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# **Odour Profiling of Blood Training Aids for Blood-detection Dogs using Comprehensive Two-dimensional Gas Chromatography (GC×GC)**

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**(Bachelor of Forensic Science (Honours) in Applied Chemistry  
Bachelor of Forensic Biology in Biomedical Science)**

A thesis submitted for the degree of  
**DOCTOR OF PHILOSOPHY (SCIENCE)**

Centre for Forensic Science  
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April 2018

# Certificate of Authorship and Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

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This research is supported by an Australian Government Research Training Program Scholarship

# DEDICATION

**In loving memory of my Dad**

**Dennis Rust**

*“Time can bring you down, time can bend your knees  
Time can break your heart, have you begging please, begging please*

*Beyond the door there's peace I'm sure  
And I know there'll be no more tears in heaven”*

-Eric Clapton

# Acknowledgements

Firstly, thank you to my primary supervisor, Prof. Shari L. Forbes for giving me the opportunity to complete this degree and inviting me into the dynamic Forbes research group. I am grateful for your support, and for sharing your knowledge in such a niche area of research. I would not have been able to complete this project without your feedback and sound advice. The many exciting projects that you have involved me in have been once in a lifetime and I have had the most memorable research journey as your student.

To my co-supervisor Dr. Katie D. Nizio, thank you for all of your support, guidance and encouragement throughout my PhD journey. I would not be the scientist and researcher I am today without your critical feedback and expertise as a GC×GC superstar. Thank you for comforting me when our lemon failed to perform, for thinking outside the box when my data needed new light and stepping in when I was ready to dive through rabbit-holes, and of course for putting up with my time-challenged personality. I am glad that I can look up to you as both a supervisor, but also a good friend. You really are the “special” emoji.

To all the dog and handler teams who participated in this research, thank you for your continued support, enthusiasm and valuable contributions to my PhD project. It has been a pleasure to work alongside your teams, and I am grateful for the expertise provided and shared. I wish the dog unit all the best and I am excited to see many successful operations in the future.

Blood, sweat and tears went into this project so a big thank you to the blood donors who participated in this research. Your donation was invaluable to the success of this project and I am thankful for the time you individually gave up. Equally, I would like to thank the phlebotomists who generously donated their time to assist this research: Kylie, Toni, Kristine and especially Anna. Your flexibility in scheduling many appointments is greatly appreciated.

I would also like to acknowledge members of UTS who contributed expertise, resources and companionship throughout my PhD journey. To the technical staff, particularly Dr. Ronald Shimmon, Dr Regina V. Taudte, Dr. David Bishop, and Zofia Winiarski. To the students and academics of the Centre for Forensic Science (CFS), it has been great to be a part of a leading group of forensic scientists. To my fellow HDR students, thank you for being a constant source of enthusiasm, support and laughter on the most difficult days of my research. Specifically to the “O-O Family”, thank you for welcoming me into your diverse family and always making my time at UTS memorable.

I have been very fortunate to be a member of many great teams throughout my PhD degree, and as such I would like to thank the past and present students of the Forbes' research group. Thank you to all the volunteers who contributed their time and energy at the dog training trials (especially on those 40 °C days!) and for the fond memories created on our road-trips. I would like to acknowledge in particular: Dr. Katelynn Perrault, Laura McGrath, Dr. Rebecca Buis, Dr. Maiken Ueland, Kate Trebilcock, Amanda Troobnikoff, Vitor Cesar Taranto, Prue Armstrong, Nicole Cattarossi, Miroslava Ross, Barea Chilcote, and Darshil Patel.

For providing financial support and consumables for my PhD I would like to thank the following: the Australian Research Council (ARC), the University of Technology Sydney (UTS), the Australian Government Research Training Program Scholarship (RTP), LECO Australia, Agilent Technologies, Restek Corporation, Sigma-Aldrich, Morris McMahon, the Australian and New Zealand Forensic Science Society (ANZFSS), and the California Separation Science Society (CASSS).

Finally, my most heart-felt thanks go to my family for their unwavering support throughout this rollercoaster. To my mum who is my inspiration for pursuing a career in forensic science, thank you for putting up with my melt-downs (mostly towards technology) and my crazy schedules that kept me away from home. I am grateful for you lending an ear to bounce ideas off, and a shoulder to cry on when things went awry. To my dad who I dedicate this thesis to, whilst you weren't able to see my PhD through to its completion I hope that I have made you proud. I have many fond memories of sharing my research with you and reflecting back on your university days. I know that I inherited my analytical problem-solving skills and stubbornness from you, and I am thankful for all the knowledge and life lessons you have taught me. Apologies that this degree came from UTS and not UNSW!

And lastly, but not least, to my fiancé Andrin, no words can really sum up how grateful I am to have you in my life. Thank you for being the level-headed and patient partner-in-crime who kept me sane throughout this whole journey. I could not have gotten through the ups and downs without your guidance, emotional support and drive to keep me pushing along till the finish line. Knowing that after all of the chaos I could come home to you was the best gift of all. Thank you for being the steel to my tempo, I love you very much.

They say that success is not a destination but a journey, and as I come to the end of this adventure I hope that all the people I have met along the way, as mentioned above, know that they have had an integral and lasting effect on my life and future career in forensic science.

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# Abbreviations

(Listed Alphabetically)

|                      |  |
|----------------------|--|
| <sup>1</sup> D:      | First dimension                                  |
| 1D:                  | One-dimensional                                  |
| <sup>2</sup> D:      | Second dimension                                 |
| 2D:                  | Two-dimensional                                  |
| 3D:                  | Three-dimensional                                |
| BTEX:                | Benzene, toluene, ethylbenzene, xylene           |
| CAR:                 | Carboxen   |
| DNA:                 | Deoxyribonucleic acid                            |
| DVB:                 | Divinylbenzene                                   |
| EPA:                 | Environmental Protection Agency                  |
| F:                   | Fish ratio or Fisher ratio value                 |
| $F_{crit}$ :         | Critical value of F                              |
| GC:                  | Gas chromatography                               |
| GC×GC:               | Comprehensive two-dimensional gas chromatography |
| HCA:                 | Hierarchical cluster analysis                    |
| HRD:                 | Human remains detection                          |
| HS-SPME:             | Headspace solid phase microextraction            |
| ID:                  | Internal diameter                                |
| IS:                  | Internal standard                                |
| K <sub>2</sub> EDTA: | Dipotassium ethylenediaminetetraacetic acid      |
| LCV:                 | Leuco-crystal violet                             |
| $m/z$ :              | Mass-to-charge ratio                             |
| MRI:                 | Magnetic resonance imaging                       |
| MS:                  | Mass spectrometry                                |
| MW:                  | Molecular weight                                 |
| NADH:                | Nicotinamide adenine dinucleotide                |
| NIST:                | National Institute of Standards & Technology     |
| NSW:                 | New South Wales                                  |
| PA:                  | Polyacrylate                                     |
| PC-1:                | First principal component                        |

|              |                                  |
|--------------|----------------------------------|
| PC-2:        | Second principal component       |
| PC-3:        | Third principal component        |
| PCA:         | Principal component analysis     |
| PDMS:        | Polydimethylsiloxane             |
| PMI:         | Post-mortem interval             |
| ppm:         | Parts-per-million                |
| ppt:         | Parts-per-trillion               |
| <i>S/N</i> : | Signal-to-noise ratio            |
| SPME:        | Solid phase microextraction      |
| STU-100:     | Scent Transfer Unit – 100        |
| TD:          | Thermal desorption               |
| TIC:         | Total ion current                |
| TOFMS:       | Time-of-flight mass spectrometry |
| US:          | United States                    |
| UTS:         | University of Technology Sydney  |
| VOC:         | Volatile organic compound        |

# Publications

Rust L, Nizio KD, Forbes SL. The influence of ageing and surface type on the odour profile of blood-detection dog training aids. *Anal Bioanal Chem.* 2016;408(23):6349-60.

Rust L, Buis RC. The scent of a crime. *Australasian Science Magazine.* 2015.



# Conferences

Rust, L, Nizio, KD, Forbes, SL, Odour profiling of aged blood training aids for cadaver-detection and blood-detection dogs using comprehensive two-dimensional gas chromatography (GC×GC), 7<sup>th</sup> European Academy of Forensic Science (EAFS) Conference, Prague, Czech Republic, 10 September 2015

Rust, L, Nizio, KD, Forbes, SL, Investigating the inter-individual variation of blood odour detected by blood-detection dogs utilising GC×GC-TOFMS, 13<sup>th</sup> GC×GC Symposium, Riva del Garda, Italy, 30 May 2016

Rust, L, Nizio, KD, Forbes, SL, The odour of foul play – validating the detection limits of scent-detection dogs to blood evidence after crime scene clean-up, Australian and New Zealand Forensic Science Society 23<sup>rd</sup> International Symposium on the Forensic Sciences, Auckland, New Zealand, 20 September 2016

Rust, L, Nizio, KD, Forbes, SL, Investigating the inter-individual variation of blood odour detected by blood-detection dogs utilising GC×GC-TOFMS, Australian and New Zealand Forensic Science Society 23<sup>rd</sup> International Symposium on the Forensic Sciences, Auckland, New Zealand, 22 September 2016

# Abstract

The use of blood-detection and cadaver-detection dogs as investigative screening tools to search for blood evidence is a contemporary addition to many law enforcement agencies around the world. The training protocols for these canines remain unstandardised and the specifics of the blood training aids implemented can vary between agencies. While there is a large field of research investigating the odour profiles of human remains, there has been very little research in a forensic context on specific tissue types such as blood, or that has linked variables in the blood odour with the response rate of these scent-detection dogs in the field.

The aim of this thesis was to investigate the chemical odour profile of blood training aids utilised for the training of blood-detection and cadaver-detection dogs and compare this directly to responses in training, in order to assist in the development of more effective and standardised training protocols. As part of this thesis, headspace solid phase microextraction (HS-SPME) was coupled with comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS), a novel technique that is also only recently being introduced into this field, to analyse fresh and degraded blood samples, from single and multiple donors, as well as latent blood samples.

Three studies were performed to investigate the chemical odour of blood under various conditions, which were compared with the responses of blood-detection and cadaver-detection dogs in scent line-ups conducted with local law enforcement. The first study examined the effect of ageing and analysed the volatile organic compound (VOC) profile of blood as it transitioned from fresh to degraded, over a 24-month period. Blood on two surfaces (non-porous aluminium tin and porous cotton) were compared and it was observed that fresh blood (collected within 24 hours) produced the most distinct profile irrespective of surface, and that as the blood degraded the odour became generalised across the two surfaces. However, comparing functional classes elucidated a common pattern between blood ages and surfaces. Similarly, results from the dog trials demonstrated that the blood-detection and cadaver-detection were more adept at locating fresh blood, with their efficacy becoming reduced as the blood aged. Notably, the samples on the non-porous surface posed a greater challenge for the canines to locate which is an important consideration for training protocols.

The second study expanded on the effect of ageing by replicating the first study over a 6-month period using four different blood donors, to determine if inter-individual differences exist in the

odour of blood. While the overall VOC profile of blood remained consistent across donors, some unique VOCs were identified between individuals as a result of variations in lifestyle, diet, health and environmental exposure. It was confirmed that the effect of ageing has a greater influence on the odour profile of blood and it was concluded that training aids should incorporate both fresh and degraded blood, with a single donor being sufficient. The blood-detection and cadaver-detection dog trials demonstrated the capability of the dogs to locate blood aged up to 6 months old regardless of the donor, but as the blood degraded, this efficacy reduced and displayed minor skewing for different donors.

The third study evaluated the baseline detection limits of the blood-detection and cadaver-detection dogs to latent blood when compared with current analytical instrumentation and a common chemical presumptive test – i.e. luminol. The experiment replicated a scenario in which a suspect may attempt to wash away a victim's blood on clothing to test anecdotal evidence of the sensitivity of scent-detection dogs. It was confirmed that the blood-detection and cadaver-detection dogs are much more sensitive than current analytical instrumentation, and are complementary screening tools to luminol for the detection of latent blood.

The overall results of this thesis provide a greater understanding of the odour of blood as a training aid for blood-detection and cadaver-detection dogs, and will assist in providing scientific support for their deployment as investigative tools if their use becomes challenged in court.