

# CLINICAL ANALYSIS OF LIVER FUNCTION

---

Development of a Novel Method for the  
Detection of Portosystemic Shunts

**Todd James Matthews BHSc (Hons)**

December 2013  
Discipline of Surgery  
School of Medicine  
Faculty of Health Science



THE UNIVERSITY  
*of* ADELAIDE

An original thesis submitted in total fulfilment of the requirements of the degree of  
Doctor of Philosophy

---

## **Abstract**

A portosystemic shunt (PSS) is defined as a congenital or acquired abnormal blood vessel that redirects blood around the liver without being filtered through hepatic parenchyma. PSS are thought to contribute to the distribution of isolated secondary metastases beyond the liver in 1.7 - 7.2% of all colorectal cancer patients without cirrhosis of the liver. No standardised clinical test for PSS yet exists and subsequently, the majority of PSS cases are detected incidentally through radiological means. To better identify PSS, a simple standardised clinical test for its detection is needed. The aim of this thesis was to develop a cost effective, non-invasive technique that can detect and measure PSS in a healthy liver model.

## **Methods**

An artificial 8mm diameter PSS was created between the portal vein and the inferior vena in a pig model with a catheter inserted in the confluence of the hepatic veins for sample collection. A spectrum of compounds including indocyanine green (ICG), <sup>13</sup>C-methacetin, sorbitol and lignocaine, were injected into the portal system. To analyse the pharmacokinetic nature of the shunt and liver, Evans blue dye and <sup>14</sup>C-sucrose were also administered. ICG was measured via a LiMON® spectrometer attached to the pig's snout, while levels of the other indicators were measured by serial blood and breath sample collection over a 40 minute period. The process was repeated with the PSS clamped as the control.

## **Results**

Of the administered compounds, only ICG had the potential to clearly identify and quantify the shunt due to the rapid serial sampling via the LiMON<sup>®</sup>. Further simulations using ICG demonstrated that the shunted fraction can be calculated using the transit times, including mean residence time, lag time and pharmacokinetic modelling.

## **Conclusion**

Although this study has not yet provided a concise method for PSS detection available for immediate clinical use, it does provide a large foundation for further exploration into a quantitative technique. A future PSS test would allow an added risk assessment for secondary cancer, and consequently individual cancer therapy may be better targeted for individual patient care.

## Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed: \_\_\_\_\_

Date: \_\_\_\_\_

## **Preface**

This thesis is the first stage toward portosystemic shunt (PSS) detection. Chapter 1 explores the range of PSS diagnosed in patients without liver disease and the associated method that was used for diagnosis, while also underlying the values for a need of a standardised clinical test. Chapter 2 replicates PSS by describing a surgical method to mimic a large PSS within a swine model. With an artificial PSS developed, chapter 3 describes the different practical dynamic techniques that may be plausible for PSS detection, with some viable techniques to be explored further. Chapter 4 studies the techniques chosen from chapter 3 in the PSS swine model and determines which technique is best suited for PSS identification and quantification. Chapter 5 reviews how the best technique from chapter 4 can quantify the shunt and what possible limits the shunt itself has with this technique. Finally, chapter 6 summarises this technique with a future outlook as to what PSS detection implications would have a clinical setting. This chapter also outlines the limitations and complications with the previous methods and what steps were used to overcome these problems. References and additional material can be found in chapters 7 and 8.

## **Acknowledgements**

The author acknowledges the involvement of those who assisted with this study. Mr Mark Hamilton, Dr Nadia Blest and Dr Joe Dawson assisted in the surgical predication of a PSS. Dr Peng Li assisted in sample collection and analysis. Professor Simon Barry, Ms Betty Zacharakis and Ms Esther Burt assisted in breath sample analysis. Dr Timothy Kuchel and Mr Matthew Smith assisted in animal anaesthesia. Professor Guy Maddern supervised the entirety of this study. A special acknowledgment to Mr Markus Trochsler, who assisted in all aspects of this study.

## Table of Contents

Abstract.....	i
Thesis Declaration.....	iii
Preface .....	iii
Acknowledgements.....	iv
Table of Contents.....	vi
List of Tables, Figures and Equations.....	xv
List of Abbreviations .....	xxv
Publications, Presentations and Competitions.....	xxviii
CHAPTER 1 Introduction .....	1
1.1 Introduction .....	2
1.1.1 Classification .....	2
1.1.2 Portosystemic Shunt Variability Within The Classifications .....	9
1.1.3 Incidence .....	10
1.1.4 Associated Malformations .....	11
1.1.5 Symptoms and Complications.....	12
1.1.6 Pathogenesis .....	14

1.1.7 Detection and Assessment.....	16
1.1.8 Treatment .....	17
1.2 Gastrointestinal Cancers .....	18
1.2.1 Circulating Tumour Cells .....	20
1.2.2 Metastases Distribution .....	20
1.3 Summary .....	22
1.4 Objectives.....	23
1.5 Question.....	24
1.6 Methodology.....	24
1.6.1 Search Strategy .....	24
1.6.2 Search Results .....	26
1.6.3 Demographics .....	28
1.6.4 Frequency of Diagnostic Procedures .....	28
1.6.5 Symptomatology and Associated Conditions .....	29
1.6.6 Other Investigations:.....	29
1.7 Discussion.....	36
1.8 Conclusion .....	41
1.9 Significance .....	42



1.10 Aim .....	42
CHAPTER 2 Development of a Portosystemic Shunt in a Swine Model.....	43
2.1 Introduction .....	44
2.2 Materials & Methods .....	47
2.2.1 Surgical Procedure .....	49
2.2.2 Shunt Flow Direction.....	57
2.2.3 Shunted Blood.....	60
2.3 Results .....	62
2.3.1 Anastomosis Material .....	62
2.3.2 Pressure Gradient .....	66
2.4 Discussion.....	69
2.5 Conclusion .....	71
CHAPTER 3 Practical Methods for Portosystemic Shunt Detection.....	72
3.1 Introduction .....	73
3.1.2 Possible Portosystemic Shunt Detection Techniques.....	76
3.1.2.1 Microspheres .....	76

3.1.2.2 Biomarkers .....	77
3.1.3 Aims & Hypothesis .....	80
3.2 Methods .....	80
3.3 Results .....	80
3.4 Discussion.....	85
3.4.1 Lignocaine and Monoethylglycinxyllidide (MEGX) .....	85
3.4.2 Sorbitol, Fructose and Ethanol.....	86
3.4.3 <sup>3</sup> H-taurocholate.....	87
3.4.4 <sup>13</sup> C-methacetin.....	87
3.4.5 Indocyanine Green and the LiMON <sup>®</sup> System .....	88
3.5 Conclusion .....	89
CHAPTER 4 Analytical Methods and Results.....	91
4.1 Introduction .....	92
4.1.1 LiMON <sup>®</sup> Spectrophotometry.....	92
4.1.2 Breath Testing .....	95
4.1.3 Plasma Sampling .....	95
4.2 Methods .....	97

4.2.1 Procedure Overview.....	97
4.2.2 Marker Preparation.....	99
4.2.3 Sample Collection.....	102
4.2.4 Analytical Methods .....	105
4.2.4.1 High Performance Liquid Chromatography (HPLC) Equipment.....	105
4.2.4.2 Sample Preparation for HPLC.....	105
4.2.4.3 Methacetin HPLC Method.....	106
4.2.4.4 Indocyanine Green HPLC Method.....	107
4.2.4.5 Lignocaine and MEGX HPLC Method .....	107
4.2.4.6 Sorbitol HPLC Method.....	107
4.2.4.7 <sup>14</sup> C-sucrose and Evans Blue Analytical Methods.....	108
4.2.4.8 <sup>13</sup> CO <sub>2</sub> Breath Analysis Method .....	109
4.2.5 Intravenous Anaesthesia.....	112
4.3 Results .....	113
4.3.1 Evans Blue and <sup>14</sup> C-Sucrose Clearance.....	113
4.3.3 Lignocaine and MEGX .....	119
4.3.4 Indocyanine Green and LiMON <sup>®</sup> .....	121
4.3.4.1 ICG Clearance – Portal System Injection Site.....	121
4.3.4.2 ICG Clearance - Systemic Injection Site.....	127

4.3.5 Methacetin .....	133
4.3.5.1 Methacetin Clearance - Portal System Injection Site .....	133
4.3.5.2 Methacetin Clearance - Systemic Injection Site .....	135
4.3.6 Breath Analysis.....	137
4.4 Discussion.....	139
4.5 Conclusion .....	145
CHAPTER 5 Determining Portosystemic Shunt Fractions .....	146
5.1 Introduction .....	147
5.2 Flow Rate Calculation.....	148
5.2.1 Flow Rate Calculation Methods .....	150
5.2.2 Flow Rate Calculation Results and Discussion .....	150
5.3 Pharmacokinetic Modelling .....	155
5.4 Portosystemic Shunt Fractional Calculations and Limits .....	161
5.4.1 Methods .....	161
5.4.1.1 Injection sites and Scenarios.....	162
5.4.2 Sampling.....	164
5.5 Results .....	164

5.5.1 LiMON <sup>®</sup> Analysis .....	168
5.5.2 Evans Blue .....	177
5.6 Discussion.....	182
5.7 Conclusion .....	185
CHAPTER 6 General Discussion .....	186
6.1 Portosystemic Shunt Detection Technique .....	187
6.2 Limitations and Complications.....	188
6.2.1 Measuring Shunt Flow .....	189
6.2.2 Breath Sampling During Anaesthesia.....	192
6.2.2.1 Breath Analysis.....	194
6.2.2.2 Isoflurane Contamination .....	197
6.2.2.3 Sampling Accuracy .....	202
6.4 Significance .....	205
6.5 Clinical Implications .....	206
6.5.1 Cancer Catagorisation and Risk Factors .....	206
6.6 Future Considerations.....	207
6.7 Conclusions .....	208

CHAPTER 7 References.....	210
CHAPTER 8 Appendices.....	249
Appendix A – List of drugs searched as a PSS marker (n= 110).....	250
Appendix B – Coding to create the model for the software ADAPT 5.....	253
Appendix C- Surgical Research Society 48 <sup>th</sup> Annual Scientific Meeting 2011	
Abstract and Presentation .....	254
Appendix D- Surgical Research Society 48 <sup>th</sup> Annual Scientific Meeting 2011	
Abstract.....	256
Appendix E- The Queen Elizabeth Hospital Research Day 2011 Abstract and	
Presentation.....	257
Appendix F – The Queen Elizabeth Hospital Research Day 2012 Abstract and	
Presentation.....	259
Appendix G – Three Minute Thesis Competition Poster .....	261
Appendix H: Review of Incidentally diagnosed congenital and acquired	
portosystemic shunts in patients without cirrhotic liver disease: <i>a need for a</i>	
<i>standardised clinical test</i> .....	262
Appendix I: Creation of a Portocaval Shunt in pigs, with a method for estimating	
shunt fractions .....	313
Appendix J: Detrimental effect of isoflurane in gas chromatography.....	337

Appendix K: Safe and inexpensive method for breath sampling and a technique for continuous intravenous anaesthesia in pigs .....	353
Appendix L: Portosystemic shunt fraction determination by pharmacokinetic modelling.....	366
Appendix M: Figure 3.1 Grant of Permission.....	390

## List of Tables, Figures and Equations

### CHAPTER 1 Introduction

<b>Table 1.1:</b> Summary of Abernethy extrahepatic and Park intrahepatic portosystemic shunt definitions.....	5
<b>Figure 1.1:</b> Extrahepatic Abernethy portosystemic shunts. Type I: All blood from the Portal Vein (PV) is diverted into the Inferior Vena Cava (IVC). Type II: a portion of blood diverted into the Inferior Vena Cava with the Portal Vein being patent, but tortuous.....	6
<b>Figure 1.2:</b> Park classification: Type I has a single, constant diameter shunt from the intrahepatic portal vein (PV) to the inferior vena cava (IVC). In Park Type II, single or multiple shunts can be found between the intrahepatic portal branches and the hepatic veins within the same segment. Park Type III has a shunt, which has formed via a portal system aneurysm connecting to a hepatic vein, and in Park Type IV, there are multiple shunts between the portal branches and the hepatic veins in multiple segments.....	7
<b>Figure 1.3:</b> Human liver divided into segments according to Couinaud’s nomenclature. The left lobe consists of segments I-IV, while the right lobe consists of segments V-VIII.....	8
<b>Figure 1.4:</b> Gastrointestinal organs with venous drainage into the portal system.....	19
<b>Search Term 1.1:</b> Search terms formatted for PUBMED used to find naturally occurring or acquired portosystemic shunts in adults without cirrhosis. ....	25
<b>Figure 1.5.</b> Flow chart of the systematic search strategy. ....	27
<b>Table 1.2:</b> Prevalence of the type of shunt in male and female patients. ....	31
<b>Table 1.3:</b> Median age at diagnosis of shunts n=104 (no data reported in eight patients)...	32



<b>Table 1.4:</b> Different methods used for detection of naturally occurring portosystemic shunts in patients without hepatic cirrhosis. CT/A – computed tomography/angiography. MRI/A – magnetic resonance imaging/angiography. n=number of patients.....	33
<b>Table 1.5:</b> Symptoms and pre-existing conditions in patients with Abernethy and Park type portosystemic shunts. ....	34
<b>Table 1.6:</b> Shunt flow rate and shunted ratio. ....	35

## CHAPTER 2 Development of a Portosystemic Shunt in a Swine Model

<b>Figure 2.1:</b> A 10 cm incision made on the lower right side of the neck. (A) The Jugular vein located (arrow). (B) Drip line inserted into the jugular vein with a 16G Braun Introcan Safety® needle. ....	51
<b>Figure 2.2:</b> A 40 cm incision was made down the midline to expose abdominal organs and vessels.....	52
<b>Figure 2.3:</b> (A) The inferior vena cava (arrow) is located and mobilised to a level at the confluence of the liver. (B) The portal vein (solid arrow) was identified and mobilised with separation of lymphatics (hollow arrow) and nodes that surround the portal vein. ....	53
<b>Figure 2.4:</b> (A) The inferior vena cava (IVC) was partially clamped using a side biting satinsky clamp and a longitudinal venotomy was performed. An end to side anastomosis of the portosystemic shunt is performed using continuous 6/0 polypropylene suture and 8 mm diameter by 10-15 cm PTFE tubing. (B) The portal vein anastomosis was similarly completed with partial occlusion clamping and end-to-side anastomosis.....	54

<b>Figure 2.5:</b> Schematic of an end-to-side anastomosis. (A and B) The graft material is trimmed. (C) A 16 mm elliptical excision is made into the portal vein or inferior vena cava with the suture starting at the ‘heel’. (D) The ‘heel’ is sutured until half way, along both sides and then suturing is started from the toe. (E) Suture from the toe is joined in the middle with any excess edges removed.....	55
<b>Figure 2.6:</b> The end to side anastomosis of the portosystemic shunt is measured and allowed to stabilise for five minutes. ....	56
<b>Figure 2.7:</b> A vacuum container consisting of a 100 mL glass jar filled with 0.9% saline solution and a portion of PTFE. A BD Connecta™ three-way tap with luer-lock has been drilled and glued into the cap so air can be withdrawn from the jar using a syringe. ....	59
<b>Equation 2.1:</b> Hagan Poiseuille equation rearranged to find the flow rate of the portosystemic shunt.....	61
<b>Equation 2.2:</b> A simplified equation to calculate the portosystemic shunt (PSS) fraction using the Portal vein pressure (PVP) when the shunt is open and closed. Adapted from Washizu et al. [210] .....	61
<b>Equation 2.3:</b> Ratio of blood volume between the portosystemic shunt (PSS) volume and the portal vein volume. ....	61
<b>Figure 2.8:</b> (A) scavenged iliac vein and (B) PTFE used to create an anastomosis between the portal vein (PV) and inferior vena cava (IVC). ....	64
<b>Figure 2.9:</b> Images of all anastomoses inserted into each of the six pigs between the portal vein (PV) and inferior vena cava (IVC). ....	64
<b>Figure 2.9:</b> Images of all anastomoses inserted into each of the six pigs between the portal vein (PV) and inferior vena cava (IVC) .....	65

<b>Table 2.1:</b> Length of anastomoses that was inserted into each pig with the ratio between the portal vein (PV) and shunt (PSS) volumes. A range is given as the portal vein length was assumed of $6.5 \pm 1.5$ cm. ....	67
<b>Table 2.2:</b> Pressure differences (mmHg) between portal vein (PV) and inferior vena cava (IVC) with the shunt open (S) and when closed/control (C).....	68

### CHAPTER 3 Practical Methods for Portosystemic Shunt Detection

<b>Figure 3.1:</b> Schematic of a hepatocyte with the location of drug transporters.....	75
<b>Equation 3.1:</b> Kety-Renkin-Crone equation ( <b>A</b> ) to find the extraction rate (E) of a substrate in a sinusoid, $x$ denotes the measurement is specific to the substrate. Rearranged Kety-Rekin-Crone equation ( <b>B</b> ) to determine the linear relationship between two substrates [226]. ....	78
<b>Figure 3.2:</b> Flow chart of pharmacological agents and compounds that may be suitable for detection of portosystemic shunts.....	82
<b>Table 3.1:</b> List of pharmacological agents and compounds that may be used as a portosystemic marker. ....	83
<b>Table 3.2</b> List of pharmacological agents and compounds with respective doses. ....	84

### CHAPTER 4 Analytical Methods and Results

<b>Figure 4.1:</b> LiMON machine by Pulsion® Medical Systems (Germany) that uses spectrophotometry to detect Indocyanine Green dilution and retention in the systemic system.....	94
---	----

<b>Table 4.1:</b> Dose and stock solution of each compound used as marker for portosystemic shunts. ....	101
<b>Figure 4.2:</b> (A) Breath sample collecting apparatus consisting of two blood transfusers and two three way taps that (B) connect to the sample line of the anaesthesia machine. ....	104
<b>Figure 4.3:</b> Pilot results of $^{13}\text{CO}_2$ ratio in the breath of a shunted and non-shunted pig.....	111
<b>Figure 4.4:</b> Concentration of Evans blue (EB) in each shunted pig and the mean when injected into the portal system .....	115
<b>Table 4.2:</b> Mean transit time (MTT) with corresponding area under the curve (AUC) from Evans blue dye in the shunted and control models .....	116
<b>Figure 4.5:</b> Concentration of $^{14}\text{C}$ -Sucrose in each pig when injected into the portal system.....	117
<b>Table 4.3:</b> Mean transit time (MTT) with corresponding area under the curve (AUC) from $^{14}\text{C}$ -Sucrose in the shunted and control models.....	118
<b>Figure 4.6:</b> Concentration of monoethylglycinexylidide (MEGX) in each pig post injection of Lignocaine into the portal system. ....	120
<b>Figure 4.7:</b> Concentration of Indocyanine green dye (ICG) in each pig in the plasma when injected into the portal system. There is an error in pig 4 in regards to concentration as it is thought that there was internal bleeding while the shunt was open, however it is still shown to reference with the LiMON <sup>®</sup> data. ....	122
<b>Table 4.4:</b> LiMON conversion factor to calibrate the LiMON <sup>®</sup> data into relative concentrations and its correlation coefficient ( $R^2$ ) when indocyanine green is injected into the portal system. ....	123
<b>Figure 4.8:</b> Concentration of indocyanine green dye (ICG) in each shunted pig and the mean from the LiMON <sup>®</sup> system when injected into the portal system. There is a error	

in pig 4 in regards to concentration, however it is still shown to determine transit times. ....	124
<b>Table 4.5:</b> Lag times (threshold > 0.01 µg/L) and time of the first peak from the portal injection using the LiMON® data.....	125
<b>Table 4.6:</b> Mean transit time (MTT) with corresponding area under the curve (AUC) from indocyanine green in the shunted and control models. ....	126
<b>Figure 4.9:</b> Indocyanine green concentration in the plasma as collected from the confluence of the hepatic veins in the inferior vena cava when injected into the systemic venous system (Jugular vein). There is an error in pig 4 in regards to concentration as it is thought that there was internal bleeding while the shunt was open, however it is still shown to reference against the LiMON® data.....	128
<b>Table 4.7:</b> LiMON conversion factor to calibrate the LiMON® data into relative concentrations and its correlation coefficient ( $R^2$ ) when Indocyanine green is injected systemically. ....	129
<b>Figure 4.10:</b> Concentration of indocyanine green dye (ICG) in each shunted pig and the mean from the LiMON® system when injected into the systemic venous system (jugular vein).....	130
<b>Table 4.8:</b> Lag times (threshold > 0.01 µg/L) and time of the first peak from the systemic injection using the LiMON® data.....	131
<b>Table 4.9:</b> Liver mean residence time data for indocyanine green dye injected into the systemic system.....	132
<b>Figure 4.11:</b> Methacetin concentration in the plasma from injecting into the portal system and collected from the confluence of the hepatic veins in the inferior vena cava..	134
<b>Figure 4.12:</b> Methacetin concentration in the plasma from injecting into the jugular vein and collected from the confluence of the hepatic veins in the inferior vena cava..	136

**Figure 4.13:** Ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  in the breath of a shunted and non-shunted pig. .... 138

**Figure 4.14:** Two compartment showing the liver and shunt in parallel with the blood flow of the portal vein ( $Q_{pv}$ ) being split into the flow through the liver ( $qQ_{pv}$ ) and the shunt  $((1-q)Q_{pv})$ . .... 141

## CHAPTER 5 Determining Portosystemic Shunt Fractions

**Equation 5.1:** Pharmacokinetic question to measure flow ( $Q$ ) by volume ( $V$ ) and the mean transit time ( $MTT$ ). .... 149

**Equation 5.2:** Adapted pharmacokinetic question to measure flow ( $Q$ ) by volume ( $V$ ) and the lag time. .... 149

**Figure 5.1:** Dispersion of Evans blue dye injected through a 17 cm length 8 mm diameter PTFE tubing *ex vivo* with flow rates set at (A) 1 mL/s, (B) 5 mL/s, and (C) 10 mL/s. .... 152

**Table 5.1:** Calculated flow rate of Evans blue in an *ex vivo* model as determined by the lag time and compared to the set pump flow rate. .... 153

**Figure 5.2:** Correlation between the set pump flow rate and the (A) lag time, and (B) the calculated flow rate based on lag time. .... 154

**Equation 5.3:** Inverse Gaussian Distribution model using the mean transit time ( $MTT$ ), the point of time ( $t$ ) and the relative dispersion ( $RD$ ). .... 157

**Equation 5.4:** Combination of inverse Gaussian distribution models for a two compartment model, with the liver ( $qf_l$ ) and the shunt  $((1-q)f_s)$ . .... 157

**Figure 5.3:** Single pass model of indocyanine green (ICG) clearance in the liver with the presence of a portosystemic shunt, when injected into the portal system. The liver and the shunt are in parallel with blood flows  $qQ$  and  $(1-q)Q$  respectively

( $Q_{pv}$  is portal flow, and  $0 < q < 1$ ). The liver and body are individually characterised by inverse Gaussian transit time density functions shown as  $f_i(t)$ . ..... 158

**Figure 5.4:** Example of a typical fit of  $f_{LS}(t)$  to data observed with an open shunt.  $R^2 = 0.99$ .159

**Table 5.2:** Estimated fractions of shunt flow ( $1-q$ ) as deemed from the model with its correlation to the fitted data ( $R^2$ ) the corresponding portal vein (PV) and shunt (PSS) volume ratio. .... 160

**Figure 5.5:** Schematic of injection plan. D1. Control 1: Into portal vein (PV) with shunt clamped. D2. Control 2: Into portal vein, above shunt (open) to capture liver function only. D3. Directly into the shunt. D4. Into portal vein below shunt, to capture both shunt and liver. .... 163

**Figure 5.6:** Portosystemic shunt created in (A) pig 7, (B) pig 8, and (C) pig 9 using 8 mm diameter PTFE. .... 166

**Table 5.3:** Length of each anastomosis that was inserted into each pig with portal vein (PV) volume and the shunt (PSS) volume ratio. .... 167

**Figure 5.7:** Mean concentration of indocyanine green dye of pigs 7, 8 and 9 in each scenario as depicted from the LiMON® system.. .... 170

**Table 5.4:** Lag times (threshold  $> 0.01 \mu\text{g/L}$ ) for different injected scenarios using the LiMON® data for all pigs. .... 171

**Table 5.5:** Relative flow rate  $Q$  (mL/s) derived from lag time for different injected scenarios using the LiMON® data in all pigs. .... 172

**Table 5.6:** Shunted ratios,  $1-q$  derived from lag time for different injected scenarios using the LiMON® data in all pigs. .... 173

**Table 5.7:** Mean residence time (seconds) for each different injected scenarios using the LiMON® data in all pigs. .... 174

**Table 5.8:** Relative flow rates, Q (mL/s) derived from mean residence time for each different injected scenarios using the LiMON® data in all pigs..... 175

**Table 5.9:** Actual, relative and maximum shunted fractions 1-q derived from mean residence time for each different injected scenarios using the LiMON® data in all pigs. .... 176

**Table 5.10:** Mean transit time (seconds) of Evans blue for each injected scenario in all pigs.179

**Table 5.11:** Relative flow rates (mL/s) derived from Evans blue mean transit time for each injected scenario in all pigs. .... 180

**Table 5.12:** Actual, relative and maximum shunted fractions (1-q) derived from Evans blue mean transit time for each injected scenario in all pigs..... 181

**Table 5.13:** D4/D1 Shunted fractions (1-q) based on indocyanine green dye lag time and mean residence time (MRT), mean transit time (MTT) of Evans blue, and the pharmacokinetic model. Included is the portal vein (PV) and Shunt (PSS) ratio as a comparison. Model data for pigs 8 and 9 could not be included due to technical difficulties..... 183

CHAPTER 6 General Discussion

**Figure 6.2:** Series of quality control references throughout sampling for <sup>13</sup>CO<sub>2</sub> in the presence of isoflurane. Each quality control should maintain similar ratio of 29.1 ± 0.2 (dashed line), however a drift occurs (solid line) as more isoflurane contaminated samples are analysed. The black dash line shows the standard deviation of the drift. .... 195

**Figure 6.3:** Series of quality control references throughout sampling for <sup>13</sup>CO<sub>2</sub> in the presence of isoflurane. Digital raw sample data was rerun in sample sets with



the analysis window being changed to correct for the drift, so each quality control should maintained similar within each set. .... 196

**Figure 6.4:** Series of quality control references samples that were not used in the presence of isoflurane. Each quality control maintained similar ratio of  $31.1 \pm 0.6\%$ . ..... 201

**Figure 6.5:** Ratio of total CO<sub>2</sub> contained within the breath samples of pigs 7, 8 and 9. .... 204

## List of Abbreviations

Abbreviation	Definition
<sup>13</sup> C-	[13]Carbon labelled
<sup>14</sup> C-	[14]Carbon labelled
1-qQ	Difference of flow rate fraction
<sup>3</sup> H-	3Hydrogen labelled
AUC	Area under curve
CF <sub>4</sub>	Tetrafluoromethane
CO <sup>2</sup>	Carbon dioxide
CT	Computed tomography
CTA	Computer tomography angiography
CTC	Circulating tumour cells
CYP1A2	Cytochrome 1A2
D1	Drug administration site into the portal vein with the shunt closed (normal control).
D2	Drug administration site into the portal vein above the open shunt flowing into the liver only (control).
D3	Drug administration site directly into the start of the shunt.
D4	Drug administration site into the portal vein below the shunt.

$E_{ICG}$	Extraction of Indocyanine green
$E_{sorbitol}$	Extraction of sorbitol
$f(t)$	Inverse Gaussian distribution function
GLUTs	Glucose transporter
ICG	Indocyanine green dye
R15	Plasma disappearance rate at 15 minutes
IRMS	Isotope-ratio mass spectrometry
IVC	Inferior vena cava
MATEs	Mammalian multidrug and toxic compound extrusion
MEGX	Monoethylglycinexylidide
MRA	Magnetic resonance angiography
MRI	Magnetic resonance image
MRT	Mean residence time
MTT	Mean transit time
N <sub>2</sub> O	Nitrogen oxide
NTCP	Sodium-dependent taurocholate co-transporting protein
OATPs	Organic anion transporting polypeptides
OATs	Organic anion transporter

OCTs	Polyspecific organic cation transporters
Ost $\alpha$ and $\beta$	Organic solute or steroid transporter alpha and beta
PSS	Portosystemic shunt
PTFE	Polytetrafluoroethylene
PV	Portal vein
Q	Flow rate
QC	Quality control
qQ	Fraction of flow
$R^2$	Correlation coefficient
RD	Relative dispersion
SD	Standard deviation
TIPS	Transjugular intrahepatic portosystemic shunt
TNM	Tumour node metastases staging system
TQEH	The Queen Elizabeth Hospital
TTD	Transit time
UV	Ultraviolet
VOC	Volatile organic compound

## **Publications, Presentations and Competitions**

Publications, papers submitted for publication and conference presentations pertaining to results relating to the thesis are listed below. Abstracts, manuscripts and presentations can be found in Chapter 8: Appendices.

### **Published Abstracts and Conference Presentations**

Matthews, T., Li , P., Hamilton, M., Trochsler, M., Butler, R., Roberts, M., and Maddern, G. J., *Clinical analysis of liver function: Can portosystemic shunts be measured?* , in *The Australasian Surgical Research Society Meeting*. 2010, ANZ Journal of Surgery: Adelaide, South Australia. p. 11. (Appendix C)

Matthews, T., Li , P., Hamilton, M., Trochsler, M., Butler, R., Roberts, M., and Maddern, G., *Clinical analysis of liver function: Can portosystemic shunts be measured?*, in *The Australasian Surgical Research Society Meeting*. 2011, ANZ Journal of Surgery: Adelaide, South Australia. p. 8. (Appendix D)

### **Conference Presentations**

Matthews, T., Li , P., Hamilton, M., Trochsler, M., Butler, R., Roberts, M., and Maddern, G. J., *Clinical analysis of liver function: Can portosystemic shunts be measured?* , in *The Queen Elizabeth Hospital Research Day*. 2011, The Hospital Research Foundation: Woodville South, South Australia. (Appendix E)

Matthews, T., Li , P., Hamilton, M., Trochsler, M., Butler, R., Roberts, M., and Maddern, G. J., *A novel non-invasive technique for the detection of portosystemic shunts*, in *The Queen Elizabeth Hospital Research Day*. 2012, The Hospital Research Foundation: Woodville South, South Australia. (Appendix F)

### **Competitions**

Matthews, T. *Find that Shunt!* Three minute thesis competition. 2012. University of Adelaide, South Australia. (Appendix G)

## Submitted Manuscripts

Matthews T, Trochsler M, Maddern G. Review of Incidentally diagnosed congenital and acquired portosystemic shunts in patients without cirrhotic liver disease: *a need for a standardised clinical test*. Submitted to British Journal of Surgery (Appendix H)

Matthews T, Trochsler M, Hamilton M, Maddern G. Creation of a portocaval shunt in pigs, with a method to estimating shunt fractions. Submitted to Journal of Surgical Research (Appendix I)

Matthews T, Barry S, Zacharakis B, Maddern G. Detrimental effect of isoflurane in gas chromatography. Submitted to Journal of Breath Research (Appendix J)

Matthews T, Kuchel T, Maddern G. Safe and inexpensive method for breath sampling and a technique for continuous intravenous anaesthesia in pigs. Submitted to Journal of Breath Research (Appendix K)

Matthews T, Weiss M, Li P, Trochsler M, Hamilton M, Roberts M, Maddern G. Portosystemic shunt fraction determination by pharmacokinetic modelling. Intended for publication (Appendix L)