

Tabloid Immunohistochemistry

Submitted by Luis Chiriboga

Immunohistochemistry (IHC) in its various forms is employed in clinical and research laboratories around the world. A search for “immunohistochemistry” in the biomedical literature (pubmed) yields close to 600,000 hits, with results dating back to 1962[†]. Despite its widespread use, there is a general neglect when it comes to writing IHC material and methods for publication (Baskin and Hewitt 2014). This pervasive indifference undermines the weight and compromises the validity of all the work that gave rise to over half-a-million manuscripts.

Those of us who routinely practice research IHC are well aware of the difficulty when tasked with optimizing and validating antibodies for IHC. The samples come from a wide variety of species, in the form of cells or tissues that may or may not have been prepared following standardized preanalytical methods. The samples are often complex, genetically engineered with unknown or unstable target protein expression levels. There may be limited amount of sample or in the case of clinical studies; specimens may come from different institutions that follow different preanalytical methodologies. The antibodies are often new and unproven, targeting low or transiently expressed molecules. They can range from highly purified to “first bleeds”. The antibody may be available only in minuscule amounts, without data from complementary techniques (western blot, flow cytometry and others) leaving little room for a wide variety of optimization tests. More often than not, a researcher cites an article from the biomedical literature or worse, an antibody manufacturer website, equating this information with an optimized, validated method or protocol. All too often the obvious implication is that IHC is a “*straightforward*” technique that is easily reproducible based on a few simple references. However, this is often not the case, here’s an example of why:

An investigator wants to perform IHC for Microglial Cystatin-F expression in frozen murine spinal cord and provides a reference to an article that claims to have an antibody and optimized protocol (Ma, Tanaka et al. 2011). A quick scan of the materials and methods section in the article reveals only a list of antibodies, their species and the relative dilutions. No protocol (retrieval, blocking, incubation times and temperature or diluent for primary or secondary antibodies, detection system type and times, positive or negative control tissue, western blot confirmation or cell line testing) information is provided. However, the author does refer, using the all too common phrase “*IHC was performed as previously described*”, to a method in an older article. This article is obtained and the material and methods section scanned (Ma, Matsumoto et al. 2006). The author again uses the same language, citing an even older article. This article is pulled (Kagawa, Ikenaka et al. 1994) and evaluation of the materials and methods section repeated. Again, the authors refer to a previously described method and cite an older article (Shiota, Ikenaka et al. 1991). Finally, in this article, the method is finally revealed: “*these primary antibodies were detected by the avidin-biotin peroxidase method*”. There is no mention of the antibody of interest and no protocol information is provided, not to mention that these references span over 20 years of biomedical research.



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Deadlines for Submission are:

November 15th – Winter

May 15th - Summer

Membership in the New York State Histotechnological Society, includes a subscription to *On Stage*. The annual membership fee is \$25.00. Please direct membership inquiries to:

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Editorial Staff

Editor-in-chief: Diana Scott

Editorial Board:

Kate Caleri, Roswell Park Cancer Institute

Advertising coordinator:

TBD

Page Layout:

Linda Chen, NYP

Inside this issue:

Tabloid Immunohistochemistry	<u>1</u>
President’s Summer	<u>3</u>
Tabloid Immunohistochemistry Continued	4-5
From your Editor	<u>6</u>
Coby Corner– News from SUNY Cobleskill	<u>7</u>
2015 NYSHS Election Results	8
Winners!	<u>9</u>
Save the date for Region I	<u>10</u>
Thank You	<u>11</u>
NYSHS Membership	<u>12</u>



Summer 2015



The NYSHS annual spring meeting “Capital Histology: What’s in YOUR Lab?” in Latham NY, was a huge success. Thank you to all attendees, speakers and vendors for making for a great meeting! I have to thank all of the board members, from planning of the meeting to executing. Tremendous job everyone!

There were 6 lectures that covered a wide variety of topics. All of the speakers gave fantastic presentations! Dr. Patricia Belair, MD from St. Peter’s Hospital spoke about interesting cases regarding upper and lower gastrointestinal biopsies. Dr. John Varrone, PhD from the NYS Department of Health Wadsworth Center spoke on the application of histology to allergens and infections disease research. Dr. Joseph Seguel, MD from Saint Peter’s Health Partners gave a lecture on Endobronchial Ultrasound. Dr. Mark Rees, PhD from StatLab Medical Products gave a very informative lecture on molecular testing in Pathology. Michelle Fuller, Diana Scott, Mary Georger and I answered questions asked by attendees during our Tech Talk. Dr. Luis Chiriboga spoke on IHC and the importance of standards being put into place.

NYSHS was happy to award 5 members with educational scholarships this year!

Jeannette Tillapaugh received the Gulf Coast Instrument Company, Inc. Scholarship. Anna Draus was awarded the Mercedes Medical Scholarship.

The Source Medical Products Scholarship was given to Leigh Oropallo. The recipient of the StatLab Medical products award was Andrew Ward. Yuri Albuquerque received the Sakura Finetek Scholarship. Congratulations! A big thank you to vendor sponsors for providing these scholarships allowing recipients to defray educational expenses.

During the general membership meeting I was very fortunate to honor Michelle Fuller with the president’s award. Michelle is dedicated to the society and its membership. Without her guidance and support my first term as president would have been overwhelming. Thank you Michelle!

Region I will be having their annual Regional I Symposium July 25th and 26th in Manchester, New Hampshire. For more information please contact Diane Weishaar at dweishaar@nmcinc.org. This year, the National Society for Histotechnology (NSH) will hold their annual Symposium/Convention August 28th through September 2nd in Washington DC.

I have sent out a Call for Constituent Society Delegates for the NSH House of Delegates. Based on the New York State NSH members, we can fill 4 seats in the 2015 NSH HOD. If you are attending NSH this year and are interested in sitting on the NSH HOD, please contact me at mack.sarah@gmail.com, asap.

The NYSHS BOD is in the process of planning next year's meeting. Check out our website, <http://www.nyhisto.com/> and our Facebook page for updates.

Happy Summer!

Sarah Mack

mack.sarah@gmail.com

. Considering this example, it is easy to see how ones' view of the IHC technique can be skewed and over simplified. The biomedical literature is full of examples such as this. More importantly, this is more than just an inconvenience. Unfortunately, this behavior perpetuates the myth that IHC is not a reliable, reproducible scientific technique. This pervasive attitude is more than just troubling. It is a trend that is receiving considerable attention from the federal government and the private sector (Wadman 2013, Akst 2014, Collins and Tabak 2014, Perkel 2014). Poor planning and execution of experiments leads to irreproducible results that can cost investigators and institutions not only hundreds of thousands of dollars but also cause substantial harm to their reputations as well as delay new and innovative research (Bissell 2013, Wadman 2013).

This lack of attention in describing IHC methods is a fundamental problem that directly contributes to the less than favorable view of immunohistochemistry and its usefulness as a research tool. In all actuality, IHC is no different than any other analytical technique. It has an operational range, with limits of sensitivity and specificity that are based on real boundaries. One cannot operate outside these parameters and expect valid results (Baskin and Hewitt 2014). As with other assays, the limits of the technique derive from all the components within the assay and therefore detailed knowledge and understanding of each step is crucial for demonstrating validity and reproducibility (Taylor 1994, Taylor 2000, O'Leary 2001, Goldstein, Hewitt et al. 2007). The often heard phrase: "leave the DAB on a bit longer" because "it wasn't dark enough" should ring alarm bells. In essence, these types of convenient solutions are part of the problem because they introduce a considerable bias to interpretation and experimental results. One cannot tailor the staining of a particular sample in order to meet ones needs. This would be viewed by many as equal to digitally enhancing a band on a western blot. This is not to imply that this behavior is scientific misconduct. Quite the opposite, it is because investigators are unaware or inexperienced, not to mention a host of other factors (Collins and Tabak 2014). Simply, if the positive and negative controls are valid and demonstrate equivalency with the optimization and validation of the marker, then the results passes muster as is, regardless of how the signal of an individual sample may have turned out (assuming all other appropriate quality control measures support the outcome).

Standardization in the clinical laboratory has been underway for well over a decade (Taylor 1994, Burry 2000, Taylor 2000, O'Leary 2001, Moskaluk 2002, Nagle and Fitzgibbons 2002). The same has not been true in research laboratories but this is now quickly changing (Baskin and Hewitt 2014, O'Hurley, Sjostedt et al. 2014). As mentioned above, a considerable amount of attention is now being paid to the reproducibility of not only IHC but of other techniques. In fact, there are major efforts underway to validate "prior art" (Wadman 2013, Collins and Tabak 2014). While this line of attack is controversial (because of funding) other groups are taking a different approach. Force11 (www.force11.org) is a group of scholars, librarians, archivists, publishers that got together to change the way scientific information is exchanged. With close to 1000 members, 20 working groups they have been endorsed by leading institutions and companies. One of the results that came out of a 2013 meeting at the NIH was a pilot project with a direct impact on the IHC community. The *Resource Identification Initiative*, is a database of resources (antibodies, model organisms, and software) with unique *Research Resource Identifiers* (RRID's) designed to expedite reproducibility (Helsby, Fenn et al. 2013, Vasilevsky, Brush et al. 2013)

Continued on Page 5



(Helsby, Fenn et al. 2013, Vasilevsky, Brush et al. 2013). More important, this effort signifies a major paradigm shift in the way scientist view the biomedical literature. Proper resource identification will result in better reporting of materials and methods that will encourage reproducible science. To this end, the working group has created the *Antibody Registry* a freely accessible and searchable database that assigns unique and persistent identifiers to antibodies (among other resources). The registry includes commercial antibodies from about 200 vendors and numerous academic labs. Finally, there is a way to universally identify and track antibodies so that they can be referenced within publications helping researchers cite the key resources used to produce the scientific findings reported in the biomedical literature

‡ Based on a keyword search string of “Immunohistochemistry” only, did not include other common names and variations of the technique name.

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From your Editor:

NYSHS Newsletter- 4/18/15 Moves to a *bi-annual* publication

Instructions to Authors/Advertisers

Authors:

The deadlines for the submission of manuscripts:

November 15th for the December 1, Winter Issue

May 15th for the June 1, Summer Issue

Manuscript files should be in Microsoft Word compatible format. We can accommodate other software, but Word will give the best results. Please follow these specifications when submitting the manuscript file:

Page and Content Layout:

- Double-spaced, 2.5 cm (1 inch) margins center-justification 12 point font
- Restrict fonts to Times New Roman, Times, or Arial throughout the manuscript.
- Do not attempt to produce a typeset look or use any special formatting. This could interfere with the layout process.
- Do not use the 'Insert Symbol' function in Word (or in other word processors). For Greek symbols such as mu and alpha, please use the Symbol Font, which is available as a standard font with most word processors.

Reference work published or in press only, and give the name of the journal.

Figures and Tables:

- Please do not embed figures into the manuscript. They must be provided as separate files for each figure with figure legend.
 - Please supply images in *jpeg* format with a resolution of at least 300 dpi.
- Each table must be appended to the end of the manuscript in either Word or Excel table format and numbered consecutively

If you have any question please do not hesitate to contact the editor-in-chief

Advertisers:

The advertisement coordinator will solicit advertisements from vendors 3 months prior to publication. Ads must be submitted 2 months prior to the newsletter release date in PDF format (see below). Once the coordinator receives an approved advertisement the coordinator will send the vendor an invoice due by the 15th of the month prior to publication. *Payment must be received by the 15th of the month before publication.* If payment is not received by the due date the advertisement will not be placed. If payment is received late the advertisement will run in the next issue of the newsletter (newsletters are published quarterly). Currently, NYSHS accepts the following forms of payment: Check, Money Order, and Cash. Once the treasurer receives payment for the ad the treasurer will notify the ad coordinator to release the ad for publication.

On stage will accept advertisements in final layout format as Adobe Acrobat (pdf) files. Please be aware that the pdf file (image) may need to be enlarged or reduced to fit with in the page layout guides. This may distort the image and lower the resolution. Please remove excess empty margins (crop) around text or images in the final pdf file (especially for business card ads) as this will reduce the quality of the image.

Please email all advertisements in final PDF format with advertisement order form to the advertising coordinator at: nyshsadcordinator@yahoo.com

Download ad order form here: [NYSHS ad_order-2012](#)



SUNY Cobleskill Summer Clinical Rotations

Classes are over, graduation is imminent and the students will be starting their summer clinicals. There will be 12 students completing their clinical rotations this summer and, since most of them live locally, there was pressure to find Affiliate sites in the Capital District. Thanks to the cooperation of our Affiliates we were able to accommodate all of the students with some hospitals accepting two students. In this situation, one student (session I) will have a rotation from mid-May until the end of July and the second student (session II) will start in early August and finish in early October. A listing of the Affiliate hospital sites and the student(s) is shown below:

Albany Medical Center: Ashley Sperbeck

Albany Memorial Hospital: Stephanie McDarby (session I); Jacob Martratt (session II)

Ellis Medicine: Amanda Oyer (session II)

Bassett Medical Center: Adnan Kadric (session I); Corinne Bowers (session II)

Columbia Memorial Hospital: Andrew Ward

Cornell University: Ashlie Louma

St. Peter's Hospital: Leigh Oropallo

SUNY Upstate Medical Center: Jill Failing

NYU Langone Hospital: Briana Zeck

University of Rochester Medical Center: Aaron Malin

SUNY Cobleskill Graduates Receive ASCP Certification

The following students successfully passed the ASCP HT National Certification exam during 2014:

Carissa Conway

Michael Faulkner

Katherine Foote

Jennifer McDowell

Neil Reynolds

Kyle Roney

Danielle Shaver

Charles Tribbley

Congratulations one and all!

Once again the graduates have performed at a high level of excellence and have allowed the Histotechnician program at SUNY Cobleskill to achieve a 100% pass rate on the ASCP national exam.



NYSHS ELECTION 2015
Kate Caleri

The ballots for the 2015 NYSHS elections have been received and tallied. Amy Farnan will serve as the Vice President and Diana Scott in the position of treasurer. Elected to the Board of Directors are Angela Fogg and Carrie Lindberg. Congratulations to all the winners and thanks to everyone who took the time to vote. This is your society and your opinion can make a difference!

It's not too early to consider a run for office for next year's election. The available positions will be President, Corresponding Secretary, Membership Secretary and 3 seats on the Board of Directors. Nominees must be a member of the Society for one year prior to the election, and willing to serve the society for a term of 2 years, beginning the first day of July following the elections.

The member elected to serve as President will act as the executive office of the Society by presiding over all Society meetings, and serve on all committees except for the Nominating Committee. These committees include but are not limited to the Awards Committee and the Education/Scientific Committee.

The Corresponding Secretary is responsible for taking the minutes at all meetings of the Society as well as Board of Directors meetings. This position also entails the maintenance of Society records, and Society correspondence including meeting notices.

As Membership Secretary, one is responsible for collecting member dues, keeping current the list of members, and issuing membership cards to those entitled.

The Board of Directors as a whole is responsible for the formation of the policies of the Society. The seats on the Board of Directors are held by the officers (President, Vice President, Secretaries and Treasurer), as well as 5 additional elected members and the immediate past-President. There shall be at least two meetings of the board of Directors called by the president each calendar year for the purpose of transacting business of the Society, including the planning of the Annual Symposium.

If holding an office or membership on the Board is not for you, you may want to consider serving on one of the many NYSHS committees. Volunteering for a committee can give you insight into the operations of the NYSHS, and familiarity enough to perhaps entice you to run for an office in the future. Also, as a committee constituent you may have the opportunity to receive reduced rates to attend meetings.

For more information on the Society, the Board of Directors and offices, or to volunteer for a committee please visit

www.nyhisto.com



2015 NYSHS Scholarships & Awards

We had a great meeting this year!

The awards committee comprised of Sara Laviska, Sarah Mack, Luis Chiriboga and I. We would like to say thank you to all who applied for one of our Educational Awards given by our sponsors.

We had eight applications submitted for the five awards. All of our applicants submitted very well thought out reasons for why they should get an award. Their reference letters all spoke highly of every applicant. We had five awards available and these are this year's award winners. Each was awarded a \$250 award to be used for educational purposes.

GULF COAT INSTRUMENT CO.

JEANNETTE TILLAPAUGH

Jeannette was a SUNY Cobleskill student.

MERCEDES MEDICAL

ANNA DRAUS

Anna works at St. Peter's Health Partners in Albany, NY

SOURCE MEDICAL

LEIGH OROPALLO

Leigh was a SUNY Cobleskill student.

STATLAB MEDICAL PRODUCTS

ANDREW WARD

Andrew was a SUNY Cobleskill Student.

SAKURA-FINETEK

YURI ALBUQUERQUE

Yuri is a Brazilian foreign exchange student and was at NYU Langone Medical Center in NYC.

We congratulate all our award winners and look forward to seeing them in the future.

Respectfully submitted,

Angela M. Fogg, Awards Chair NYSHS



Where: Radisson, Manchester, NH
Wine tasting on Saturday evening

Everyone is welcome! Registration and more details coming soon!

Presenter spots are still available!

If you would like to present contact Diane Weishaar at dweishaar@nmcinc.org

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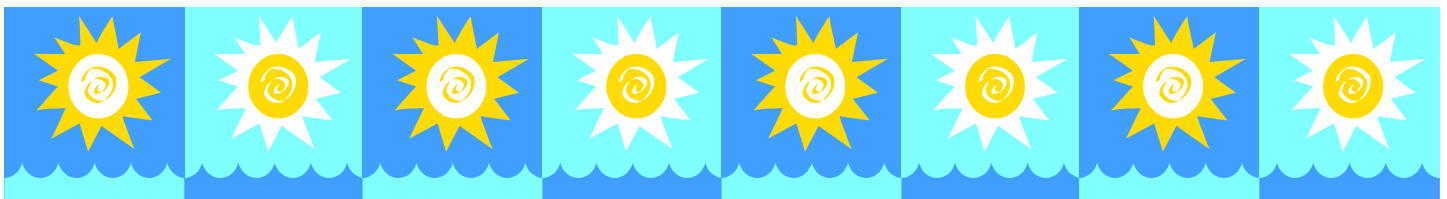


The Board members and our members of the NYSHS would like to thank all of the vendors that supported our annual meeting once again. Your continual support is very much appreciated. Through your support it helps our organization to provide valuable knowledge to all of our members through our annual conference and without your support our conferences would not be possible.

Again, thank you to:

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Sincerest gratitude and we look forward to working with all of you next year.



Have a wonderful summer everyone!!

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Registration: (check one) Membership year runs from July 1 to June 30

Education Annual Membership Fee (tax deductible): \$25.00

Student Full Time Student Fee: \$7.00

Membership will expire June 30th,

Please send applications & check payable to NYSHS to:

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NYSHS Membership Secretary
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