

Immunohistochemistry in Forensic Pathology

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In this month's Focus, we share ProPath's experience at delving into forensic pathology, with an immunohistochemistry (IHC) slant (of course).

On August 25-26, 2000, I gave a series of lectures on IHC in Palmerston North, New Zealand, at the annual meeting of the the Royal College of Pathologists of Australasia. Three days later, 38 year-old Christine Lundy and her 7-year-old daughter Amber were found brutally murdered (by multiple blows to the face and head with a hatchet) in their Palmerston North home. As you can imagine, the gruesome murder scene had blood and brain tissue splattered throughout. Mark Lundy, the husband and father of the victims (who came to be the prime suspect), claimed to be out-of-town on business at the time of the murders.

On the morning the murder was discovered, Mark Lundy was contacted by a friend who noted police vehicles outside of his home. On his way back into Palmerston North, Lundy was intercepted by police three blocks from the murder scene, and gave police permission to examine his car and retain any of its contents for evidence. One of the items found was a gym bag containing a blue polo shirt. The shirt was placed into an evidence bag, sealed, and securely stored. The officers who collected the shirt had not been to the scene of the murder.

58 days after the murder, the blue polo shirt was examined by a forensic scientist with the use of ultraviolet light, and two tiny faint stains were found. The stains tested positive for blood, and subsequent DNA analysis documented the presence of Christine Lundy's DNA. The forensic scientist then moistened a glass slide and touched it to one of the stains, and this slide was stained with H&E and brought to pathologists in

Palmerston North for examination. They felt that the slide might contain some glial or neuronal cells, but were unsure, and suggested to the officer in charge of the investigation, Detective Sgt. Ross Grantham, that IHC for GFAP would be needed to confirm brain.

Detective Grantham contacted multiple forensic laboratories in New Zealand, Great Britain, and the United States, but was told by all that the chance of success was near zero, and that they could not assist. A New Zealand pathologist who attended my IHC lectures suggested to Detective Grantham that he contact ProPath, which he did in mid-January 2001. Despite my inability to guarantee him success, he decided to bring the evidence from New Zealand to ProPath to see if we could prove that there was brain tissue on Mark Lundy's shirt.

The week before Detective Grantham came to ProPath, I was rinsing off a fresh chicken to be used for dinner, and noticed spinal cord tissue protruding from the severed neck of the chicken. I thought this presented an excellent opportunity to see whether we could use IHC to detect tissues smeared on shirts, so I smeared chicken spinal cord, kidney, and liver on portions of an old shirt. The following day, I placed small pieces of shirt containing the smeared tissue into plastic tissue processing cassettes, and placed them in the tissue processor. The shirt fragments were then cut into 3 mm longitudinal strips and embedded on edge in paraffin blocks. Subsequent H&E sections taken from this block showed identifiable tissue, which was readily classified using appropriate IHC stains. Little did the chicken know that she would be contributing greatly to putting a guilty man behind bars.

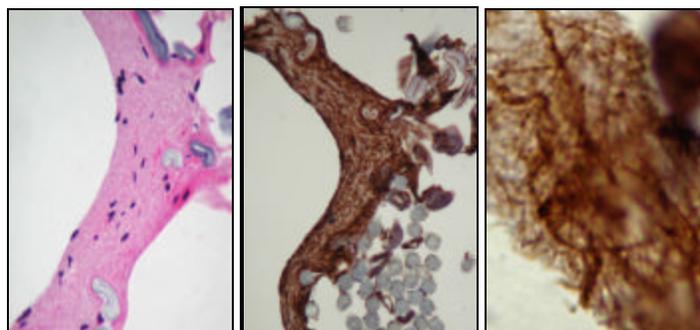
Detective Grantham arrived in Dallas on Saturday

afternoon February 4, 2001, along with the evidence. He and I went into ProPath on Sunday, photographed and prepared the evidence, and placed the portions of shirt containing the stains (as well as portions of shirt away from the stain and portions of a different shirt) into tissue processing cassettes, placed them in the tissue processor, and subsequently embedded strips from the shirt on edge in paraffin blocks. Examination of H&E stained sections on Monday from the stained area of Mark Lundy's shirt showed unequivocal tissue in the areas of the stain, and a subsequent battery of immunostains showed that this tissue reacted positively with GFAP, S100 protein, neurofilament, and synaptophysin, but was negative for cytokeratin 5/6, cytokeratin LMW, and CD45 (LCA). Individual nerve fibers and astrocytic processes were apparent on the neurofilament and GFAP stains, respectively. This provided unequivocal evidence that Mark Lundy had brain tissue on his shirt, from an area that also contained Christine Lundy's DNA. This was the critical piece of evidence that allowed an arrest to be made.

Several days after Detective Grantham returned to New Zealand, Mark Lundy was arrested and charged with the murder of his wife and child. The lengthy trial started on February 4, 2002, and over 160 witnesses testified. I traveled to New Zealand and gave evidence during the latter part of the trial on March 8, 2002, and the jury subsequently found Mark Lundy guilty of double murder on March 20, 2002, after a seven-week trial. The jury deliberated less than seven hours before delivering a verdict, and the definitive identification of brain tissue on Mark Lundy's shirt using immunohistochemistry was the most important piece of evidence pointing toward Mark Lundy's guilt. He received a sentence of life in prison, which in New Zealand means 10 years, although due to the horrific nature of the crime, prosecutors and the judge were able to lengthen the sentence to 17. The verdict was appealed by the defense, based on the contention that the prosecution failed to prove the case. Upon appeal, the guilty verdict was upheld, and the appellate judge added 3 more years to the sentence, making the total sentence 20 years. I think we all know that if he committed this crime in Texas, his punishment would be a bit different.

To our knowledge, immunohistochemistry has not been widely used in analysis of forensic evidence, but

hopefully this case will highlight the utility of this technique in the forensic pathology community. To that end we reported our findings in the December 2002 issue of *The American Journal of Forensic Medicine and Pathology*.



LEFT: High power (400x) photo of H&E from the stained area of the shirt, showing cell nuclei and pink fibrillar tissue. The shirt fibers are visible as the rounded refractile structures. CENTER: Strongly positive GFAP immunostain. RIGHT: Individual nerve fibers are apparent on the neurofilament immunostain (1000X).



The Lundy Family

Dr. Miller and Detective Ross Grantham in the Lab at ProPath

REFERENCE: Miller RT, Grantham R, Lockett B et al: Immunohistochemical Analysis of Forensic Evidence from a Double Homicide. *Am J Forensic Med Pathol* 23(4): 321-325, 2002.

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