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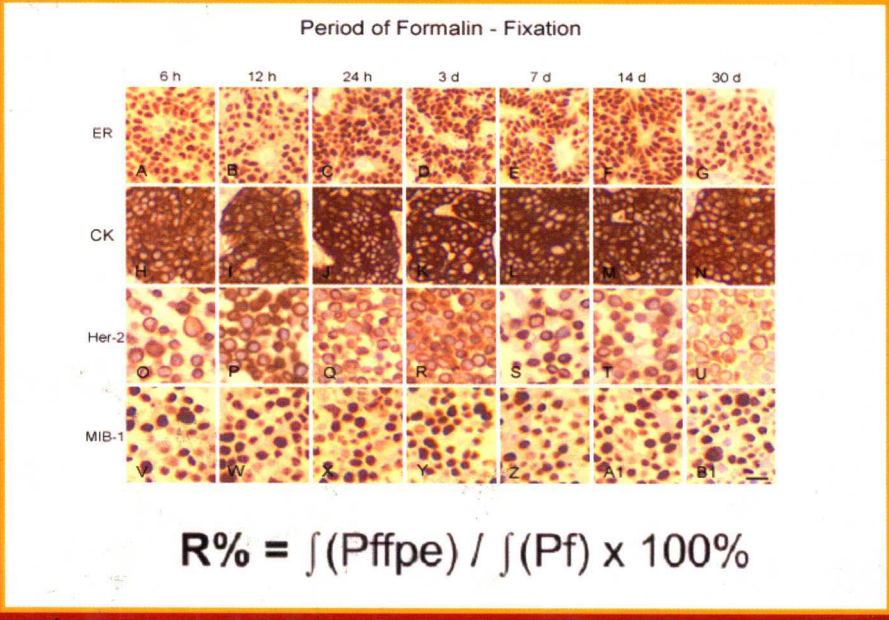
Antigen Retrieval Immunohistochemistry Based Research and Diagnostics



Edited by
SHAN-RONG SHI • CLIVE R. TAYLOR

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ANTIGEN RETRIEVAL IMMUNOHISTOCHEMISTRY BASED RESEARCH AND DIAGNOSTICS

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ANTIGEN RETRIEVAL IMMUNOHISTOCHEMISTRY BASED RESEARCH AND DIAGNOSTICS

WILEY SERIES IN BIOMEDICAL ENGINEERING AND MULTI-DISCIPLINARY INTEGRATED SYSTEMS

Kai Chang, Series Editor

Advances in Optical Imaging for Clinical Medicine *Nicusor Iftimia, William R. Brugge, and Daniel X. Hammer*

Antigen Retrieval Immunohistochemistry Based Research and Diagnostics *Shan-Rong Shi and Clive R. Taylor*

PREFACE

The purpose of this collection of contributions by experts in the field is to set forth current knowledge with respect to antigen retrieval (AR) and immunohistochemistry (IHC). In so doing, we hope to contribute to the ongoing evolution of these methods, and the development of greater reliability and reproducibility of IHC. Effective standardization of AR and IHC would lend improved capabilities to IHC when employed in a "special stain" capacity. In addition, effective standardization would allow the development of IHC methods into tissue-based immunoassays, having true quantitative capabilities, analogous to the ELISA method. In order to attain this latter capability, quantifiable reference standards are required to calibrate the IHC method and assessment of proper tissue preparation. This book deals with all of these complex issues in a manner designed both to inform and to stimulate further research, particularly with respect to how AR methods might be employed for improved test performance.

The two of us (Shan-Rong Shi and Clive Taylor) have worked towards these goals, together for two decades, coming to the problem from different directions, but walking down a common path.

I (Shi) have been asked many times the same question: "What made you think of boiling a slide in a microwave oven before doing immunostaining?" There is no short answer for this question. I would like to share my story of AR to honor those people who touched my life and helped me meet my career goals.

My interest in IHC began in 1981 when I went to Massachusetts Eye and Ear Infirmary (MEEI) and Massachusetts General Hospital in Boston as a research fellow under the guidance of Drs. Harold F. Schuknecht, Max L. Goodman, and Atul K. Bhan. One of my projects was focused on IHC staining using archival formalin-fixed paraffin-embedded (FFPE) tissue sections of nasopharyngeal carcinoma obtained from China. I was deeply impressed by the sharp staining contrast between the cancer cells and the background inflammatory cells highlighted by a series of cytokeratin markers. Without IHC, not a single malignant cell would be identified. Because of the great diagnostic potential of IHC demonstrated by this project, I decided to exploit the application of this technique on thousands of valuable samples of human temporal bone collected by Professor Schuknecht, a world-renowned Otologist

at MEEI. Although I tried many different IHC protocols with enzyme digestion for these archival formalin-fixed celloidin-embedded temporal bone sections, only moderate positive results were achieved with one antibody tested. This experience made me realize that the key point for successful IHC on archival formalin-fixed tissue sections was to find a method for the recovery of formalin-masked antigenicity, in the search for an AR approach.

In 1987, I had a research opportunity for a newly developed monoclonal antibody at InTek Laboratories, Inc., in Burlingame, California. This antibody was effective only on frozen sections, and I was asked to try to adapt it to FFPE tissue. At that time, enzyme digestion was the only option of choice, and it was not successful. As a result, I lost my job. I moved to a small room close to San Jose State University (SJSU), and in order to make a living, I started to work at a Chinese supermarket. I was insulted regularly by the sales manager, but these poor working conditions in a way inspired a strong feeling that I have never had before. I spent days and nights searching the literature at the library of SJSU, in order to answer what had become an obsession: "was formalin-masked antigenicity reversible or irreversible?" At that time online searching was not available. I read numerous volumes of the "index" page by page, taking notes line by line. I then looked for the journals one by one. In this way I searched all related literature regarding formalin and proteins starting from the most recent year back to 1940s. Finally, I found key clues to the answer in a series of studies published by Fraenkel-Conrat in the 1940s.[1–3] Their studies indicated that cross-linkages between formalin and protein could be disrupted by heating above 100°C or by strong alkaline treatment. However, I did not think of using high-temperature heating of FFPE tissue sections because I believed so strongly that high temperature denatures the protein.

In 1989, after much trying, I obtained a job interview at BioGenex Laboratories, San Ramon. That was a sunny afternoon. I met Dr. Marc E. Key, Director of Research, in his office. As soon as I sat down, he asked me: "What can you do for BioGenex?" I answered: "I intend to develop a new method which enables IHC to be performed on archival FFPE tissues." He was interested in my answer, and told me: "Many people have tried to find such a way but they all failed. If you could succeed, you would become world-famous." I was hired. Today, when I look back, I appreciated Marc and Dr. Krishan L. Kalra, President of BioGenex, for giving me the opportunity that made it possible for my dream to come true.

Shortly thereafter, Marc gave me an abstract[4], and suggested that I drop zinc sulfate solution on FFPE tissue sections prior to IHC staining for enhancing IHC staining results. After multiple attempts following the reported protocol, I did not observe any improvement. At this most frustrating moment, a microwave oven sitting at the table near my desk caught my attention and reminded me of those long forgotten studies performed by Fraenkel-Conrat. Even though I still doubted their conclusions and worried that high temperature might destroy all the antigens on the tissue sections, I decided to give it

a try. I covered the FFPE sections with a few drops of zinc solution and heated them in the microwave oven for a few minutes. Unfortunately this attempt was not successful, because the solution evaporated. I decided to immerse the slides in a Coplin jar containing zinc solution and heated them twice in the oven for five minutes, in order to avoid drying the artifact during the boiling process. To my great surprise, I observed a significantly improved IHC staining signal with a clean background. I could not believe my eyes! I repeated the same experiment several times with similar results. This was "antigen retrieval (AR)."

The President of BioGenex, Dr. Kalra, invited three distinguished experts of IHC, Drs. Clive R. Taylor, Ronald A. DeLellis, and Hector Battifora to evaluate AR. They repeated this heat-induced AR protocol at their labs, and were all impressed by the great effects of this simple method. The first landmark article of AR was quickly accepted by Dr. Paul Anderson, Editor of the Journal of Histochemistry and Cytochemistry and published in 1991.[5]

At that time I started to work with Dr. Clive R. Taylor, Professor and Chairman of Pathology at the University of Southern California, Keck School of Medicine. Clive is a world renowned pioneer in archival IHC used for pathology since the early 1970s. With his kind help and support, I have been conducting a series of research projects on basic principles, further development, standardization and mechanisms of the AR technique. This work has yielded more than 40 peer reviewed articles and a book. Our AR research has been funded by NIH grant since 2001.

In 2000, we published *Antigen Retrieval Techniques: Immunohistochemistry and Molecular Morphology* attempting to summarize major achievements in this interesting field with a wish to stimulate further development of AR-IHC.[6] Since then, the AR technique has been accepted not only by pathologists who routinely apply AR-IHC for daily pathologic diagnosis in surgical pathology, but also by all scientists who work with cell/tissue morphology worldwide. Because of the expanded application of AR-IHC, the philosophy embedded in this simple technique has created several approaches for further study. For this second AR-IHC book, we categorize the recent literature concerning the AR technique into five sections: recent advances of AR techniques and their application, standardization of IHC, tissue/cell sample preparation, molecular mechanism of the AR technique, and proteomic analysis of proteins extracted from tissue/cells. Our goal is to summarize current key issues in these five fields, to stimulate future studies. It is our intention to initiate research projects addressing several critical issues such as standardization and quantifiable IHC, a desired topic for targeted cancer treatment as emphasized by the American Society of Clinical Oncology/College of American Pathologists Guideline for human epidermal growth factor receptor 2 testing in breast cancer documented in 2007.

Our plan for editing this book was enhanced by the Histochemical Society Annual Meeting held at the Experimental Biology 2007 Meeting in Washington, DC. Several interesting workshops with respect to tissue fixation for molecular

analysis in pathology and cell biology, as well as tissue banking and sample preparation, were presented by world-renowned experts from Europe, the United States and Japan. We greatly appreciated all valuable presentations at these workshops that have been driving us in editing this book.

I (Taylor) find Shan-Rong Shi's story to be interesting in many ways, not least because during its course the conventional scientific dogma of the day, was overturned, by experimental evidence. When Shan-Rong first spoke to me, in his early days at BioGenex, of the notion of boiling deparaffinized sections in buffer, I assured him that, based on what I know of proteins (which turned out to be remarkably little) the method was unlikely to work. After all if one heats complement to just 56 degrees, it is inactivated. But lurking in the back of my mind there was just enough of my own experience, to temper that initial judgment. Almost two decades earlier, when I had first tried to "stain" immunoglobulins in formalin fixed paraffin embedded tissues, I too had been assured by those senior to me that it would not work. Examination of the literature also supported the view that it was doomed to failure, but with just a few glimmers of hope. Cold alcohol processing of paraffin embedded tissues (Sainte-Marie) did allow demonstration of some antigens by immunofluorescence.

I was then working on my D. Phil thesis in Oxford, under the mentorship of Alistair Robb-Smith, murine models of lymphoma and Hodgkin's disease. And I had problems. Already after just a year in the pathology department I was disconcerted to find that histopathology was not the definitive discipline that I had imagined, that it was subjective and that senior experienced pathologists could disagree vehemently with the diagnosis of a single slide. Recognition of the individual cells contributing to the development of "reticulum cells sarcomas'" in my murine models was even more of a challenge, with differing criteria offered by almost every expert whom I consulted, or every paper that I read. I resolved to try immunologic identification of cells using the specificity of antibodies. Like Shan-Rong, I was inspired by the literature of the 1940s, Albert Coons, and Astrid Fagraeus, and the genesis of the immunofluorescence method. A long story, cut short, by switching from fluorescein to peroxidase labeled antibodies we circumvented the problem of "background" fluorescence in FFPE sections, greatly simplifying the task. With Ian Burns, we obtained our first positive results. The late Dr. David Mason joined me in Oxford shortly thereafter. With his healthy disbelief of most of what was written, we did, what I encouraged Dr. Shi to do 20 years later, we did the experiments, and they worked. This was "immunoperoxidase."[7]

In an exhilarating 2-year period we multiplied the world literature in the field, and then watched it grow exponentially. With the distant collaboration of Ludwig Sternberger we improved the "sensitivity." All that was left then, was to try multitudes of new anti-sera (polyclonal antibodies) and the new monoclonal antibodies that began to pour from labs worldwide. Some of these gave results on FFPE tissue sections, most did not, or at least gave poor or inconsistent results after prolonged tissue manipulations. Thus the world of

IHC was ripe for Dr. Shi's equally unconventional idea, and the time was ripe to perform the experiment. The outcome we all now know. Many antigens can be "retrieved." I have come to think of AR as "unfixation," and by the use of AR, IHC has become more straightforward and more widespread.

The very success of AR has, however, added to the problems of performing IHC in a reliable and reproducible manner. Less care is taken, than once it was, with fixation, processing, antibody selection and titration, because with AR the stain "works." In addition, many different labs perform IHC, treating it much like an H&E stain, without fully controlling the method, all because AR allows that to happen. Then the AR protocol itself has inevitably changed as others have sought to improve upon Shan-Rong's original formula. The result has been a proliferation of different AR methods, that allow the staining of many antigens, in diverse ways that certainly are not standard, and are difficult to reproduce exactly. While AR unarguably has improved the overall qualitative results of IHC, it has in some ways hindered the development of more quantitative methods that are necessary for "measuring" prognostic or predictive markers. For example ER or HER2 results can be converted from negative to positive, from weak to strong and back again, by different AR protocols. Thus for any particular analyte, where the goal is measurement, AR also must be standardized. This book presents the views of many experts with broad and diverse experience in AR and IHC, about how to consolidate the gains that have been made, and how to extend them for diagnosis and research.

Antigen Retrieval Immunohistochemistry Based Research & Diagnostics is intended for clinical pathologists, molecular cell biologists, basic research scientists, technicians, and graduate students who undertake tissue/cell morphologic and molecular analysis and wish to use and extend the power of immunohistochemistry. It is our hope that the readers will find it informative and useful.

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We greatly appreciate those people who have contributed to or are working on the development of the AR technique. We express our sincere appreciation to all contributors for writing excellent chapters for this book. Our appreciation also goes to Dr. Richard J. Cote, for his support and collaboration of research, and to Chen Liu, Lillian Young, Leslie K. Garcia, Carmela Villajin, and William M. Win for their technical assistance. The editors wish to express our deep gratitude for the active support of George J. Telecki, Lucy Hitz, Kellsee Chu, Stephanie Sakson, and the production and sales teams at John Wiley & Sons, and Best-Set Premedia. We also appreciate Lindsey Gendall and Wayne Yuhasz of Artech House, Inc. We are grateful for permission to reproduce illustrations and data of published materials from all publishers appearing in every chapter of this book.

I (Shi) greatly appreciate valuable clinical and research training in Sichuan Medical College (currently Huaxi Medical School of Sichuan University, Chengdu, China), and I also would like to thank those who have helped me during the most difficult time in my life, especially Drs. Iwao Ohtani, Masahiro Fujuta, Andrew C. Wong, Jimmy J. Lin, as well as Susan Price, and Victor Jang. It would have been impossible for me to develop this technique without their kindness.

Shan-Rong Shi, MD

Clive R. Taylor, MD, PhD

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