Cited 260 times.)

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