Sheron Lear on Leica BOND III™ and Leica BOND RTUs: "Phenomenal"

After 50 years in histology, science veteran Sheron Lear has "seen a half century of technical change" in the preparation and examination of stains. From the early days of manual preparation to today, Sheron shares her experiences on how laboratory efficiency has improved, especially since adopting Leica Microsystems' BOND III and BOND ready-to-use antibodies (RTUs).

Sheron Lear is the Manager of Immunohistochemistry (IHC) at the CPA Laboratory in Louisville, Kentucky, USA, which provides comprehensive anatomic and cytopathology services to physicians and clinics throughout Central Kentucky and Southern Indiana. In response to increased needs for specialization, CPA maintains a professional staff with expertise in cytopathology, dermatopathology, hematopathology and gastrointestinal, urolological, gynecological, molecular and pediatric pathology.

Before joining CPA, Sheron was Manager of the IHC laboratory for the University of Louisville. When Sheron commenced her IHC work in the 1970s, all staining was done manually using the peroxidase-antiperoxidase (PAP) method. Later she used the avidin biotin and Streptavidin Biotin techniques, and now uses polymer technology. While at the University of Louisville, Sheron moved to semi-automation as soon as it became available, however she continued to experiment with epitope retrieval methods. At that time, keeping tissue samples on the slides was a challenge. "I experimented heavily with steamers, autoclaves, microwaves, pressure cookers in a microwave oven, I just can't tell you all the ways we experimented," says Sheron, "I even tried many ways to double stain and triple stain slides with various results."

According to Sheron, the introduction of both Leica BOND and the Leica BOND RTUs to the world of IHC staining has resulted in a "phenomenal" increase in quality. Slide turnaround times have significantly improved too, with Sheron now able to "beat the basket" and have slides ready for pick-up every time. "Beat the basket" is what Sheron refers to as reducing the time for getting the IHC slides cut, stained, examined for quality, wrapped, recorded and placed in the box ready for the courier service to pick up and take to the client.

When the fully automated Leica BOND max™ system became available, Sheron discovered it offered online epitope retrieval. Always an early adopter, Sheron recalls thinking, "I have to try this. I have to see if it works."

In late 2005, Sheron obtained two Leica BOND max stainers to introduce full IHC automation to the University of Louisville. Sheron quickly put Leica BOND max to work on some of her hardest cases. "We had some really tough and very bloody and necrotic cases that we ran on BOND. We stained a number

of these cases with citrate and EDTA. We achieved fantastic staining and all of the tissues stayed on instead of blistering off. The stains were beautiful, too." Sheron also decided to develop protocols for Cytology cases on Leica BOND as the Covertile™ technology meant gentle treatment for the more delicate tissues and cell preparations.

By the end of 2008, Sheron was using three Leica BOND max instruments to keep up with the heavy workload, while saving her and the techs a lot of manual work: "Every day I thanked the inventors of the beautiful BOND because it meant better quality and less labor than manual staining. That's why all my techs loved it and I do too."

When Sheron became Manager of IHC at CPA Laboratory they increased the number of Leica BOND max instruments to six. However many of the primary antibodies validated for Leica BOND were not Leica Novocastra™ antibodies. The laboratory also continued to use an older, semi-automated system. That was until early 2009, when CPA Laboratory was invited to trial the new Leica BOND III system. As with Leica BOND max, Leica BOND III offers full automation of IHC and *In Situ* Hybridization (ISH) plus additional innovative features that decrease the turnaround time for single and double IHC and ISH stains.

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After a short trial of Leica BOND III, CPA Laboratory were so impressed with the slide quality, the turnaround time, and the waste reduction, that they purchased four additional Leica BOND III instruments when they were released in October 2009. Within months, the lab had converted the majority of the 200 primary antibodies onto Leica BOND III and took this opportunity to begin switching to Bond RTUs for better QA assurance and increased efficiency. Converting to Leica BOND III was smooth since so much had already been validated. With a workload of between 4000 to 5000 IHC stains per month and approximately 200 primary antibodies in the CPA menu, the introduction of Leica BOND III has hugely increased the efficiency of the laboratory.

"To me the beauty of the BOND is the perfect consistency," explains Sheron. "The instrument will dewax, hydrate, retrieve the antigens with an enzyme, citrate or EDTA buffer, stain with any marker selected and complete the system with such consistency the finished slide looks like beautiful art work

under the microscope. The automation also allows the techs to complete other projects and reduces injury from repetitive motion that manual staining causes."

As CPA Laboratory offers comprehensive anatomic and cytopathology services, it manages a wide range of tissues that arrive with varying methods of specimen preparation. Specimens for IHC and ISH are either processed on site or may come in paraffin blocks or as unstained slides. CPA now uses Leica BOND RTUs for approximately 70% of their primary antibodies. In use almost daily are antibodies like MelanA, S-100, HMB45, MITF, Factor XIIIa, CD3, CD5, CD15, CD20, CD19, CD10, CD30, CD61, CD38, CD138, CD163, Bcl-2, Bcl-6, *Helicobactor pylori*, Cytokeratin 7, Cytokeratin 20, TTF-1, p16, ER and PR. All patient slides have a positive control on the slide, and in one day there may be between 50 and 100 controls used for lymphoma cases alone.

Sheron says the high number of RTUs available on Leica BOND III has greatly improved CPA's efficiency. And with no need to dilute the antibodies, preparation is also faster, even with the very diverse specimen preparation including different methods for fixation and tissue processing.

Today, using Leica BOND III and Leica BOND RTUs, Sheron is able to cut, stain, QC and release an IHC slide to a pathologist in under three and a half hours – faster than the courier's pick-up/drop-off cycle – so the basket is well and truly beaten.