Variability in neonatal gentamicin administration influencing drug delivery kinetics

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Abstract

Introduction: Intravenous gentamicin is frequently prescribed for the empiric treatment of early-onset sepsis in premature neonates\(^1\). The effective delivery of gentamicin may be influenced by infusion rate and flush volume\(^2\). Additional parameters affecting drug delivery kinetics may include route of administration, background infusion rate and drug measurement variability which we aimed to further quantify in this study.

Methods: A self-administered questionnaire was completed by Dunedin hospital Neonatal Intensive Care Unit (NICU) nurses to investigate: the site of administration, comparing peripheral intravenous line (PIV) or umbilical venous catheter (UVC); and which would be used for a dose of gentamicin given two clinical scenarios describing babies of 24 and 32 weeks gestation. Secondary information was collected regarding flush volume. Intravenous infusions were then designed to simulate gentamicin delivery through UVCs with a constant background flow rate of 0.5 mL/hr. Intended doses of gentamicin (2 mg or 5 mg) in syringes were weighed before and after administration and given by bolus injection over 3-5 minutes followed by a flush of 0.9% saline (1 mL or 2 mL). Samples were collected at 5 minute intervals for 1 hour and analysed by high pressure liquid chromatography. Additionally, congo red dye (1% w/v) was used to mimic the drug administration phase during one replication of each dose/flush volume combination.

Results: There were 42 nurses employed in Dunedin NICU during the survey period, of whom 37 (88%) responded. For a 24-week gestation baby, 34 nurses (92%) would administer into the primary lumen (20 ga), containing 10% dextrose (0.5 mL/hr), compared to 3 (8%) who would use the secondary lumen (23 ga), containing parenteral nutrition fluid (2.1 mL/hr). For a 32-week gestation baby 35 nurses (95%) would
administer through the slow-flowing primary lumen. If a PIV was present this would be used preferentially by 35 nurses (95%) to reduce the risk of infection. Smaller flush volumes were documented following administration through the UVC compared with PIV (1.17-1.35 mL vs 2.4 mL at 24 weeks and 1.42-1.74 mL vs 3.2 mL at 32 weeks). Complete recovery of 2 mg and 5 mg intended gentamicin doses was observed following administration of both 1 mL and 2 mL flush volumes when administered via a UVC. Of the 2.15 mg administered dose recovered when a 1 mL flush is used, 85% (standard deviation, SD, 3.1%) was collected by 10 minutes and 93% (SD 1.4%) over the first 30 minutes. When a 2 mL flush was given, 99% (SD 0.5%) of the 1.88 mg administered dose was recovered in 10 minutes. Following a 5 mg intended dose, 93 % (SD 3.4%) was recovered at 10 minutes and 97% (SD 2%) in 30 minutes after a 1 mL flush, compared to 99% (SD 0.6%) recovered at 10 minutes with a 2 mL flush.

Conclusion: Variability in neonatal gentamicin pharmacokinetics may be attributable to a number of factors. Clinical variability in the route of intravenous delivery was documented by means of a survey. Experimental evidence showed that simulated gentamicin delivery by bolus injection into slow-flowing neonatal central lines resulted in >90% dose recovery at 1 hour. Additionally, variation in the volumes of drug and flush prepared for administration, and retrograde flow of dye were observed.
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# Table of Contents

Abstract........................................................................................................................................... i  
Acknowledgements ......................................................................................................................... iii  
List of figures................................................................................................................................... vii  
List of tables..................................................................................................................................... viii  
Glossary of terms .............................................................................................................................. ix  
  Clinical ........................................................................................................................................... ix  
  Pharmacological .............................................................................................................................. ix  
List of abbreviations ......................................................................................................................... x  
1  Introduction ................................................................................................................................... 1  
  1.1  Neonatal morbidity and mortality ......................................................................................... 2  
    1.1.1  Epidemiology .................................................................................................................. 2  
    1.1.2  Aetiology ........................................................................................................................ 3  
    1.1.3  Survival rates .................................................................................................................. 4  
    1.1.4  Early onset sepsis ........................................................................................................... 5  
    1.1.5  Complications of prematurity related to infection ....................................................... 7  
    1.1.6  Summary ....................................................................................................................... 8  
  1.2  Principles of intravenous drug administration to neonates ............................................... 10  
    1.2.1  Venous access devices .................................................................................................. 10  
    1.2.2  Intravenous tubing and connections .......................................................................... 11  
    1.2.3  Formulation and dilution of medications .................................................................... 12  
    1.2.4  Fluid dynamics .............................................................................................................. 13  
    1.2.5  Current understanding of drug delivery kinetics .......................................................... 14  
    1.2.6  Summary ....................................................................................................................... 17  
  1.3  Gentamicin ............................................................................................................................... 18  
    1.3.1  Pharmacokinetics .......................................................................................................... 18  
    1.3.2  Pharmacodynamics ...................................................................................................... 20  
    1.3.3  Indications and dose recommendations ...................................................................... 21  
    1.3.4  Adverse effects .............................................................................................................. 23  
    1.3.5  Therapeutic drug monitoring ...................................................................................... 24  
    1.3.6  Summary ....................................................................................................................... 26  
  1.4  What is not known about this topic ...................................................................................... 27  
  1.5  Objective of this research ..................................................................................................... 28  
    1.5.1  Specific aims: ................................................................................................................ 28  
2  Materials .................................................................................................................................... 29
5.1 Overview ......................................................................................................................... 73
5.2 Variability in current clinical practice ............................................................................... 73
5.3 Peripheral line infusion variables ..................................................................................... 76
5.4 Central line infusion variables ........................................................................................ 77
5.5 Measurement variability ................................................................................................... 80
5.6 Strengths and weaknesses ............................................................................................... 81
5.7 Summary ........................................................................................................................ 82
5.8 Implications for further research ..................................................................................... 83
6 Conclusion .......................................................................................................................... 85
7 References .......................................................................................................................... 87
8 Appendices ......................................................................................................................... 93
  8.1 Dunedin Hospital gentamicin dosing protocol ................................................................. 93
  8.2 NICU nurses survey questions ....................................................................................... 96
  8.3 Published journal article ............................................................................................... 98
List of figures

Figure 1: Double lumen umbilical venous catheter ................................................................. 32
Figure 2: Survey question 1 ........................................................................................................ 33
Figure 3: Peripheral IV line simulation ....................................................................................... 38
Figure 4: Components of PIV set-up ........................................................................................ 38
Figure 5: UVC simulation set-up ............................................................................................... 40
Figure 6: UVC simulation pump ............................................................................................... 40
Figure 7: Nursing response regarding flush volumes ............................................................... 45
Figure 8: Examples of gentamicin sulphate standard curves prepared for analysis ............. 49
Figure 9: Retention time vs concentration of acetonitrile in HPLC mobile phase ................. 51
Figure 10: HPLC chromatogram of tobramycin (internal standard) ...................................... 52
Figure 11: Recovery of gentamicin after administration via PIV line. .................................... 55
Figure 12: Cumulative gentamicin recovery and percentage of intended dose recovered following administration of 2mg gentamicin dose via UVC ........................................ 60
Figure 13: Recovery of 2 mg administered gentamicin dose (followed by 1 mL and 2 mL flush volumes) over 1 hour .......................................................................................... 61
Figure 14: Cumulative gentamicin recovery and percentage of intended dose recovered following administration of 5 mg gentamicin via UVC ......................................................... 65
Figure 15: Recovery of 5 mg administered gentamicin dose (followed by 1 mL and 2 mL flush volumes) over 1 hour .......................................................................................... 66
Figure 16: Cumulative gentamicin recovery over 2 hours after UVC administration ........... 68
Figure 17: Photographs of congo red dye administration (0.2 mL of 1% w/v) followed by 1 mL and 2 mL flush volumes ..................................................................................................... 70
Figure 18: Photographs of congo red dye administration (0.5 mL of 1% w/v) followed by 1 mL and 2 mL flush volumes ..................................................................................................... 71
List of tables

Table 1: Neonatal gentamicin pharmacokinetic studies ................................................................. 16
Table 2: Dunedin Hospital NICU gentamicin dose calculator ......................................................... 22
Table 3: Dunedin Hospital NICU gentamicin dosing interval ......................................................... 22
Table 4: Survey question 2 - flush volumes .............................................................................. 34
Table 5: PIV gentamicin dose/flush volume combinations ......................................................... 37
Table 6: UVC gentamicin dose/flush volume combinations ........................................................ 39
Table 7: Average flush volumes given to neonates at different sites of administration .............. 46
Table 8: Low (5 µg/mL) concentration validation data ............................................................... 50
Table 9: Medium (40 µg/mL) concentration validation data ..................................................... 50
Table 10: High (90 µg/mL) concentration validation data ............................................................ 50
Table 11: Measured drug and flush volumes in 2 mg PIV experimental series ......................... 53
Table 12: Measured drug and flush volumes in 5 mg PIV experimental series ......................... 53
Table 13: Measurements of drug and flush administered for 2 mg UVC experimental series .... 57
Table 14: Cumulative amount of gentamicin recovered during each 5 minute interval for one hour following 2 mg nominal dose administration via UVC ........................................... 58
Table 15: Measurements of drug and flush administered for 5 mg UVC experimental series ... 62
Table 16: Cumulative amount of gentamicin recovered during each 5 minute interval for one hour following 5 mg nominal dose administration via UVC ........................................... 63
Table 17: Cumulative amount of gentamicin recovered during each 30 minute interval for two hours following UVC administration .................................................. 68
Glossary of terms

Clinical
Early onset sepsis event: clinically suspected or proven infection in the first 72 hours after birth
Extremely low birth weight (ELBW): < 1000 grams
Gestational age: number of days/weeks of completed in-utero growth
Low birth weight (LBW): < 2500 grams
Neonatal period: first 28 days after birth
Post-conceptual age: gestational age plus postnatal age
Postnatal age: number of days/weeks after birth
Preterm delivery: < 37 weeks completed gestation
Very low birth weight (VLBW): < 1500 grams

Pharmacological
Administered dose: Gentamicin dose as determined by HPLC gentamicin concentration derived from standard curve on each day of analysis
Dead volume/dead space: volume of fluid contained within the tubing of the intravenous system
Intended dose: Prescribed 2 mg or 5 mg dose of gentamicin in this study
Measured dose: Volume of 10 mg/mL solution of gentamicin drawn up in experimental syringe, based on weight
Minimum inhibitory concentration (MIC): the lowest amount of drug required to limit the growth of an organism
Total parenteral nutrition (TPN): nutritionally-complete solution containing glucose, electrolytes, amino acids, minerals, vitamins and fat
List of abbreviations

$C_{\text{max}}$: maximum concentration of an antibiotic

CV: coefficient of variation

IV: intravenous (usually referring to therapy or venous access device)

mg: milligrams

mL: millilitres

NICU: Neonatal Intensive Care Unit

PICC: Peripherally Inserted Central Catheter

PIV: Peripheral intravenous line

SD: standard deviation

UVC: Umbilical Venous Catheter

w/v: weight per volume
1 Introduction

The use of medication for preterm babies is a frequent event in the modern neonatal intensive care unit (NICU), however the pharmacokinetics of drug administration are not well understood. This is a clinically focused dissertation and is principally concerned with the administration of gentamicin, an aminoglycoside antibiotic, in the NICU setting. Drug administration research in the neonatal population is extremely difficult and there is a paucity of evidence regarding the pharmacokinetics and efficacy of many medicines, despite their regular use. The following literature review will take the approach of introducing the population and clinical relevance of the topic first, followed by a discussion about drug administration in general and previous drug delivery kinetics work in the neonatal population and finally a section dedicated to gentamicin. A large number of questions remain unanswered in the area of neonatal drug administration, but this work hopes to contribute to an increasing understanding of the most effective method of administration and monitoring of gentamicin for the treatment of early-onset neonatal sepsis.
1.1 Neonatal morbidity and mortality

With advances in neonatal care, the survival of premature infants has greatly improved in the last decades of the 20th century³. Premature babies are defined as those born before 37 weeks completed gestation, though these can be subcategorised further into degrees of prematurity based on gestational age and birth weight. Babies born weighing <1000 g are classed as extremely low birth weight, those 1000 g-1499 g considered very low birth weight and 1500 g-2499 g is low birth weight. The average weight of a full-term male baby is 3.55 kg and a female is 3.4 kg. In this section the epidemiology of premature births in Australia and New Zealand will be discussed, followed by a general discussion regarding the aetiology and survival of these babies. The primary indication for gentamicin therapy in premature neonates is in the treatment of early-onset sepsis, which will be discussed in due course, along with the consequences of early infection in premature babies. The aim of this section of the literature review is to present the clinical relevance of this topic in the wider context of neonatal hospital care.

1.1.1 Epidemiology

The epidemiology of premature births in Australia and New Zealand is well documented in data collected by the Australian and New Zealand Neonatal Network (ANZNN), a collaboration of 28 Level III and 26 Level II neonatal intensive care units (NICUs) across these two countries. Babies are eligible for inclusion in this audit process if they are born at <32 weeks gestation or weighing <1500 g or received assisted ventilation (including mechanical ventilation and continuous positive airways pressure) for 4 hours or more, including those who died earlier than 4 hours while receiving ventilatory support, or babies who received major surgery or therapeutic hypothermia⁴. This care is generally provided at Level III NICUs for more specialised interventions, or Level II units where supportive and convalescent care is delivered. In the lower South Island of New Zealand
the Southern District Health Board provides Level III care at Dunedin Hospital and Level II at Kew Hospital in Invercargill.

The most recent published ANZNN data set is from 2011\textsuperscript{4}, and contains epidemiological data regarding 7,412 Australian and 1,770 New Zealand babies who met criteria for inclusion. These births account for 2.5\% of the total births in 2011 in Australia and 2.9\% of births in New Zealand in that calendar year, of whom 40.6\% of the Australian births and 29.9\% of the New Zealand cohort were born before 32 weeks gestation\textsuperscript{4}. There are an increasing number of births after each week of completed gestation up to 32 weeks, ranging from 0.6\% of all ANZNN-registered births occurring at <24 weeks to 9.5\% at 31 weeks\textsuperscript{4}. Similarly, birth weights rise with increasing gestational age.

1.1.2 Aetiology
The aetiology of premature birth can vary by gestational age and is largely unknown even though worldwide the rate of premature birth is increasing\textsuperscript{5}. Overall rates of premature delivery are greatest in non-caucasian ethnic groups\textsuperscript{5,6} although again the mechanisms for this remains unclear. Maternal predispositions to premature delivery include a personal or family history of premature birth\textsuperscript{6,7}; multiple gestation pregnancies\textsuperscript{7}; maternal medical disorders such as thyroid disease, asthma, diabetes and hypertension\textsuperscript{7}; and smoking\textsuperscript{8,9}. Intrauterine infection is commonly described as predisposing to preterm birth\textsuperscript{10}, possibly due to the activation of inflammatory cytokines and chemokines which lead to prostaglandin synthesis and subsequent stimulation of uterine contraction\textsuperscript{7}. Cervical insufficiency has also been postulated as a predisposing factor, but the contribution that this makes to premature deliveries is uncertain\textsuperscript{3}. Extremely preterm births (23\textsuperscript{+0} to 26\textsuperscript{+6} weeks) have been commonly observed due to idiopathic reasons, whereas births at late gestation (between 34 and 36 weeks) are more frequently due to medical indications for conditions such as pre-eclampsia and gestational diabetes\textsuperscript{5}. 
As well as maternal and obstetric reasons for premature delivery there are likely to be infant-related factors in the aetiology of this condition. For example, pregnancies affected by a single gene disorder such as neurofibromatosis, myotonic dystrophy, Ehlers-Danlos syndrome or Smith-Lemli-Opitz syndrome are at increased risk of premature delivery.

1.1.3 Survival rates
Hospital discharge data from New Zealand and Australia is reported in the ANZNN audit, with 94.8% of registered babies born in 2011 surviving to discharge home after a median length of stay of 29 days (ranging from 6 days at 41 weeks completed gestation to 129 days at 24 weeks)\(^4\). Mortality is highest in babies born at less than 27 weeks gestation compared with those born after this time (52.7% survival to discharge in babies born at <24 weeks completed gestation versus 97.5% survival in babies born at 29 weeks\(^4\)). Increasing rates of survival to discharge home are also observed with increasing birth weight\(^4\).

The long-term impact of premature birth must not be overlooked. Disability rates (including cerebral palsy, developmental delay, visual and hearing impairment) are approximately 20% in babies born weighing <1000 grams at any gestation and rates are highest in those born earlier than 25 weeks gestation\(^11\). These children are likely to have ongoing health and educational needs related to their prematurity and associated complications. There are also significant social and economic costs for the family related to the length of stay their babies require in NICU settings; an Australian paper from 2009 estimated the financial burden to amount to 27% of the gross weekly income\(^12\) during their hospital stay.
1.1.4 Early onset sepsis

One of the most frequent problems encountered by newborn premature babies is sepsis, which has a 3-10 fold greater incidence in preterm low birth weight babies than in those of full-term gestation\(^{13}\) and can have a significant effect on morbidity and mortality. Traditionally this is divided into episodes of early or late-onset sepsis, though both are variably defined in the literature. Early-onset sepsis events are most commonly defined as occurring in the first 72 hours after birth\(^{1 14 15 16 17 18 19 20 21}\) but other definitions such as the within the first 48 hours after birth\(^4\) are sometimes used. Early-onset characteristically refers to the first significant infection that is encountered by a neonate, and can refer both to suspected and proven infections. In 2011 6.3% of ANZNN babies had an episode of culture-proven early-onset sepsis, of which 72.6% occurred in those born at less than 32 weeks gestation\(^4\). This prevalence is higher than in other large studies from North America (1997–2010)\(^{17}\) and Taiwan (2005 – 2009)\(^{18}\) which report a 1% prevalence of early-onset sepsis, and Israel (1995–2005) who report a 2.4% risk of early onset sepsis in very low birth weight neonates\(^{15}\). In the next section the clinical manifestations and laboratory investigations for early-onset sepsis will be discussed, followed by the common causative organisms and treatments used in this condition.

A. Investigations

Episodes of sepsis can be clinically suspected, blood-culture proven or both. The signs and symptoms vary by gestational age and severity of infection and include non-specific findings such as lethargy, hypothermia (more commonly than fever); and poor feeding\(^{20}\). Investigations including laboratory tests and imaging are often initiated at the onset of suspicion of infection and empiric antibiotics are started promptly. Blood samples for culture, white blood cell count and differential and acute-phase reactants such as C-reactive protein (CRP) are frequently requested, though microbiological identification of
bacterial pathogens on culture is often not known for hours or days after the sample has been obtained. As yet there is no definitive test which will predict culture-positive sepsis at an early stage, nor any that will rule it out\textsuperscript{14}. Positive bacterial cultures (from blood, urine or cerebrospinal fluid) in addition to clinical suspicion determine whether an episode of sepsis is proven or not. Because respiratory signs such as tachypnoea are a common presentation of sepsis in the neonate a chest x-ray is often taken as part of the initial investigation into the underlying cause of an illness. Additional tests including abdominal x-ray, lumbar puncture, sterile urine collection, tracheal aspirate and samples from the tip of catheters such as umbilical lines or endotracheal tubes are performed according to the clinical presentation and timing of illness.

B. Risk factors
Risk factors for early-onset sepsis include maternal factors such as dietary intake of contaminated food (predisposing to \textit{Listeria monocytogenes}), procedures during pregnancy such as amniocentesis and labour-related factors such as prolonged (>18 hour) rupture of membranes, fever and vaginal colonization with group B streptococcus\textsuperscript{20}. Additionally, there are infant factors such prematurity, low birth weight, low Apgar scores, instrumental delivery and congenital anomalies which are also risk factors for early-onset development of sepsis\textsuperscript{20}. Exposure to antenatal antibiotics and the need for mechanical ventilation on Day 1 were additionally shown to be significantly more likely to be associated with an episode of early onset sepsis (Odds Ratio, OR, 1.87 and 1.76 respectively) in a North American study\textsuperscript{17}.

C. Common organisms
The pathogens most commonly isolated from blood cultures in cases of early onset sepsis are gram positive group B streptococcus\textsuperscript{18,19,20} and gram negative \textit{Eschericia coli}\textsuperscript{22}, which are responsible for the majority of infections in term and preterm infants
respectively\textsuperscript{21}. Additional pathogens to consider are \textit{Staphylococcus aureus} and other \textit{Streptococcus} species. The use of maternal prophylactic intrapartum antibiotics has reduced the incidence of early-onset group B streptococcal disease by 80\%\textsuperscript{20}, but despite this the incidence of early-onset sepsis has remained unchanged over time\textsuperscript{21}.

D. Treatment

Empiric antibiotic therapy is frequently given to neonates early in their infective course, with rationalisation of antibiotic choices occurring when sensitivities of the isolated organism are known. Frequently, the combination of gentamicin with a beta-lactam antibiotic is used to provide synergistic activity against gram positive and gram negative bacteria\textsuperscript{23}, and ampicillin or penicillin in combination with gentamicin have been shown to be equivalent\textsuperscript{1}. There is a lower threshold for starting empiric antibiotic therapy in premature neonates when sepsis is suspected than term babies because of their immature immune system, vulnerable physiology, and potential for significant complications which will be discussed in section 1.1.5. A detailed description of gentamicin will be provided in section 1.3 as it is the use of this antibiotic for the treatment of early-onset sepsis that is fundamental to this dissertation. Gentamicin is a frequently used antibiotic in the Dunedin Hospital NICU, with 415 single use 20 mg/2 mL vials stocked by the unit in 2013\textsuperscript{24}. In this calendar year there were 250 admissions, 112 (45\%) of them for preterm babies, but the number of babies who received gentamicin (either one dose or multiple) was not able to be determined from this data set.

1.1.5 Complications of prematurity related to infection

The significance of early onset infection in the neonatal population should not be underestimated. In the very low birth weight (VLBW) population, these infants are at a 3-fold risk of death or major neurological morbidity\textsuperscript{15}. Examples of the morbidity associated with early onset sepsis include grade 3 or 4 intraventricular haemorrhage
(Odds Ratio, OR, 2.24 compared to those without episodes of sepsis), grade 3 or 4 retinopathy of prematurity (OR 2.04) and bronchopulmonary dysplasia (OR 1.74)\textsuperscript{15}. Neonatal sepsis is also associated with an increased risk of chronic lung disease\textsuperscript{15}, possibly from the increased ventilator-dependency of these premature babies during times of illness. In a 5-year follow-up study, the presence of both early and late onset sepsis events in VLBW babies was associated with an increased risk of cerebral palsy\textsuperscript{22}. The pathological mechanisms underlying these complications of prematurity related to infection is not completely understood, but may include the response of the developing brain to an inflammatory insult\textsuperscript{25}.

Additionally, the consequences for the wider family and the health system can be considered as complications related to infection. For the family, there is commonly a restriction on their ability to hold (and hence to bond with) the baby when they are septic as neonates are often fragile on handling in these circumstances. It is difficult to quantify the impact that this has in the long term, but this is important to be cognisant of when nursing a sick premature baby in the NICU. Furthermore, the liberal use of antibiotics in extremely preterm babies is worth reflecting on, as the increasing antibiotic resistance of bacteria grows within our population and awareness of the importance of antibiotic stewardship becomes more apparent in the medical community.

1.1.6 Summary
Premature neonates are a vulnerable patient population whose clinical outcomes continue to be greatly enhanced by developments in modern technology. However, despite the most advanced equipment and environs, these patients remain susceptible to bacterial infections acquired in a multitude of different ways which require timely and effective treatment to ensure good outcomes. The consequences of not successfully treating early-onset infections can be devastating, and so knowledge of predisposing factors and
causative organisms is important for preventing infection as far as that is possible to do, and an understanding of the most efficient way to use antibiotics wisely in this setting will be needed to eradicate the infection. While premature babies only account for a small percentage of births in Australia and New Zealand each year, they require a considerable investment of resources, finance and emotion to ensure they have the potential to thrive once they leave hospital.
1.2 Principles of intravenous drug administration to neonates

There are a variety of different methods for administration of medications to neonates. For the purposes of this dissertation only the intravenous (IV) route will be discussed, but other methods include oral, intramuscular, subcutaneous and rectal, each of which has their own set of unique challenges in this population. There are a variety of factors to consider when administering IV medications to neonates, including the placement and construction of the venous access device and tubing; the formulation and dilution of medications; and the ways in which alterations to other fluids or medications interact with the drug of interest. Many of the studies on which dosing recommendations are made for neonates are adult or paediatric-based, but there is an increasing body of work specifically related to neonatal drug delivery kinetics which will be discussed in due course.

1.2.1 Venous access devices

Placement of the intravenous line for drug administration is often a challenging task in extremely low birth weight premature babies. They have fragile skin, small vessels and physical access to the baby is difficult given the thermoregulation that is provided by incubators and radiant warmers, particularly in the hours after delivery. Intravenous lines can take the form of peripheral or central lines. Peripheral IV lines are inserted into veins in the hands, arms or feet of the child and are short (14 or 19 mm in length) (BD Insyte 24GA, Singapore, used in Dunedin NICU). Central IV lines are intended to reach larger vessels located more central to the circulatory system. These can take the form of umbilical venous catheters (UVC), particularly in the first 24 hours, which can remain in situ for approximately one week; or peripherally inserted central catheters (PICC), inserted distally but with extended lengths of tubing which reach the central circulation. Central lines require confirmation of placement (ideally at the superior vena cava/right
atrial junction if placed in the upper extremities, or at the inferior vena cava/right atrial junction if placed in the lower extremities) with an x-ray due to their potential for complications (the most severe being myocardial perforation causing pericardial effusion, cardiac tamponade or arrhythmia), however technology is evolving to make this process more efficient, including the use of bedside ultrasound to confirm position of the tip of the catheter.

1.2.2 Intravenous tubing and connections
The construction of IV tubing for neonatal lines is often overlooked when considering medication administration in the neonatal population. The potential space within the line or tubing is often referred to as the "dead volume" or "dead space", and it can be a reservoir of drug or fluid which is able to be unintentionally administered, or inadvertently not administered at all. This could result in a drug's continued administration despite the clinical effect no longer being required, or administration of the initial drug at a later time when the line is accessed to give another medication. The tubing used in neonatal IV infusion sets has been designed to minimise this dead volume of tubing and improvements continue to evolve over time.

In regard to connections within the line, the configuration of the angle at which they attach (T or Y junction) and the angle of the primary infusion tubing can also have an impact on the delivery of medications in the NICU setting. Experimental evidence has shown poor mixing and retrograde flow when a coloured solution diluted with water is administered into a primary continuous fluid infusion of 10% dextrose with the T connector angled 15° upwards because of the difference in specific gravities between the drug solution for infusion and that of the carrier fluid. Inline filters are used in the IV tubing configuration as a way to decrease the risk of phlebitis by firstly reducing the
potential for particulate matter to be infiltrated and secondly reducing the risks of microorganism contamination\textsuperscript{31}, but these may also interfere with the delivery of medications. Additionally, drug adsorption onto components of the IV tubing is an important factor, an example of which is insulin binding onto the PVC tubing in neonatal infusion studies\textsuperscript{32} resulting in deactivation of the drug. Similarly, amiodarone is recommended to be given through a set-up that is free of polyvinyl chloride (PVC) and does not contain diethyhexyl phthalate (DEHP) because of the risk respectively of drug adhering to the bag\textsuperscript{33} and plasticiser leaching from the infusion bag\textsuperscript{34}.

1.2.3 Formulation and dilution of medications

There are few medications that are specifically formulated for administration to neonates. Because of this, dilutions of adult or paediatric medicines are frequently required when these are prescribed for preterm babies, some of which require serial dilution steps making the potential for error in calculations even greater. The preparation of medicines varies between units, but in most there will be at least some of this done by nursing staff working in the unit at the time a specific drug is required. Preparation of morphine has been shown to have a significantly greater variability in accuracy when completed in the NICU, in comparison to preparation in the pharmacy (19.2\% vs 7.8\% respectively for solutions outside of the acceptable error range of $\pm 7.5\%$)\textsuperscript{35}. This has been partly attributed to calculation errors and wrong volume measurements\textsuperscript{35}.

Similarly, in paediatric and general anaesthesia an error rate of 29\% (drug doses outside the acceptable error range of $\pm 10\%$ in comparison with the declared concentration) has been found and the greatest errors are shown to occur with non-standard dilutions or when medications are highly diluted\textsuperscript{36}. 
Suggested improvements to the way that paediatric and neonatal medications are formulated include manufactured standard concentrations appropriate for neonates\textsuperscript{35, 36}, standardising IV infusion concentrations within units\textsuperscript{37} and standardising dosing protocols for common medications (such as gentamicin) between units\textsuperscript{38}.

1.2.4 Fluid dynamics

The total daily fluid requirement of a neonate, which is then translated into mL/hour, varies depending on the postnatal age, weight and clinical condition of the baby, but 150 mL/kg/day is considered necessary for growth\textsuperscript{13}. Because of the dead space and slow flow rates of neonatal infusions, it can take longer than expected for medicines administered upstream to reach the baby, as has been shown by \textit{in vitro} experiments of continuous gentamicin infusions delivered over 30 minutes to extremely low birth weight babies, where less than 60% of the intended dose would be received by a 0.5 kg baby 60 minutes after the infusion was started\textsuperscript{2}. Similarly, if changes are made to the flow rate of a continuous infusion, including stopping the infusion, this also may take longer to register at the patient-end of the line than anticipated, especially at slow flow rates\textsuperscript{39}. These considerations are especially important for clinicians to be cognisant of when medications such as inotropes are being administered.

Also worth considering is the composition of the primary fluid that is being infused and the effect that mixing this with a drug being administered intermittently will have. The compatibility issues between these might give rise to problems such as precipitation or inactivation, drug trapping or retrograde flow\textsuperscript{29, 30}.

One way to ensure the stable, forward flow of neonatal intravenous infusions is with the use of anti-reflux valves. At low adult flow rates, when the carrier fluid is being driven
by gravity rather than a pump, these have been shown to improve drug delivery by 10% at 10 minutes when compared with faster flow rates and no anti-reflux valve.

1.2.5 Current understanding of drug delivery kinetics
There is a paucity of specific information regarding IV medication delivery to neonates, but some recent in vitro studies have attempted to explain this with respect to gentamicin in particular. Table 1 summarises experimental work completed at the University of Otago regarding gentamicin administration in the NICU.

Different background carrier fluid rates have been investigated in simulated experiments designed to approximate neonatal clinical scenarios. The carrier fluid rate is determined by the chronological age and weight of the baby and experiments have been based on flows of 180 mL/kg/day for ELBW (500 g) and LBW (2.5 kg) babies. These experiments assume that the medication is given into a venous access device which concurrently has the nutrition-containing fluid running, such as when only one lumen is available (single lumen PICC) or when only a single PIV line is in situ. The composition of the carrier fluid has also been investigated, with 10% dextrose commonly used as a proxy for TPN solution. Primary infusion solutions of a different specific gravity to the drug solution were also investigated and retrograde flow (away from the patient) seen more frequently when the glucose concentration was 20% w/v.

Different flush volumes of 0.9% sodium chloride (saline) were also used during these experiments which found that the smallest babies (with the smallest corresponding doses) were not likely to receive all of their intended dose within 1 hour due to the slow primary fluid flow rate and small flush volume when both drug and flush were given as a continuous infusion.
Drug administration through different venous access devices (peripheral or central lines) has been considered over the course of these studies. As no direct comparison between the proportions of gentamicin recovered when it is administered by different methods has yet been made, there is no strong evidence to direct recommendations for clinical practice. This is an important consideration as the location for administering a dose of gentamicin may affect the measured peak and trough levels, and hence have implications for dose adjustments in NICU.

It has been shown in these circumstances that lower carrier flow rates, given to smaller babies as a result of their limited fluid requirements, resulted in slower delivery of gentamicin than when a corresponding dose was given to a larger baby who received a faster background infusion rate. Continuous infusions of medication resulted in less of the dose recovered when given to ELBW neonates, but complete recovery of both small and large doses when the medication was given as a bolus instead. There was increased dose recovery with larger flush volumes.
## Table 1: Neonatal gentamicin pharmacokinetic studies

<table>
<thead>
<tr>
<th>Year of study (and method of drug administration)</th>
<th>Carrier flow rate</th>
<th>Gentamicin dose (volume)</th>
<th>Flush volume (0.9% NaCl)</th>
<th>Significant results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013&lt;sup&gt;41&lt;/sup&gt;, (UVC, bolus injection)</td>
<td>2 mL/hr</td>
<td>2 mg (0.2 mL)</td>
<td>1 mL at drug administration site over 5 mins or 0.5 mL at site and 0.5 mL further upstream</td>
<td>• At 2 mL/hr: 74-87% of drug was delivered at 10 mins; 95-97% delivered at 30 mins • At 10 mL/hr: 97% of drug delivered at 10 mins</td>
</tr>
<tr>
<td></td>
<td>10 mL/hr</td>
<td>5 mg (0.5 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012&lt;sup&gt;30&lt;/sup&gt; (PIV, bolus injection or infusion over 30 mins)</td>
<td>3.8 mL/hr</td>
<td>2 mg (0.4 mL)</td>
<td>1 mL over 5 mins or 1 mL over 30 mins or 2 mL over 5 mins or 3 mL over 45 mins</td>
<td>• 30 min infusions resulted in &lt;30% of 2 mg drug dose recovered during the infusion but at a higher dose and flow rate 80-90% recovery was seen during the infusion • Complete dose recovery (2 mg and 10 mg) seen when bolus doses were given over 5 mins</td>
</tr>
<tr>
<td></td>
<td>18.7 mL/hr</td>
<td>10 mg (1 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009&lt;sup&gt;2&lt;/sup&gt; (PIV, infusion over 35 mins)</td>
<td>3.8 mL/hr</td>
<td>0.5 mg (0.2 mL)</td>
<td>1 mL or 2 mL Over 35 mins</td>
<td>• Larger neonates had a higher percentage of administered dose recovered at 60 and 75 mins compared to ELBW babies • 2.5 kg neonates received only 80% of the intended dose by 60 mins, increasing to 90-95% at 75 mins • Increased dose recovery with larger flush volume</td>
</tr>
<tr>
<td></td>
<td>18.7 mL/hr</td>
<td>2 mg (0.2 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5 mg (1 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mg (1 mL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.2.6 Summary
Administration of IV medications to neonates is a complex and incompletely understood aspect of neonatal care. This route is commonly used for medication delivery in the NICU and for preterm babies who are unable to tolerate enteral feeding for a variety of reasons, the IV route fully sustains them. The devices and tubing through which fluids are administered are important to consider, both in terms of the volume of fluid within them and the materials that they are constructed from, as both can have an impact on the drug of interest and the clinical condition of the baby. Similarly, the dynamics of fluid flow within this tubing are an important parameter to be aware of so that medication delivery can be optimised in these extremely premature babies. The standard manufacturing of medications for adult and sometimes paediatric patients results in a frequent need for dilution, reconstitution and preparation for their use to be translated into the NICU, thereby creating the potential for dosing errors or ineffective medicines. Work has started already with the aim of better understanding gentamicin drug delivery kinetics in neonates, and this experimental study will add to this growing body of knowledge.
1.3 Gentamicin

Gentamicin is an aminoglycoside antibiotic which was first developed in the 1940s “by screening soil actinomycetes for antibiotic activity”\(^{42}\). It has the advantages of being low cost, chemically stable and with low potential for developing resistance\(^{42}\). However, due to its narrow therapeutic window requires more investigation before optimal use in the neonatal population can be firmly established. This section firstly introduces the principles of pharmacokinetics and pharmacodynamics with respect to gentamicin, including the added pharmacokinetic parameter of the drug delivery system in neonates, and then progresses to discussion regarding indications, dosing recommendations, adverse effects and therapeutic drug monitoring. There has been a lot of work done regarding gentamicin therapy in older populations but firm evidence of its specific use in premature neonates is sparse, although its use remains almost universal.

1.3.1 Pharmacokinetics

Pharmacokinetics describes the movement of drugs into and around the body. The pharmacokinetic properties of gentamicin include distribution and elimination, which are affected by infusion rate and have the potential to change significantly with age. In the neonatal population these parameters are complex and much of the current body of knowledge is a reflection of adult studies which have been extrapolated into younger populations. For the purposes of this work only intravenous administration of gentamicin will be considered, although in addition to a solution which can be administered by the intravenous, intramuscular and intrathecal routes it is also available as a cream, liquid and ointment for topical, ophthalmic and otic (auricular) administration, as well as a bone cement.

Although assimilation of gentamicin is 100% when given by the IV route, the process of drug administration is important to consider. In the case of neonates, the tubing and
connections within the intravenous delivery system contain a significant dead space volume in comparison to the size of the baby and several different fluids and medications are frequently given simultaneously. The method of delivery of gentamicin to neonates, whether via central line or peripheral IV line is not often considered as a pharmacokinetic parameter, but does have a significant impact on the amount of drug that is successfully administered, especially in low birth weight babies. This is illustrated by Sherwin et al., where it was shown that in extremely low birth weight babies only 60% of the intended gentamicin dose was recovered from the end of a simulated intravenous line after 60 minutes\(^2\). Additionally, work has been done which shows the effect that the angle of connections within the tubing has on the direction of anterograde or retrograde flow of the infusion, and also the effect of different fluid densities on the successful administration of medications given intravenously\(^3\).

Absorption following intravenous administration of gentamicin is considered to result in peak serum concentrations in 30-60 minutes in adults and older children\(^4\), but this time frame is yet to be confirmed in the neonatal population, particularly premature neonates.

Distribution is concerned with the dispersion of gentamicin throughout body compartments – that of blood stream where it is administered, and to the primary site of action in the tissue compartment. The volume of distribution varies with age, and for water soluble drugs such as gentamicin it is significantly larger in neonates than adults as they have a proportionally greater body water content\(^4\) and immature renal function\(^4\). In the case of septic neonates this volume of distribution increases further still, possibly because of fluid retention, interstitial oedema and capillary leakage\(^5\).

Gentamicin is renally eliminated. This is important to consider in the context of the relative renal insufficiency of premature neonates at birth, though renal function as
determined by glomerular filtration rate improves rapidly thereafter\textsuperscript{46}. Additionally, gentamicin is often prescribed on the first day of life when renal function is unknown, as serum creatinine is more reflective of the mother’s renal function than the child’s at this time. This poses unique problems in this vulnerable population and results in dosing recommendations being made based on gestational age, post-conceptual age (gestational age plus postnatal age) and weight as these have the best predictive capacity for clearance in premature neonates\textsuperscript{47}.

1.3.2 Pharmacodynamics

The pharmacodynamic properties, as well as describing the effect of gentamicin on the body, relate to the drug’s effects on the bacteria that they are prescribed to treat. Gentamicin is a bactericidal aminoglycoside antibiotic and exerts its effects on the 30s ribosomal subunit of bacterial cells, resulting in impaired protein synthesis. Gentamicin works synergistically with the beta-lactam antibiotics it is commonly prescribed with, as these disrupt the bacterial cell wall making it easier for gentamicin to reach its site of action.

The relationship of gentamicin to bacteria, in particular to the minimum inhibitory concentration (MIC, the lowest amount of drug required to limit the growth of an organism) of each organism, is another essential pharmacodynamic factor to consider. When dosing concentration-dependent drugs such as aminoglycosides, the ratio of maximum concentration of antibiotic ($C_{\text{max}}$) to MIC ($C_{\text{max}}$/MIC) and the area under the concentration-time curve over a 24 hour period ($\text{AUC}_{0-24}$) are important PK/PD measurements of efficacy\textsuperscript{48}, however the most appropriate $C_{\text{max}}$/MIC ratio has not been established\textsuperscript{49,50}.
1.3.3 Indications and dose recommendations

Gentamicin is indicated and commonly used for the treatment of suspected or proven neonatal sepsis, as discussed in section 1.1.4 Part D. It has wide gram negative coverage, including activity against *E. coli, Pseudomonas, Klebsiella* and *Serratia species* as well as activity against gram positive organisms such as *Staphylococcus*. Gentamicin is widely used in neonatal units across New Zealand and Australia as first line treatment for early onset sepsis, in conjunction with a beta-lactam antibiotic, though there is no consensus on the most appropriate dose or monitoring protocol and a wide variety of different regimes are in use currently\(^{51}\), as is also the case in the UK\(^{38}\).

It has now been established that extended interval dosing is safe and effective in the neonatal population as compared to the previous multiple daily dosing schedule\(^{42\ 52\ 53}\). This dosing regimen has benefits that include the ability to achieve higher peak concentrations (thus greater concentration-dependent bactericidal activity), along with being able to maximise the post-antibiotic effect of gentamicin, which is the continued ability to suppresses bacterial growth after concentrations drop below the MIC of the organism\(^{48}\). With a longer dosing interval there is also less potential for toxicity, as the trough concentrations are able to fall to an appropriately low level, and the development of adaptive resistance is minimised. Adaptive resistance occurs as the bacteria become more exposed to a particular antibiotic and are able to down-regulate transport of the antibiotic into their cells\(^{42}\), but it is thought that this ability decreases with a longer time interval between doses. However, individualised dosing, including extending the dosing interval is possibly a more reliable strategy for infants born at less than 32 weeks gestation\(^{53}\). In this way Alshaikh *et al.* based the dosing interval for an extended-interval dosing schedule on levels at 22 hours in infants <28 weeks gestation and showed that this

21
was able to achieve higher peak levels and no increase in trough concentrations when compared with traditional dosing\textsuperscript{54}.

The current recommendations in Dunedin hospital are based on the Neofax\textsuperscript{®} 2011 guidelines\textsuperscript{33} and are dosed according to Table 2, varying by gestational age, postnatal age and body weight.

Table 2: Dunedin Hospital NICU gentamicin dose calculator\textsuperscript{55}

<table>
<thead>
<tr>
<th>Gestational Age (Weeks)</th>
<th>Postnatal Age (days)</th>
<th>Dose (mg/kg)</th>
<th>Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;29*</td>
<td>0 to 7</td>
<td>5</td>
<td>Determined by level at 24 hours: See below</td>
</tr>
<tr>
<td></td>
<td>&gt;7</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>30 to 34</td>
<td>0 to 7</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>≥35</td>
<td>ALL</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Dose interval is determined by drug levels at 24 hours according Table 3 and peak and trough levels are monitored after the first and third doses.

Table 3: Dunedin Hospital NICU gentamicin dosing interval\textsuperscript{55}

<table>
<thead>
<tr>
<th>Level at 24 hours</th>
<th>Dose Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤2mcg/mL</td>
<td>24hr</td>
</tr>
<tr>
<td>2.1 - 3mcg/mL</td>
<td>36hr*</td>
</tr>
<tr>
<td>3.1 - 4mcg/mL</td>
<td>48hr*</td>
</tr>
<tr>
<td>&gt;4mcg/mL</td>
<td>Consider alternative antibiotic e.g. cefotaxime</td>
</tr>
</tbody>
</table>

* If interval >24h, discuss with paediatrician whether an alternative antibiotic is required

In many units, it is standard practice to administer gentamicin intravenously over a 30-minute infusion time. However, administration by bolus injection has been shown in \textit{in}
vitro studies to result in a greater proportion of gentamicin being recovered when prescribed in extremely low birth weight babies when compared with administration by continuous infusion, thus it is administered as a slow IV push over 3 – 5 minutes in Dunedin NICU.

1.3.4 Adverse effects
In 1972 the World Health Organisation defined an adverse drug reaction as any response to a drug which is “noxious, unintended and occurs at doses used in man”\(^\text{56}\). The two potentially significant adverse effects of gentamicin are nephrotoxicity and ototoxicity, which predominantly occur due to impaired clearance, resulting in an accumulation of the drug in the patient. In a 2011 Cochrane review comparing once daily dosing with multiple doses per day of gentamicin there were no nephrotoxic results seen in either group and no statistically significant difference in auditory toxicity found\(^\text{53}\).

Nephrotoxicity develops predominantly as the result of accumulation of gentamicin in the proximal tubules of the kidney, inducing cellular apoptosis and necrosis, but can also occur due to reduced glomerular filtration by induction of mesangial contraction in the glomerulus, and reduced renal blood flow due to increased vascular resistance in the renal vascular bed\(^\text{57}\). The observed physiological manifestations of this nephrotoxicity are described as non-oliguric renal failure with a decrease in glomerular filtration rate as measured by creatinine clearance\(^\text{58}\). Based on adult studies Wargo et al. argue that the empiric use of aminoglycosides for less than 7 days has minimal risk of nephrotoxicity, especially when extended interval dosing and therapeutic drug monitoring are used\(^\text{57}\). The cumulative effects of gentamicin resulting in nephrotoxicity are thought to arise from high trough concentrations due to accumulation of high concentration of the drug in the tissues\(^\text{59}\), and thus checking serum levels before the administration of the next dose, once steady state has been established, is an important practice. Nephrotoxicity is also felt to
be more of an issue when gentamicin is prescribed concurrently with other nephrotoxic drugs such as furosemide. The renal impairment that can be noted on routine blood monitoring is considered reversible on stopping the nephrotoxic drug(s)\textsuperscript{33}.

Conversely, ototoxicity, is irreversible and harder to screen for. This is felt to develop as a result of free radical production and damage to cochlear and vestibular hair cells\textsuperscript{60}. The cochlear cells that are damaged first are associated with high-frequency hearing loss, and though audiology screening is done as part of the newborn hearing testing before an infant leaves the NICU, it is often difficult to know what the clinical significance of findings is, particularly if they are mild, until months or years later as the child's language development progresses. A Christchurch study found no evidence of any increased risk of hearing impairment in neonates treated with gentamicin for early onset sepsis over a 5 year period\textsuperscript{61}; nevertheless ototoxic drugs remain a risk factor which is highlighted in the universal newborn hearing screen. Another risk factor for the development of sensorineural hearing loss is the presence of a mitochondrial A1555G mutation\textsuperscript{62}, suggesting that care should be taken when prescribing aminoglycosides to babies in whom a family history of progressive hearing loss exists\textsuperscript{42}. The vestibular effects of gentamicin toxicity are more subtle and include symptoms such as balance disturbance, visual disturbance and tinnitus\textsuperscript{63}. Gentamicin is felt to be more vestibulotoxic than cochleotoxic, and the risk of toxicity increases with duration of exposure to the drug\textsuperscript{60}, though no current screening test exists to detect this potential damage in newborns.

1.3.5 Therapeutic drug monitoring

The purpose of therapeutic drug monitoring is to monitor the efficacy and toxicity of gentamicin. The peak serum concentration is used as a proxy for tissue concentration and clinical efficacy correlates well with this level\textsuperscript{43}. A $C_{\text{max}}/\text{MIC}$ ratio of 8-10 is targeted to ensure maximum bactericidal activity\textsuperscript{43, 50} as a $C_{\text{max}}/\text{MIC}$ ratio of $>10$ has been shown to
have an Odds Ratio of response to treatment of 8.41 (as compared to Odds Ratio of response to treatment of 1.83 when a $C_{\text{max}}$/MIC ratio of 4-6 was targeted). In order to get accurate measurements of the peak concentration, it is imperative that pharmacokinetic aspects such as drug administration are accurately understood. Thus far, assumptions have been made for neonates based on extrapolated data from adults, and peak levels are routinely taken 30 to 60 minutes after the drug is administered. Whether this is an accurate reflection of the true serum peak concentration of gentamicin in neonates remains to be seen.

Trough levels are felt to reflect the potential to develop toxicity as a result of gentamicin accumulation. These are traditionally taken ½ hour before the next dose is due, and the practice in Dunedin NICU is to withhold the dose until the trough level is $<2 \, \mu g/mL$. With changing the dosing recommendations from traditional interval dosing to extended interval dosing there have been a number of studies comparing gentamicin dosing schedules in premature babies which have generally shown that low birth weight babies receiving a high dose every 48 hours attain therapeutic peak levels and low trough concentrations and are therefore safe to use.

Steady state concentrations are traditionally considered to exist after 4 half-lives of the drug have passed which, in the case of gentamicin, is generally accepted as after the third dose. Though therapeutic drug monitoring usually occurs around this dose, it is the experience of practitioners at Dunedin NICU that considerable variability exists in aminoglycoside pharmacokinetics in neonates so levels for therapeutic drug monitoring are taken after both the first and third doses.
1.3.6 Summary
Gentamicin is a frequently used antibiotic in the NICU setting. Its traditional pharmacokinetic and pharmacodynamic properties are well understood in the older population, but in neonates there is more still to be learned. Additionally, the different methods of IV gentamicin delivery (peripheral or central line; differences in flow rate, type of primary infusion fluid; and volume of flush) are likely to result in further alterations to the pharmacokinetics of the drug in this population. Gentamicin toxicity and efficacy correlate with measurements of trough and $C_{\text{max}}$ concentrations respectively. In these circumstances, the trough concentration is useful for therapeutic drug monitoring to prevent adverse effects such as nephrotoxicity and ototoxicity occurring, but further research is needed to accurately determine aspects of efficacy.
1.4 What is not known about this topic

Gentamicin for intravenous administration in the treatment of early-onset neonatal sepsis is well established, though there are many aspects of its use that are incompletely understood. Currently a range of dosing and monitoring protocols exist, which is likely a reflection of the paucity of evidence in the literature regarding the administration of this antibiotic. This is especially true in the case of extremely preterm neonates where PK/PD parameters are different from those in the older paediatric and adult populations.

The following are examples of knowledge gaps in the administration of gentamicin in the NICU setting:

- The dose in mg/kg needed to consistently achieve a therapeutic peak
- Target $C_{\text{max}}$ and $C_{\text{max}}/\text{MIC}$ ratio
- The most appropriate location to give a dose of gentamicin (PIV, UVC or PICC) and where in the intravenous architecture to administer this dose
- The size of the flush to give following a dose of gentamicin
- The effect that very slow flow rates have on drug delivery

These factors need research evidence and clinical correlation before a robust dosing and monitoring protocol for gentamicin can be proposed.
1.5 Objective of this research
The overall objective of this research project is to understand the influence of background infusion rate and flush volume on recovery of gentamicin when it is administered by UVC to premature neonates and to compare this to PIV administration of gentamicin in NICU.

1.5.1 Specific aims:
1. To investigate the current neonatal nursing practice for UVC administration of gentamicin in the first 48 hours after birth and to determine the size of the flush volume administered following the medication.
2. To describe what flush volume is needed to recover the entire gentamicin dose when it is administered as a bolus through a peripheral IV line.
3. To find out how long it takes a dose of gentamicin to be given in its entirety when administered into a UVC with a slow flowing background infusion rate.
4. To appreciate what percentage of a dose of gentamicin is delivered 1 hour after administration through a UVC with different flow-rates.

It is beyond the scope of this dissertation to use the information gained here to predict the appropriate time for measuring peak serum gentamicin concentrations in premature babies, but it is hoped that this work will be used in conjunction with previous studies to contribute to this model development. In time the information gained here will be used to more accurately dose, monitor and treat preterm babies with early-onset sepsis.
2 Materials

2.0 Chemical compounds
The antibiotic for injection was gentamicin 20mg/2mL Pediatric injection vial (APP pharmaceuticals, USA) followed by a flush of 0.9% sodium chloride [NaCl] (Ajax Finechem, New Zealand). The internal standard was tobramycin (Sigma-Aldrich, China), and standard curve was prepared from gentamicin sulphate (Sigma-Aldrich, China). The derivatisation reaction was completed with boric acid [H$_2$BO$_3$] (Ajax Finechem, New Zealand), sodium tetraborate [Na$_2$B$_4$O$_7$] (Ajax Finechem, New Zealand), 9-fluorenylmethyl chloroformate [C$_{15}$H$_{11}$ClO$_2$] (Fluka, Switzerland) and glycine [NH$_2$CH$_2$COOH] (BDH AnalR, England). The mobile phase was 90% acetonitrile (HPLC grade LiChrosolv, Germany) in distilled deionised water (MilliQ, 18.2 Ohms), degassed by vacuum filtration through a 0.45 µm filter.

2.1 Clinical equipment
The following is a description of the clinical equipment used for the simulation experiments, identical to the Dunedin NICU setup. CareFusion NZ are gratefully acknowledged for their loan of the Alaris® GH plus pump.

The peripheral IV line was 24GA 0.75IN BD Insyte ™ IV catheter (BD, Singapore) and the UVC was 3.5Fr 15IN polyurethane dual-lumen umbilical vessel catheter (Tyco Healthcare, USA). The components of IV tubing were SmartSite ® Needle-Free Valve Port (CardinalHealth), Baxter Interlink System T-connector extension set (Baxter Healthcare Corporation USA), Safti-ject SV® (Mini bore leur lock extension set with a Safti-ject SV® swabable valve needleless connector and a back check valve and 0.2 micron air eliminating filter)(CODAN US Corporation for REM Systems Ltd, New Zealand). This was plugged for the PIV experimental series, and attached to a 140 cm
minimum volume extension tubing (B. Braun, Germany) connected to an Alaris® GH plus pump (CareFusion) for the UVC experiments. Dextrose 10% (Baxter, Australia) was the primary fluid in the tubing. Syringes used for administration of gentamicin and 0.9% saline flush were 1 mL leur lock tip (BD, Singapore) and 5 mL leur lock, latex free (BD, Singapore) respectively.

2.2 Laboratory equipment
Samples were collected in 1.7 mL microtubes (Axygen, USA) or 5 mL polypropylene screw top tubes (Sarstedt, Australia). Dilutions and preparation for HPLC analysis was made using eppendorf pipettes and 200 µL and 1000 µL pipet tips (Axygen, USA).

A SHIMADZU HPLC instrument was used. This consisted of a RF-10Axl fluorescence detector (λ excitation 260 nm, λ emission 315 nm), CTO-20A column oven (set at 30°C), SIL-20AC autosampler, LC-20AD liquid chromatograph and DGU-20A5 degasser. The column was a C18 (2) Luna, 4.6 mm x 150 mm, particle size 5 microns, pore size 100 angstroms. Flow rate was 1 mL/hr. The HPLC mobile phase was 90% acetonitrile in distilled deionised water, degassed by vacuum filtration through a 0.45 µm filter and administered with a gradient system.
3 Method Part A: Neonatal nurses survey

3.1 Overview
Part A of this project was completed with the purpose of gaining baseline information about how gentamicin was currently being given in clinical practice. Previous work in this department\(^2\)\(^{30}\)\(^{41}\) has simulated gentamicin delivery using a variety of different approaches as discussed in section 1.2.5, but for this current work to be clinically relevant it was felt that the next set of experiments should reflect contemporary clinical practice. Reflection on current nursing care practice will also be useful as a baseline for the development of a ‘best practice’ approach to gentamicin administration for premature neonates.

3.2 Study design
A questionnaire (Section 6.2) was designed with guidance from medical and research nursing staff in the Dunedin NICU. This was a self-administered written survey and responses were recorded anonymously. There were a range of question types, with multi-choice, free text written and numerical information sought.

Ethics approval was discussed but not formally requested due to the quality improvement nature of this work, and no reference to patient notes or direct impact on patient care occurred.

3.3 Participants
All nurses working at Dunedin NICU were invited to participate, in writing and verbally, by the primary investigator and associate charge nurse manager on each shift. Discussion was permitted within the nursing team and reference to the unit drug administration protocol (Section 6.1) may have occurred. Information was gathered over a 17 day period from 23/04/14 to 09/05/14.
3.4 Objectives

The primary aim was to determine which lumen of a double lumen umbilical venous catheter (UVC) would be used for administration of gentamicin to a premature baby in the first 24 – 48 hours after birth as depicted in Figure 1.

Figure 1: Double lumen umbilical venous catheter
with thanks to A/Prof Natalie Medlicott

The primary lumen contained an infusion of total parenteral nutrition (TPN) and lipid at a rate of 90 mL/kg/day (minus 0.5 mL/hr to account for the fluid running through the second lumen) and the second lumen contained 10% dextrose at a consistent rate of 0.5 mL/hr as is standard practice in this neonatal unit. Locations for bolus drug administration were labelled B on the primary lumen and A on the secondary lumen.

Secondary information regarding the flush volume of normal saline that would be given following the dose of gentamicin at different sites of administration (both on the UVC and also when given peripherally) was also collected.
3.5 Survey questions

Three questions were asked in the survey in order to determine where a dose of gentamicin would be given to a premature baby in their first days of life. Two different scenarios – that of an extremely low birth weight baby (700 g, which is on the 50th centile for babies born at 24 weeks gestation) and a low birth weight baby (1800 g, on the 50th centile for babies born at 32 weeks gestation) – were used as clinical examples. The total fluid volume of 90 mL/kg/day was chosen as the baseline flow rate because it represents a common fluid prescription for premature babies in the 48 hours after birth.

In the first question (Figure 2) only the option of using the UVC for administration of gentamicin was offered.

![Figure 2: Survey question 1](image)

In the second question, nurses were asked whether the presence of a peripheral IV line in addition to the UVC would alter the answers they gave in the first question. A free text response was invited to allow them to expand on reasons behind these answers.

The third question asked for the nurses to fill in the table below with the volume of 0.9% saline flush that they would use in each of the scenarios listed.
Table 4: Survey question 2 - flush volumes

<table>
<thead>
<tr>
<th>Case</th>
<th>Plugged peripheral intravenous line <em>in situ</em></th>
<th>Gentamicin bolus dose given at point A</th>
<th>Gentamicin bolus dose given at point B</th>
</tr>
</thead>
<tbody>
<tr>
<td>700g 24-weeker receiving 90ml/kg/day of total fluid (2.6ml/hr)</td>
<td>............mL</td>
<td>............mL</td>
<td>............mL</td>
</tr>
<tr>
<td>1800g 32-weeker receiving 90ml/kg/day of total fluid (6.7ml/hr)</td>
<td>............mL</td>
<td>............mL</td>
<td>............mL</td>
</tr>
</tbody>
</table>

3.6 Analysis

Results of the data received were analysed using Microsoft Excel 2013. They have been discussed by departmental medical and research staff in addition to presentation and discussion with neonatal unit nursing staff. Future practice may be informed by the results of experimental data that was found as a result of this nursing survey.
3 Method Part B: Experimental Work

3.7 Overview
The second part of this project intended to more fully explore the influence that flush volume and flow rate had on the amount of gentamicin that was successfully delivered to a premature baby. Two studies were designed to illustrate the recovery of gentamicin when it was delivered into a peripheral IV line and when the same dose was delivered into an umbilical central line (UVC). Analysis of these simulation experiments was completed in the laboratory using reverse-phase high pressure liquid chromatography.

3.8 Study design
Two experimental studies were designed, based on the results of the aforementioned NICU nurses questionnaire and with previously performed studies as a model. Both experiments were run in the laboratory and based on a set-up of how gentamicin would be administered intravenously to premature infants in the Dunedin Hospital NICU.

The first of these experiments was designed to simulate gentamicin given via a peripheral intravenous line (PIV) and the second with a central line (an umbilical venous catheter, UVC). Different flush volumes were used within each experiment to compare the effect that this had on the percentage of the intended dose of gentamicin that was recovered in each series of experiments. The first simulation was completed to ascertain what flush volume was needed to recover the entire gentamicin dose when administered through a peripheral IV line. The second simulation was designed to appreciate what percentage of a dose of gentamicin was delivered over 1 hour after administration through a UVC with a slow flow-rate primary infusion line.
3.9 Aim
The aim of both parts of this experimental study was to ascertain whether different flush volumes affected the proportion of gentamicin dose recovered after administration through a simulated intravenous line.

3.10 Preparation of stock solutions
The following solutions were prepared and used for gentamicin analysis as described in section 3.12:

- Gentamicin sulphate stock solution (1 mg/mL): 5 mg gentamicin sulphate powder was dissolved in deionised water to 5 mL using a volumetric flask. Aliquots of 1 mL were transferred into eppendorf tubes and stored at -20 °C.

- Internal standard: Tobramycin (0.5 µg/mL): 1 mg tobramycin powder was dissolved in deionised water to 1 mL using a volumetric flask. This stock solution was diluted to a working solution in stages by: 50 µL of 1 µg/mL solution diluted to 1000 µL with deionised water (=50 µg/mL), then 100 µL of 50 µg/mL solution diluted to 10 mL with deionised water (=0.5 µg/mL). Stored in fridge at 4 °C.

- 9-Fluorenlymethyl chloroformate (FMOC-Cl) (2.5 mM in acetonitrile): 12.935 mg FMOC-Cl (MW 258.7 g/mol) powder was dissolved in acetonitrile to 20 mL using a volumetric flask. Stored in fridge at 4 °C, protected from light.

- Glycine (0.1 M): 150.14 mg glycine (MW 75.07 g/mol) powder was dissolved in deionised water to 20 mL in a volumetric flask. Stored in fridge at 4 °C.

- Borate buffer (0.2 M, pH 8.9): 50 mL solution A + 42.5 mL solution B, diluted to 200 mL with deionised water. pH confirmed 8.9
Solution A: 0.2 M boric acid (MW 61.83 g/mol = 61.83 g/1000 mL): 1.24 g boric acid powder diluted to 100 mL with deionised water in a volumetric flask.

Solution B: 0.05 M sodium tetraborate (MW 381.37 = 381.37 g/1000 mL): 1.9069 g sodium tetraborate powder diluted to 100 mL with deionised water in a volumetric flask.

- (v/v) Acetonitrile: Borate buffer (0.2 M, pH 8.9): 200 mL solution prepared by combining 100 mL acetonitrile + 100 mL borate buffer

3.11 Simulation experiments

3.11.1 Peripheral intravenous line

For the peripheral IV line component, six gentamicin dose/flush volume conditions were proposed as described in Table 5:

Table 5: PIV gentamicin dose/flush volume combinations

<table>
<thead>
<tr>
<th>2 mg dose</th>
<th>5 mg dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mL flush</td>
<td>1 mL flush</td>
</tr>
<tr>
<td>2 mL flush</td>
<td>2 mL flush</td>
</tr>
<tr>
<td>4 mL flush</td>
<td>4 mL flush</td>
</tr>
</tbody>
</table>

Each was completed with six repetitions using the same intravenous line components, with a fresh set-up for each dose/volume combination. The order of testing was determined using a random number generator in Microsoft Excel 2013. Each component of the peripheral intravenous line was weighed, both empty and full of distilled water to allow calculation of the volume within the IV line. Numbered syringes with drug for administration and 0.9% saline flush were prepared. These were weighed empty, after
filling with the drug or flush solution and again after administration through the line to determine how much of the drug and flush was successfully administered. The line was flushed with 5 mL normal saline (and discarded) before each drug dose was administered and new components were used for each dose/flush volume combination.

The model peripheral intravenous line was assembled on a flat workspace, with numbered plastic tubes to collect samples in as shown in Figures 3 and 4.

Figure 3: Peripheral IV line simulation

Figure 4: Components of PIV set-up
Gentamicin was administered by slow push over 2-3 minutes, followed by a saline flush over the same duration. Samples were stored in the freezer at -20°C until analysis by reverse-phase high performance liquid chromatography (HPLC).

3.11.2 Umbilical Venous Catheter (UVC)

For the UVC simulation series four dose/volume combinations were proposed as described in Table 6:

Table 6: UVC gentamicin dose/flush volume combinations

<table>
<thead>
<tr>
<th>UVC Flow rate</th>
<th>Gentamicin Dose</th>
<th>Flush Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mL/hr</td>
<td>2 mg</td>
<td>1 mL</td>
</tr>
<tr>
<td></td>
<td>2 mg</td>
<td>2 mL</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
<td>1 mL</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
<td>2 mL</td>
</tr>
</tbody>
</table>

Each was completed with 6 repetitions, with the order of experiments determined using a random number generator in Microsoft Excel 2013. Each component of the line (UVC, smart site, filter, tubing) was weighed both empty and full of distilled water to allow calculation of the volume within the IV line. Numbered syringes with drug for administration and 0.9% sodium chloride flush were also prepared and were weighed empty, after filling with the drug or flush, and again after administration. New UVC components were used for each dose/flush volume combination experiment.
The model UVC was assembled on a flat workspace, with the carrier fluid pump containing 10% dextrose slightly raised on a pump stand as documented in Figures 5 and 6.

Figure 5: UVC simulation set-up

Figure 6: Pump and UVC simulation

Numbered plastic tubes in which to collect samples every 5 minutes for an hour after drug administration were prepared. Gentamicin was administered by slow push over 2-3 minutes, followed by a saline flush over the same duration. A timer was set for 5 minute intervals and the end of each increment the catheter was moved to the next tube. The line was purged with 10% dextrose (and discarded) between each repetition. Samples were
stored in the freezer at -20°C until analysis by reverse-phase high pressure liquid chromatography.

In order to ascertain how long the full dose of gentamicin took to give, one further experiment was done for each of the 4 gentamicin dose/flush volume combinations. This involved delivery of dose and flush as previously described, with a continuous background infusion rate of 0.5 mL/hr. Fluid was collected from the end of the UVC in half-hour increments for 2 hours and the line was purged with 10% dextrose through the pump after this and collected to be analysed.

3.12 Analysis of samples
Reverse-phase high pressure liquid chromatography (HPLC) analysis was conducted using a derivatisation reaction previously described and validated by Sherwin et al.\textsuperscript{2}. Derivatisation was needed in order to add a fluorescent tag to the amine groups of the gentamicin molecule to allow it to be detected and depicted on a chromatograph.

A separate standard curve of gentamicin sulphate stock solution (1 mg/mL) was prepared on each day of HPLC analysis in concentrations of 2 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 50 µg/mL and 100 µg/mL in 10% dextrose. For the derivatisation reaction, 20 µL of each gentamicin standard (or experimental sample after dilution) was added to 20 µL of internal standard solution (tobramycin 0.5 µg/mL) and 980 µL of acetonitrile:borate buffer (v/v pH 8.9). 200 µL FMOC-Cl (2.5 mM in acetonitrile) was added after mixing and the samples were then mixed again and incubated at 30°C for 20 minutes in a water bath. Following incubation, 50 µL of glycine (0.1 M) was added to stop the derivitisation reaction and samples were analysed in the HPLC instrument.

The area under the C1a component peak of gentamicin which appeared on the chromatograph was measured and recorded as a ratio to the area under the tobramycin
internal standard peak. The concentration of gentamicin present in each sample was derived from the slope of the standard curve for each day.

Quality control samples (at low, medium and high concentrations – 5 µg/mL, 40 µg/mL and 90 µg/mL respectively) were prepared and analysed each day also to ensure intraday and interday variability.
4 Results: Neonatal nurses survey

4.1 Overview: NICU nurses survey

In order to clarify the actual routes and methods of intravenous gentamicin administration in Dunedin NICU a self-administered questionnaire was performed. This was done as a quality improvement exercise to document current nursing care practice. Thirty-seven out of forty-two nurses (88%) currently employed at Dunedin NICU returned the questionnaire. This study highlighted variations in clinical practice for gentamicin delivery within our unit which may impact on the pharmacokinetics of gentamicin in neonates.

4.1.1 Site of gentamicin dose administration

When asked about an extremely low birth weight 24-week gestation baby, 92% (34 nurses) responded that they would administer a dose of gentamicin through the second lumen of the UVC which had a slower flow rate (0.5 mL/hr) compared to 8% who administered the drug through the primary lumen (2.1 mL/hr). In the second scenario the same drug (gentamicin) and options of site to administer were offered and in this case 95% (35 nurses) answered that they would also administer the drug through the second lumen (flow rate 0.5 mL/hr vs 6.2 mL/hr through primary lumen).

Free text answers were invited when the scenario was altered to include a peripheral intravenous line in addition to the double lumen UVC. The majority of responses indicated that the peripheral line would be used in preference to the UVC for gentamicin administration. The most common reason justifying this choice was to lessen the infection risk to the baby that may occur with repeated interruptions to the sterile central line.
4.1.2 Flush volume to follow gentamicin dose

Secondary information gained from this survey demonstrated that larger flush volumes were likely to be used when gentamicin was administered through the peripheral IV line and smaller flush volumes when it was given via the UVC as shown in Figure 7. In both scenarios the most premature, lowest birth weight neonate was likely to receive a smaller saline flush following antibiotic administration.
Figure 7: Nursing response regarding flush volumes that would be administered following a dose of gentamicin given into (A) a plugged PIV and (B) point A in the UVC set up.
As these data were collected in free-text format there were additional flush volumes that were also documented. In administering gentamicin via a peripheral IV line variable flush volumes were 0.5 mL before/1 mL after at 24 weeks gestation, and 0.5 mL before/2 mL after the gentamicin at 32 weeks gestation. Additionally, five ‘other’ volumes were described in the free text response box for PIV administration – four of “would discuss with medical team” and one “wouldn’t use PIV”. For the UVC, respondents replied that saline flushes of 1 mL before and after gentamicin, 0.5 mL before and after, and 0.5 mL before/1 mL after would be used at both gestations and in one case (in the 24-week gestation infant) the flush volume was unclear.

Table 7 shows the average flush volumes used in each of the scenarios given in the survey. Despite the volume of the flush appearing smaller in the most premature baby, as percentages of total daily fluid intake (based on 90 mL/kg/day calculations) these flush volumes are greatest for the more premature baby. For example, when given via the PIV line, the flush volume of 2.4 mL equates to 3.8% of the total fluid allowance for a 24 hour period in the 700 g 24-week gestation baby. The flush volumes estimated by NICU staff in this survey represent 1.9 – 3.8% of the 90 mL/kg/day fluid amount for the 24 week gestation baby and 0.9 – 2% of the total daily requirement in the 32 week gestation baby.

Table 7: Average flush volumes given to neonates at different sites of administration

<table>
<thead>
<tr>
<th>Site of administration of gentamicin</th>
<th>24-week gestation, 700g</th>
<th>32- week gestation, 1800g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral intravenous line</td>
<td>2.4 mL</td>
<td>3.2 mL</td>
</tr>
<tr>
<td>Point A (secondary lumen, 0.5mL/hr flow rate)</td>
<td>1.35 mL</td>
<td>1.74 mL</td>
</tr>
<tr>
<td>Point B (primary lumen, 90 mL/kg/day total fluids)</td>
<td>1.17 mL</td>
<td>1.42 mL</td>
</tr>
</tbody>
</table>

4.1.3 Summary

The findings of this study demonstrated that the slow-flowing, relatively free arm of the UVC was most commonly used to administer gentamicin when prescribed in extremely low birth weight premature neonates in Dunedin NICU. If a peripheral intravenous line was also
available this was frequently used in preference to the UVC due to nursing staff reluctance to compromise the sterile integrity of the central line.

Flush volumes following the administration of gentamicin were smaller for the most premature baby but represented a greater proportion of total daily fluid intake in this group.
4 Results: Experimental work

4.2 Overview

The experimental work in Part B of this thesis builds on the background work from Part A in order to construct clinically-relevant simulation experiments for gentamicin administration to premature neonates. Two aspects of gentamicin administration are considered here – by peripheral IV line and through an umbilical central line – with different saline flush volumes used following antibiotic administration.

4.2.1 Validation

The gentamicin assay and HPLC method was validated with three separate runs on consecutive days by preparing quality control samples to detect precision and accuracy. Six quality control samples, at nominal concentrations of 5 µg/mL (low), 40 µg/mL (medium) and 90 µg/mL (high) of gentamicin sulphate in 10% dextrose were prepared fresh each day. Examples of standard curves generated in duplicate with 6 data points (intended concentrations of 2 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 50 µg/mL and 100 µg/mL) are shown in Figure 8. The average retention time was 4.3 minutes for the internal standard and 11.3 minutes for the gentamicin C1a peak. This peak was used as the reference point in order to maintain consistency with previous studies\textsuperscript{2,41}. Additionally, the change in retention time with three different concentrations of acetonitrile:water mobile phase was investigated separately using gradient channels on the HPLC.
Figure 8: Examples of gentamicin sulphate standard curves prepared in 10% dextrose for analysis of (A) UVC 2 mg dose, 1 mL flush 3rd repetition (B) PIV 2 mg dose, 1 mL flush and (C) UVC 2 mg dose, 1 mL flush 4th and 5th repetition samples
4.2.2 Gentamicin assay

Tables 8, 9 and 10 show results for the validation data at each of the nominal values of low, medium and high concentrations. The inter-day variability is consistently <15% but intra-day variability fluctuates from 0.37% to 21.29%, with the greatest variability observed on one replication at the lowest concentration.

Table 8: Low (5 µg/mL) concentration validation data; concentration of gentamicin sulphate derived by day and replication attempt

<table>
<thead>
<tr>
<th>Replication</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>4.61</td>
<td>5.74</td>
<td>4.51</td>
<td>4.12</td>
<td>4.76</td>
<td>5.1</td>
<td>4.81</td>
<td>0.56</td>
<td>11.61</td>
</tr>
<tr>
<td>Day 2</td>
<td>4.48</td>
<td>4.94</td>
<td>4.95</td>
<td>5.88</td>
<td>5.38</td>
<td>4.67</td>
<td>5.05</td>
<td>0.51</td>
<td>10.06</td>
</tr>
<tr>
<td>Day 3</td>
<td>4.06</td>
<td>4.3</td>
<td>4.78</td>
<td>4.16</td>
<td>4.34</td>
<td>4.03</td>
<td>4.28</td>
<td>0.28</td>
<td>6.44</td>
</tr>
<tr>
<td>Mean</td>
<td>4.38</td>
<td>4.99</td>
<td>4.75</td>
<td>4.72</td>
<td>4.83</td>
<td>4.60</td>
<td>4.28</td>
<td>0.28</td>
<td>6.44</td>
</tr>
<tr>
<td>SD</td>
<td>0.29</td>
<td>0.72</td>
<td>0.22</td>
<td>1.00</td>
<td>0.52</td>
<td>0.54</td>
<td>1.00</td>
<td>0.52</td>
<td>0.54</td>
</tr>
<tr>
<td>%CV</td>
<td>6.56</td>
<td>14.45</td>
<td>4.67</td>
<td>21.29</td>
<td>10.84</td>
<td>11.70</td>
<td>21.29</td>
<td>10.84</td>
<td>11.70</td>
</tr>
</tbody>
</table>

Table 9: Medium (40 µg/mL) concentration validation data; concentration of gentamicin sulphate derived by day and replication attempt

<table>
<thead>
<tr>
<th>Replication</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>40.78</td>
<td>42.34</td>
<td>43.06</td>
<td>43.54</td>
<td>42.27</td>
<td>42.25</td>
<td>42.37</td>
<td>0.94</td>
<td>2.21</td>
</tr>
<tr>
<td>Day 2</td>
<td>41.7</td>
<td>39.35</td>
<td>42.77</td>
<td>42.81</td>
<td>44.9</td>
<td>42.16</td>
<td>42.28</td>
<td>1.81</td>
<td>4.27</td>
</tr>
<tr>
<td>Day 3</td>
<td>39.35</td>
<td>39.96</td>
<td>43.02</td>
<td>40.99</td>
<td>40.27</td>
<td>39.3</td>
<td>40.48</td>
<td>1.39</td>
<td>3.44</td>
</tr>
<tr>
<td>Mean</td>
<td>42.77</td>
<td>40.55</td>
<td>42.95</td>
<td>42.45</td>
<td>42.48</td>
<td>41.24</td>
<td>42.77</td>
<td>1.39</td>
<td>3.44</td>
</tr>
<tr>
<td>SD</td>
<td>1.18</td>
<td>1.58</td>
<td>0.16</td>
<td>1.31</td>
<td>2.32</td>
<td>1.68</td>
<td>1.18</td>
<td>1.58</td>
<td>0.16</td>
</tr>
<tr>
<td>%CV</td>
<td>2.92</td>
<td>3.90</td>
<td>0.37</td>
<td>3.09</td>
<td>5.47</td>
<td>4.07</td>
<td>2.92</td>
<td>3.90</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Table 10: High (90 µg/mL) concentration validation data; concentration of gentamicin sulphate derived by day and replication attempt

<table>
<thead>
<tr>
<th>Replication</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>92.55</td>
<td>95.47</td>
<td>94.71</td>
<td>98.23</td>
<td>102.39</td>
<td>89.12</td>
<td>95.41</td>
<td>4.58</td>
<td>4.80</td>
</tr>
<tr>
<td>Day 2</td>
<td>90.7</td>
<td>92.23</td>
<td>101.28</td>
<td>92.95</td>
<td>92.12</td>
<td>93.57</td>
<td>93.81</td>
<td>3.78</td>
<td>4.03</td>
</tr>
<tr>
<td>Day 3</td>
<td>85.65</td>
<td>91.2</td>
<td>83.91</td>
<td>88.27</td>
<td>89.75</td>
<td>91.16</td>
<td>88.32</td>
<td>3.00</td>
<td>3.40</td>
</tr>
<tr>
<td>Mean</td>
<td>89.63</td>
<td>92.97</td>
<td>93.30</td>
<td>93.15</td>
<td>94.75</td>
<td>91.28</td>
<td>89.63</td>
<td>3.00</td>
<td>3.40</td>
</tr>
<tr>
<td>SD</td>
<td>3.57</td>
<td>2.23</td>
<td>8.77</td>
<td>4.98</td>
<td>6.72</td>
<td>2.23</td>
<td>3.57</td>
<td>2.23</td>
<td>8.77</td>
</tr>
<tr>
<td>%CV</td>
<td>3.98</td>
<td>2.40</td>
<td>9.40</td>
<td>5.35</td>
<td>7.09</td>
<td>2.44</td>
<td>3.98</td>
<td>2.40</td>
<td>9.40</td>
</tr>
</tbody>
</table>
4.2.4 HPLC gradient method

The retention time for gentamicin sulphate shortened with increasing concentrations of acetonitrile (ACN) in the HPLC mobile phase as shown in the Figure 9. To ensure reproducibility on successive days of experiments a gradient system was used throughout this project. This was done in order to decrease the potential for error arising from inaccurate preparation of the mobile phase that may occur when using a premixed solution.

Figure 9: Retention time vs concentration of acetonitrile in HPLC mobile phase for both internal standard (lower line) and gentamicin sulphate (upper line).

Examples of the chromatogram generated by HPLC with fluorescence is shown in Figure 10 at three different concentrations. On the left, the tobramycin (0.5 µg/mL) internal standard peak is visible and further to the right of the chromatogram the three components of gentamicin (C1a, C2 and C1 respectively) are visible as separate peaks in this order. The area under the C1a peak as a ratio to the area under the internal standard was used as a marker of the total gentamicin concentration in the samples.
Figure 10: HPLC chromatogram of tobramycin internal standard (0.5 µ/mL) on left and the 3 components of gentamicin sulphate in concentrations of (A) 5 µg/mL (B) 20 µg/mL and (C) 100 µg/mL on the right of the x axis.
4.3 PIV infusion studies

Six experimental studies were completed during the peripheral IV line simulation series. As described in section 3.11.1 (Table 5), these consisted of combinations of two nominal gentamicin doses (2 mg and 5 mg) with three flush volumes (1 mL, 2 mL and 4 mL). Tables 11 and 12 illustrate the actual dose of gentamicin and flush volume that was administered in these experiments as measured by weight of the syringes. For the 2 mg series, the actual gentamicin dose administered is consistently greater than the nominal dose, by 0.1–1.3%, whereas in the 5 mg series the dose is less than 0.25% below the nominal dose. Flush volumes vary also, from -0.03% of the nominal volume to +1.73%.

Table 11: Measured drug and flush volumes for six replications of each drug/flush combination in 2 mg PIV experimental series with flush volumes of 1 mL, 2 mL and 4 mL.

<table>
<thead>
<tr>
<th></th>
<th>1 mL</th>
<th>2 mL</th>
<th>4 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug (mg)</td>
<td>Flush (mL)</td>
<td>Drug (mg)</td>
</tr>
<tr>
<td>1</td>
<td>2.01</td>
<td>0.01</td>
<td>2.02</td>
</tr>
<tr>
<td>2</td>
<td>2.02</td>
<td>0.01</td>
<td>2.03</td>
</tr>
<tr>
<td>3</td>
<td>2.01</td>
<td>0.01</td>
<td>2.02</td>
</tr>
<tr>
<td>4</td>
<td>2.02</td>
<td>0.01</td>
<td>2.03</td>
</tr>
<tr>
<td>5</td>
<td>2.01</td>
<td>0.01</td>
<td>2.02</td>
</tr>
<tr>
<td>6</td>
<td>2.02</td>
<td>0.01</td>
<td>2.03</td>
</tr>
<tr>
<td>Mean</td>
<td>2.01 mg 1.02 mL</td>
<td>2 mg 2.01 mL</td>
<td>2.03 mg 4 mL</td>
</tr>
<tr>
<td>SD</td>
<td>0.01</td>
<td>0.04</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 12: Measured drug and flush volumes for six replications of each drug/flush combination in 5 mg PIV experimental series with flush volumes of 1 mL, 2 mL and 4 mL.

<table>
<thead>
<tr>
<th></th>
<th>1 mL</th>
<th>2 mL</th>
<th>4 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug (mg)</td>
<td>Flush (mL)</td>
<td>Drug (mg)</td>
</tr>
<tr>
<td>1</td>
<td>5.04</td>
<td>0.07</td>
<td>4.99</td>
</tr>
<tr>
<td>2</td>
<td>5.04</td>
<td>0.07</td>
<td>5.04</td>
</tr>
<tr>
<td>3</td>
<td>5.04</td>
<td>0.07</td>
<td>5.04</td>
</tr>
<tr>
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<td>5.04</td>
<td>0.07</td>
<td>5.04</td>
</tr>
<tr>
<td>5</td>
<td>5.04</td>
<td>0.07</td>
<td>5.04</td>
</tr>
<tr>
<td>6</td>
<td>5.04</td>
<td>0.07</td>
<td>5.04</td>
</tr>
<tr>
<td>Mean</td>
<td>5.04 mg 1.01 mL</td>
<td>5 mg 2.01 mL</td>
<td>5 mg 4.01 mL</td>
</tr>
<tr>
<td>SD</td>
<td>0.07</td>
<td>0.05</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Figure 11 displays the amount of gentamicin recovered (and standard deviation) from the end of the peripheral IV line after gentamicin doses of 2 mg and 5mg were administered, followed by 1 mL, 2 mL or 4 mL flushes of normal saline. The gentamicin dose was given as 0.2 mL or 0.5 mL of a 10 mg/mL solution, and the mean internal volume of the PIV tubing (cannula, extension, smart site and filter) was 1.19 mL. The dotted line indicates the mean intended dose. These results indicated that a 4 mL flush was required to recover all of the 2 mg dose administered into a peripheral IV line, whereas a 2 mL or greater flush was required in order to ensure complete recovery of the 5 mg administered gentamicin dose from the end of the peripheral IV line.
Figure 11: Recovery of gentamicin after administration via a peripheral intravenous line with flush volumes of 1 mL, 2 mL and 4 mL. (A) 2 mg gentamicin dose administered; (B) 5 mg gentamicin dose administered. Dotted line indicates mean intended dose over 6 replications of each dose/volume combination.
4.4  UVC infusion studies

4.4.1  One hour infusions
Fluid discharged from the end of the UVC infusion was collected at 5 minute intervals for an hour following each dose/flush volume combination experiment and will each be discussed in turn. The mean volume of dead space of the IV line tubing from the point of drug administration to the patient end of the line (UVC 20 guage primary lumen, smart site and filter) was 1 mL. Experiments were repeated 6 times and standard deviations are shown on each figure.

4.4.1.1  2 mg dose
In the experiments where a 2 mg dose was intended, Table 13 shows the actual amount of drug and flush administered in each replication based on the weight of the syringes. The gentamicin preparation used clinically and in these experiments is 10 mg/mL, therefore 0.2 mL was required for administration in this series. As the result of variable measurements, on average a 3.9% greater dose was administered in the 2 mg/1 mL series and 6.3% greater dose in the 2 mg/2 mL series. The actual volume of flush administered was not substantially different from the nominal volume.
Table 13: Measurements of drug and flush administered for 2 mg experimental series

<table>
<thead>
<tr>
<th>Intended drug &amp; flush volume</th>
<th>2 mg dose, 1 mL flush</th>
<th>2 mg dose, 2 mL flush</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug dose (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 2.29</td>
<td>2.02</td>
<td></td>
</tr>
<tr>
<td>2. 2.08</td>
<td>2.06</td>
<td></td>
</tr>
<tr>
<td>3. 2</td>
<td>2.23</td>
<td></td>
</tr>
<tr>
<td>4. 2</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>5. 2.03</td>
<td>2.06</td>
<td></td>
</tr>
<tr>
<td>6. 2.03</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.07 mg</td>
<td>2.13 mg</td>
</tr>
<tr>
<td>SD</td>
<td>0.11</td>
<td>0.09</td>
</tr>
</tbody>
</table>

| Flush volume (mL)           |                      |                      |
| 1. 1                        | 1.98                 |                      |
| 2. 1.01                     | 2.01                 |                      |
| 3. 1.01                     | 2.01                 |                      |
| 4. 0.98                     | 1.95                 |                      |
| 5. 0.97                     | 1.96                 |                      |
| 6. 0.98                     | 1.98                 |                      |
| Mean                        | 0.99 mL              | 1.98 mL              |
| SD                          | 0.02                 | 0.02                 |

Fluid expelled from the end of the UVC tubing was collected at 5 minute intervals (timed from t=0, the start of drug administration) and analysed for gentamicin content by HPLC. Table 14 shows the cumulative amount of gentamicin recovered at each of these intervals for the 6 repetitions of each flush volume in combination with a 2 mg administered dose. Differences in the amount recovered as compared with the intended dose may be partly explained due to the administration of pharmaceutical-grade gentamicin as compared with the dry powdered stock solution that was used for to prepare the standard curves.
Table 14: Cumulative amount of gentamicin recovered during each 5 minute interval for one hour following 2 mg nominal dose administration

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>Dose</th>
<th>Flush</th>
<th>Replication</th>
<th>Cumulative amount of gentamicin recovered during each 5 minute interval for one hour (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mL/hr</td>
<td>2 mg</td>
<td>1 mL</td>
<td>1</td>
<td>1.73 1.81 1.85 1.89 1.92 1.95 1.98 2.03 2.05 2.07 2.09 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>1.54 1.83 1.86 1.92 1.95 1.98 2.0 2.03 2.05 2.07 2.08 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>1.29 1.55 1.58 1.62 1.65 1.68 1.71 1.74 1.76 1.79 1.80 1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>0.66 1.80 1.88 1.94 2.00 2.06 2.09 2.14 1.18 2.02 2.23 2.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>1.6 1.86 1.91 1.95 1.99 2.02 2.05 2.08 2.11 2.12 2.14 2.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0 2.14 2.20 2.26 2.64 2.31 2.35 2.39 2.42 2.44 2.46 2.47</td>
</tr>
<tr>
<td></td>
<td>2 mL</td>
<td></td>
<td>1</td>
<td>0.41 1.49 1.49 1.50 1.50 1.50 1.50 1.50 1.51 1.51 1.51 1.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>1.89 2.06 2.07 2.07 2.08 2.08 2.08 2.09 2.09 2.10 2.10 2.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>1.97 2.10 2.10 2.10 2.10 2.11 2.11 2.11 2.11 2.12 2.12 2.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>1.70 1.99 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00 2.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>1.57 1.85 1.86 1.86 1.86 1.86 1.86 1.87 1.87 1.87 1.87 1.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0 1.62 1.62 1.63 1.63 1.63 1.63 1.64 1.64 1.64 1.65 1.65</td>
</tr>
</tbody>
</table>
For a 2 mg dose (0.2 mL of 10 mg/mL solution) Figure 12 illustrates that the amount of gentamicin recovered rises dramatically with both a 1 mL flush and a 2 mL flush from time=0. There was a gradual increase in the gentamicin successfully recovered from the end of the UVC over the course of an hour when a 2 mg dose was given followed by a 1 mL flush. After one hour, all of the 2.08 mg measured dose (calculated based on weight of syringes) had been recovered when gentamicin was followed by a 1 mL flush. When a 2 mL flush was used 92% of the dose (1.88 mg out of 2.13 mg calculated by weight) was recovered from the end of the UVC after 1 hour.
Figure 12: Cumulative gentamicin recovery and percentage of intended dose recovered following administration of 2mg gentamicin (0.2 mL of 10 mg/mL solution) via a UVC with a consistent background infusion rate of 0.5 mL/hr. (A) Dose followed by 1 mL flush volume; (B) Dose followed by 2 mL flush volume. Mean and SD of 6 replications each illustrated.
Figure 13 shows the administered dose recovered over time during the one hour UVC infusion. Of the 2.15 mg administered dose recovered when a 1 mL flush was used, 85% of this was recovered by 10 minutes and 93% in the first 30 minutes. In comparison, when a 2 mL flush was used 99% of the 1.88 mg administered dose was recovered within the first 10 minutes. The greatest variability was seen in the first 5 minutes for both flush volumes, with standard deviations of 21.07% and 40.72% in the 1 mL and 2 mL flush volume series respectively. This variability was much smaller after 10 minutes had elapsed, with standard deviations of between 0.09% and 3.1%.

![Figure 13: Recovery of 2 mg administered gentamicin dose over 1 hour infusion duration when followed by 1 mL and 2 mL flush volumes. Mean and SD of 6 replications for each flush volume.](image)

4.4.1.2 5 mg dose

In the experiments where a 5 mg dose was intended, Table 15 shows the actual amount of drug and flush administered in each replication based on the weight of syringes. These experiments required 0.5 mL of the 10 mg/mL gentamicin solution to be administered and there was variability in the dose administered even though the same investigator prepared every syringe for both the drug and flush. In the series with a 1 mL flush, the gentamicin dose was 4.5% greater than intended, whereas in the series with a 2 mL flush only a 1.4% measurement error was observed. As seen in the 2 mg experiments, the
volume of flush was closer to the nominal volume in this 5 mg experimental series as well.

Table 15: Measurements of drug and flush administered for 5 mg experimental series

<table>
<thead>
<tr>
<th>Intended drug &amp; flush volume Experiment number</th>
<th>5 mg dose, 1 mL flush</th>
<th>5 mg dose, 2 mL flush</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug dose (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>5.01</td>
<td>5.03</td>
</tr>
<tr>
<td>2.</td>
<td>5.2</td>
<td>5.14</td>
</tr>
<tr>
<td>3.</td>
<td>5.81</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>5.42</td>
<td>5.01</td>
</tr>
<tr>
<td>5.</td>
<td>5.01</td>
<td>5.21</td>
</tr>
<tr>
<td>6.</td>
<td>4.93</td>
<td>5.03</td>
</tr>
<tr>
<td>Mean SD</td>
<td>5.23 mg</td>
<td>5.07 mg</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>0.08</td>
</tr>
<tr>
<td>Flush volume (mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>1</td>
<td>1.96</td>
</tr>
<tr>
<td>2.</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3.</td>
<td>1.02</td>
<td>2.01</td>
</tr>
<tr>
<td>4.</td>
<td>1.01</td>
<td>1.99</td>
</tr>
<tr>
<td>5.</td>
<td>1</td>
<td>1.96</td>
</tr>
<tr>
<td>6.</td>
<td>1</td>
<td>1.98</td>
</tr>
<tr>
<td>Mean SD</td>
<td>1.01 mL</td>
<td>1.98 mL</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

As with the 2 mg experimental series, fluid expelled from the end of the UVC tubing was collected at 5 minute intervals and analysed for gentamicin content by HPLC. Six repetitions of each dose/volume combination were completed, but replication 1 of the 5 mg dose/2 mL flush combination was excluded from the analysis due to pump malfunction. Table 16 shows the cumulative amount of gentamicin recovered at each of these intervals for following 5 mg dose administration.
Table 16: Cumulative amount of gentamicin recovered during each 5 minute interval for one hour following 5mg nominal dose administration

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>Dose</th>
<th>Flush</th>
<th>Replication</th>
<th>Cumulative amount of gentamicin recovered during each 5 minute interval for one hour (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mL/hr</td>
<td>5 mg</td>
<td>1 mL</td>
<td>1</td>
<td>0  4.13  4.15  4.23  4.32  4.41  4.49  4.53  4.59  4.63  4.67  4.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>4.29  5.30  5.39  5.46  5.54  5.60  5.68  5.73  5.77  5.82  5.84  5.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>1.43  4.36  4.42  4.48  4.53  4.57  4.61  4.64  4.67  4.69  4.70  4.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>3.29  5.74  5.79  5.83  5.87  5.89  5.92  5.94  5.95  5.97  5.97  5.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>4.74  5.89  5.93  5.97  6.00  6.02  6.04  6.06  6.07  6.08  6.09  6.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>2.56  5.67  5.72  5.79  5.86  5.92  5.96  5.99  6.00  6.02  6.03  6.04</td>
</tr>
<tr>
<td>2 mL</td>
<td>1 mL</td>
<td></td>
<td>1</td>
<td>Excluded due to pump failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>3.70  4.39  4.41  4.42  4.43  4.44  4.45  4.45  4.46  4.47  4.47  4.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>4.28  5.41  5.42  5.42  5.43  5.43  5.44  5.45  5.45  5.45  5.46  5.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>0.96  5.48  5.49  5.49  5.50  5.50  5.51  5.51  5.51  5.51  5.52  5.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>4.59  6.01  6.01  6.02  6.02  6.02  6.02  6.03  6.03  6.03  6.04</td>
</tr>
</tbody>
</table>
For a 5 mg dose (0.5 mL of 10 mg/mL solution given over 3-5 minutes, followed by a flush over the same duration) Figure 14 shows that the amount of gentamicin recovered rises dramatically with both a 1 mL flush and a 2 mL flush from time 0. When the 5 mg dose is followed by both a 1 and 2 mL flush all of the intended dose is recovered after one hour.
Figure 14: Cumulative gentamicin recovery and percentage of intended dose recovered following administration of 5 mg gentamicin (0.5 mL of 10 mg/mL solution) via a UVC with a consistent background infusion rate of 0.5 mL/hr. (A) Dose followed by 1 mL flush volume (mean and SD for 6 replications). (B) Dose followed by a 2 mL flush volume (mean and standard deviation of 5 replications)
Figure 15 shows the administered dose over the course of the hour-long infusion. When a 1 mL flush was administered following the 5 mg (0.5 mL) dose of gentamicin, 93% of the total 5.56 mg was recovered in the first 10 minutes, and 97% in the first 30 minutes. With a 2 mL flush, 99% of the 5.6 mg administered dose was recovered at 10 minutes. As seen in the 2 mg gentamicin dose series, the greatest variability in percentage of administered dose recovered was evident in the first 5 minutes. In this 5 mg series, the standard deviation of samples at 5 minutes was 28.99% with a 1 mL flush and 27.83% with a 2 mL flush, but after 10 minutes this standard deviation was between 0.09% and 3.36% across both simulations.

Figure 15: Recovery of 5 mg administered gentamicin dose over 1 hour infusion duration when followed by 1 mL and 2 mL flush volumes. Mean and SD for 6 replications of 1 mL flush and 5 replications of 2 mL flush experimental series.

4.4.2 Two hour infusions
In order to determine how long the total amount of gentamicin took to be recovered from the end of the UVC, one further experiment was completed in which each dose/volume combination was administered separately and samples collected at half-hour intervals for 2 hours. This was followed by a large bolus of dextrose through the IV line to ensure all of the drug was flushed through and could be recovered. This showed that 87% of a 2 mg
dose (followed by a 1 mL flush) was recovered within 30 minutes and 93% by the end of 1 hour, whereas 95–97% of the other doses were recovered within 30 minutes when each is accompanied by a stable carrier fluid flow rate of 0.5 mL/hr. Each dose/flush volume combination is shown in Figure 16 with the dotted horizontal lines indicating the average measured dose of gentamicin (2.14 mg and 5.14 mg).
### Table 17: Cumulative amount of gentamicin recovered during each 30 minute interval for two hours following administration with large flush at 150 minutes

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>Dose</th>
<th>Flush volume</th>
<th>Cumulative amount of gentamicin recovered during each 30 minute interval (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>0.5 mL/hr</td>
<td>2 mg</td>
<td>1 mL</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 mL</td>
<td>1.91</td>
</tr>
<tr>
<td>5 mg</td>
<td></td>
<td>1 mL</td>
<td>4.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 mL</td>
<td>5.38</td>
</tr>
</tbody>
</table>

**Figure 16:** Cumulative gentamicin recovery over 2 hours after bolus dose administration of 5 mg or 2 mg followed by 1 mL and 2 mL flush volumes. Dotted line indicates mean intended dose for each dose/volume experimental combination.
4.5 Dye experiments
Retrograde flow up the intravenous line (leading away from the patient) was observed when dye was used to mimic the drug administration phase during one replication of each dose/flush volume combination as shown in the series of photographs in Figures 17 and 18. These illustrate dye flowing up the line when it was first administered and then clearing at different speeds depending on the volume of flush that follows it. When a 2 mL flush was instilled, the line below the port was clear of drug before the syringe was detached, but dye remained in the tubing above this point. The port was then seen to back-fill with dye as the flush-containing syringe was detached, and then the remainder of the line was cleared by the carrier flow. When a 1 mL flush was instilled, a slower clearing of the line below the drug injection site was observed in addition to dye collecting in the port after detachment of the syringe.
Figure 17: Injection of 0.2 mL 1% (w/v) congo red dye followed by (A) 1 mL flush and (B) 2 mL flush. Photos at intervals of (i) end of dye administration, (ii) beginning and (iii) end of flush administration, (iv) after disconnecting syringe used to administer flush, (v) 5 minutes and (vi) 10 minutes after end of flush administration.
Figure 18: Injection of 0.5 mL 1% (w/v) congo red dye followed by (A) 1 mL flush and (B) 2 mL flush. Photos at intervals of (i) end of dye administration, (ii) beginning and (iii) end of flush administration, (iv) after disconnecting syringe used to administer flush, (v) 5 minutes and (vi) 10 minutes after end of flush administration.
4.6 Summary

The findings of Part B, the experimental phase of this thesis, have focused on investigating the impact that two different flush volumes have on the amount of gentamicin recovered after administration of two different doses by peripheral IV line and umbilical central line. The experimental conditions for these simulations have been based on conditions which are used in the Dunedin hospital NICU so as to make the results of this experiment applicable and clinically relevant to premature babies receiving intensive care.

The first set of results from this experimental work showed that a flush volume that was at least as large as the internal volume of the peripheral IV line was required for the full dose of gentamicin to be recovered after administration. This was especially noticeable with the smaller dose as it was administered in a correspondingly small volume of fluid.

The second set of results from this part confirmed that >80% of both 2 mg and 5 mg doses of gentamicin given via a UVC was able to be recovered from the end of the line 10 minutes after it was administered as a bolus when followed by a flush of either 1 mL or 2 mL of normal saline. The percentage of gentamicin recovered was lowest for a 2 mg dose with a 2 mL flush under these experimental conditions, where the flow of the carrier fluid (10% dextrose) remained constant at 0.5 mL/hr. Retrograde flow of dye was noted under all four experimental conditions.
5 Discussion

5.1 Overview
The survey and the subsequent laboratory investigations completed for this thesis suggest that to increase successful gentamicin dose recovery in the NICU greater consideration should be given to the methods of drug and flush administration. Variability has been illustrated in the site of administration, volume of drug and flush volume prepared and in the infusion itself over time. Issues such as individual measurement techniques, dead space volume of the intravenous line components and retrograde flow all contribute to successful recovery of gentamicin and may consequently affect treatment of early-onset sepsis in NICU babies.

5.2 Variability in current clinical practice
The significant findings revealed as part of the NICU nurses survey were:

- That the 2nd lumen of a UVC was favoured by nurses in Dunedin NICU to administer a dose of gentamicin to a premature baby, but that a PIV line would be used preferentially if this was available.

- A wide variation in flush volume occurred in Dunedin NICU when gentamicin is administered by both UVC and PIV methods.

- The most common reason for using a PIV in preference to a UVC, amongst NICU nurses, was to reduce the infection risk to the baby by preserving the sterile integrity of the TPN line.

These contemporary findings will be discussed in turn in the following paragraphs and contextualised. There has not been a direct comparison between different methods of gentamicin administration or of nursing preference for different routes of administration published in the literature.
One survey of a similar nature, completed by neonatal and paediatric nurses regarding gentamicin delivery, also displays a large amount of variation in the preferred site of administration and 75% of these respondents varied their site of administration depending on the flow rate of the infusion\textsuperscript{66}. The variation described in the current survey reveals differences in nursing practice across the unit, and may also represent modifications to the care of the same baby in different shifts over the course of the day. This could result in fluctuating systemic levels of gentamicin which might be clinically significant over the course of their sepsis event. The pharmacokinetics of gentamicin are anticipated to differ depending on where and how the drug is administered as this impacts on the time it takes for the drug to reach the circulation as discussed in section 1.3.1. As there are often long lengths of tubing used in the intravenous line architecture for preterm babies the delivery of the drug is unpredictable. It has been observed that the time from administration to the drug appearing at the patient-end of an infusion can be predicted based on the flow of the line and the dead space volume of the tubing, but the actual time taken for the infusion to be completed is longer than would be expected based on these calculations\textsuperscript{66}. This is a particularly important feature to be aware of when changes to the drug dose or interval are required based on serum measurements, as occurs with gentamicin, as these decisions may be based on the incorrect assumption that the whole of a dose has been delivered within a specified time period.

A flush is instilled following a dose of medication given intravenously in order to ensure that the drug is cleared out of the infusion system, though there are no studies with recommendations of minimum flush volume required for different infusion configurations\textsuperscript{29}. Previously it has been shown that administration of a 2 mL saline flush following a dose of gentamicin administered by continuous infusion improved the amount of drug recovered by 19% in extremely low birth weight neonates\textsuperscript{2}. The flush
volume that is administered needs to be considered in the context of the total fluid requirements of the baby over a 24 hour period, especially if medications are administered multiple times each day or if numerous medications are prescribed concurrently, in order to prevent fluid overload. In the NICU survey completed here it was indicated that the flush volumes used following the administration of gentamicin would be smaller for the most premature baby, but these represented a greater proportion of the total daily fluid intake in this group. The potential implication for medications given multiple times per day and for babies receiving numerous medicines is that babies would be receiving 0.9% saline as a flush at the expense of nutrition-containing fluids.

Parenteral nutrition solutions are commonly prepared under sterile conditions in the hospital pharmacy prior to their use in the NICU. To maintain the sterility of the fluid, extended lines which require changing every 48 – 96 hours are attached to existing intravenous devices, though the optimal timing for making these changes taking into account colonisation, infection, mortality and nursing time is not definitively understood. Within the intravenous line configuration it has become routine practice to include an in-line filter (such as the Pall ELD96) in order to prevent contamination and microemboli from microparticles that may be found in nutrition or medication solutions. However, medications such as gentamicin can be administered downstream of this endotoxin-retentive filter (above a 0.2 micron air-eliminating filter only), which could result in bacterial contamination of the sterile line. Of greater concern is the potential for proliferation of bacteria once they are introduced, because of the nutritional components of the fluid, which could then result in iatrogenic bacteraemia and neonatal sepsis. This concern is felt to relate primarily to the lipid emulsion component, though a 1998 prospective study exposing samples of TPN fluid to 12 nosocomial pathogens resulted in growth of only *C. albicans* and *S. saprophyticus* at 24 – 48 hours, concluding that total
nutrient admixture was a poor growth medium for most nosocomial infections and no better than 5% dextrose\textsuperscript{72}.

In a paediatric ICU setting, there was an increased risk of bloodstream infection seen with receipt of parenteral nutrition (OR 3.12) and with UVC placement in particular\textsuperscript{73, 74}. However no significant difference was found in the systemic infection rates when comparing TPN delivered by PICC line versus PIV line in a Cochrane review\textsuperscript{75}. The concerns raised by nursing staff in this Dunedin survey regarding iatrogenic complications related to parenteral nutrition are echoed in the literature\textsuperscript{76, 77} and should be considered when writing drug protocols for infants who require parenteral nutrition.

5.3 Peripheral line infusion variables

The main finding from the PIV experimental series was:

- A flush that is at least as large as the internal volume of the line is required in order to deliver all of the intended dose when gentamicin is administered by a peripheral IV line.

The flush volume required following drug administration by PIV line has not been definitively established in the scientific literature. This may be because different configurations of intravenous line components are used in different hospitals but it is an important pharmacokinetic parameter to consider.

The internal volume of the intravenous line architecture needs to be taken into consideration when administering medications by bolus injection or continuous infusion as well as at different sites along the line. Neonatal lines have been designed to minimise the dead space within them by methods such as low-volume tubing though there are likely to be different configurations used in different hospitals\textsuperscript{29}. As well as minimising the internal volumes, low-volume tubing decreases the risk of fluid layering occurring\textsuperscript{29}. In
the current study, average measured dead space from the port where the drug was administered to the patient end of each line was 1.19 mL for the PIV experiments (range 1.12 – 1.29 mL) and 1 mL for the UVC series (range 0.96 – 1.02 mL). While these measurements are similar to the reported capacity measurements on the packaging, relying exclusively on these may underestimate the interal volume, as that of the Smart Site was not published and some variability in capacity of each individual component may exist. Given the small internal IV line volume, it could be expected that complete recovery of both 2 mg (0.2 mL) and 5 mg (0.5 mL) doses of gentamicin would occur after a 2 mL flush as this would completely displace the volume of the line. However, although this was found to be true for the larger 5 mg dose, it was not so for the smaller 2 mg dose. It is possible that the smaller dose requires a larger flush for complete recovery because it is dispersed along the length of the tubing more in keeping with a “well-mixed” drug delivery model than the proposed alternative “plug-flow” model. It is also possible that some of the drug remained adhered to components of the line such as the filter, thus making it unrecoverable. Previous experiments have shown gentamicin recovery to be complete when a 2 mg (0.2 mL of 10 mg/mL solution) dose was infused through a Poisyne Neo filter with a background carrier flow rate of 3.8 mL/hr, but have not investigated the Safti-ject SV® filter which was used in these experiments.

5.4 Central line infusion variables
The most notable findings from the UVC experimental series were:

- Retrograde flow of dye away from the patient was noted under all conditions.
- A larger flush (2 mL) resulted in faster recovery of the administered gentamicin dose than a small flush (1 mL).
When gentamicin was administered into the slow-flowing second lumen of a UVC by bolus injection it appeared to be recovered as well as previous studies where the dose has been administered into a faster flowing primary line.

The dye model experiments help to explain some of the findings for the UVC series. With a larger flush, the line below the port is completely clear of drug before the syringe is detached, leaving only a small amount of drug above the port to be cleared by the carrier flow whereas with a smaller flush volume, dye remains above and below the port after the flush is completed, and requires the carrier flow rate to deliver a visibly greater proportion of the dose. Additionally, some of the dye that remained above the port after the flush was completed was seen to back-fill the port itself when the empty syringe was removed, and thus was not cleared by the carrier fluid. The proportion of dye remaining in the hub is likely to be more significant when a smaller dose is intended, but this has not been formally quantified in these experiments. This may help to explain why there is a discrepancy between the intended dose and recovered dose for the 2 mg/2 mL series, where less was recovered than was intended to be administered, and may also contribute to the apparent increased recovery of drug dose with subsequent replications of the same flush/volume combination as the port is primed with additional drug volume in these experiments. This observation is consistent with the work of Lovich et al.\textsuperscript{78}. Although this apparent loss of drug is not seen in the 2 mg/1 mL series when gentamicin recovery was measured, only one replication of the dye experiment was completed for each dose/volume combination, and it is to be expected that a degree of variation would exist in these experiments as was seen in the gentamicin recovery experiments. Previous studies have also investigated retrograde flow of dye in various configurations of neonatal IV line giving sets and it has been observed that retrograde flow was more pronounced in infusions with a flow rate of 2 mL/hr whereas it was not seen in infusions
at 10 mL/hr\textsuperscript{41}, and was more notable when the primary infusion fluid was of a different specific gravity from the solution being instilled or when the T-connector was angled upwards by 15°\textsuperscript{30}. At an even slower rate, as was used in these experiments, the retrograde flow effect could become even more pronounced.

Previous simulation experiments have shown a correlation between slow carrier flow rates with small drug doses and poor recovery of the administered dose from the end of a peripheral intravenous catheter when the dose and flush are given as continuous infusions\textsuperscript{2}. In this study, at a slower carrier flow rate, it has been shown that this recovery can be improved by administering the gentamicin dose as a bolus. This is consistent with other work using the same doses (2 mg and 5 mg) at higher flow rates (2 mL/hr and 10 mL/hr)\textsuperscript{41} and additionally with larger drug doses\textsuperscript{30}. However, in contrast, earlier work showed that the longest drug infusion time occurred with slow IV flow rates in combination with large dose volumes\textsuperscript{79}. In addition to carrier fluid rate, the site of injection and volume of drug to be injected also significantly influence the length of infusion time\textsuperscript{80}. The rapidity of recovery is greatest in the current study when a larger flush volume (2 mL in comparison to 1 mL), is used but there is a large degree of variation in the amount of gentamicin recovered within the first 5 minutes in all of the dose/flush volume combinations. Beyond 10 minutes there was only a small amount of drug expelled at each five minute interval (<1% of the intended dose), which became difficult to detect with the current assay, but this method could be optimised for future research.

Although neonatal infusion configurations have evolved to enable drugs and nutrition-containing fluid to be connected together and run simultaneously, the ideal system which enables independent delivery of each is not yet known\textsuperscript{69,80}. Interventions such as anti-reflux valves and very low volume deadspace may contribute to more consistent and timely drug administration in the NICU.
5.5 Measurement variability

One further observation that can be made from this experimental work is:

- That variability in measured drug and flush volumes for administration was identified and was more pronounced with smaller volumes of fluid.

In this work, inter-individual variability was not quantified because one investigator prepared all of the syringes of drug and flush for administration. However, there was considerable intra-individual variability, even under controlled conditions in a laboratory, with up to a 6.3% greater than intended dose administered (in the 2 mg drug/2 mL flush UVC series). This variability is likely to increase in situations of high workload and stress in the intensive care environment and is particularly concerning because a number of drugs requiring small volumes of fluid are used in the NICU. These findings support previous research illustrating the greater variability in drug doses prepared in the NICU as compared to those prepared in pharmacy settings and when doses requiring dilution are needed. Previous simulation experimental work by Godden in Dunedin also confirms the inconsistency between the actual and intended doses of gentamicin. In that study it was felt that as the administered drug doses were consistently lower than intended (-7.78% for the 2 mg dose and -5.2% for the 5 mg dose) this may have contributed to the low dose recovery seen in the infusions with a slower background flow rate. This is not likely to be the case in the present work however, as all of the intended doses were recovered for 3 of the 4 experimental dose/volume combinations. In the series where less than the intended dose was delivered (2 mg dose/2 mL flush volume), it is possible that serial or inaccurate dilutions contributed to the low recovery seen. The inconsistency in drug dose administered may contribute to errors in serum drug level interpretation which could result in changes being made to drug doses or dosing interval that have the potential to result in clinically significant adverse effects (due to drug
accumulation resulting in nephrotoxicity or ototoxicity), or to potentiate treatment failure for septic premature infants. Although there might not be ways to eliminate the risk of inconsistent drug dosing completely as these are subject to human error, it may be possible to mitigate the effect that these have by raising awareness of the problem and encouraging further research, particularly in the area of neonatal pharmacology where small volumes are routinely required.

5.6 Strengths and weaknesses

The NICU nurses survey is limited by several factors, in particular that it contains information from one tertiary NICU only and as such may not be generalisable to other units. During the completion of the questionnaire nurses were permitted to read the unit drug administration manual and to discuss the questions amongst themselves. Although this has the potential to create bias it reflected routine practice and emphasis was placed on what would actually be done, not what was recommended in the protocol. A significant strength of the survey is the excellent response rate from nursing staff.

The laboratory work is limited by its nature as a simulation in a controlled environment and as such may not reflect true physiological processes. Additionally, there were no measurements done on peak or trough concentrations – it is assumed that the gentamicin administered remains physiologically active but this may actually be being excreted before the peak serum level is obtained in routine clinical practice since the drug enters the patient’s circulation over an extended period of time. The strengths of this experimental work are that there were many repetitions done and that one investigator did all the drug/flush measurements during the experiment and dilutions in the HPLC analysis. Both of these factors ensure the consistency of the results, so the variability that is noted can be attributed to the infusion itself rather than an individual. The experiments use current clinical practice as the basis for the simulation in that the components of the
line and syringes used for administration of the drug and flush are the same as those used in Dunedin NICU. This ensures that the results are able to be generalised from the laboratory to this clinical setting.

5.7 Summary
This body of work has demonstrated several findings relevant to clinical practice in the NICU. Firstly, that there is variability in the way that gentamicin is currently given in the clinical setting, which may differ from the way that researchers and clinicians assume that it is given (and in the way that doctors interpreting gentamicin levels also imagine that it is given). A common concern in the care of premature neonates is maintaining the sterility of the UVC lumen dedicated to parenteral nutrition fluid which can affect the choice of location to administer a dose of gentamicin. Secondly, it is possible to reveal that a flush of 4 mL is required to ensure complete delivery of a 2 mg dose through a peripheral IV line with the current configuration used in Dunedin NICU, whereas a flush of 2 mL only is required when it follows a dose of 5 mg administered peripherally. The reasons for this discrepancy in required flush volume are not fully understood. Thirdly, there is a degree of variation in the measured volumes in syringes that are used for drug and flush administration. The clinical significance of this is difficult to quantify, but this variation may contribute to the differences observed in serum levels that is noted when peak and trough measurements are taken, which in turn has implications for the interpretation of these results and subsequent dose adjustments to prevent adverse events occurring. Fourthly, with regard to UVC administration, this work has demonstrated that when a dose of gentamicin is given into the 2nd lumen of a UVC it is successfully administered faster when followed by a larger flush than a smaller one. This is notable because the 2nd lumen typically has a slower carrier fluid rate than the primary (parenteral-nutrition-containing) line and although the dose is recovered slower with a
smaller flush it does not seem to take dramatically longer. The implications of this are that doses can be recommended to be given as a bolus injection (as opposed to previously when continuous infusion of dose and flush over 30 minutes were advised) and recovery of 90% of the administered gentamicin dose can be anticipated within 30 minutes regardless of the flush volume that is used. Retrograde flow and back-filling of the port used for UVC gentamicin administration was noted in these experiments and may be under-appreciated in the clinical NICU setting.

It is not possible to say from this research, however:

- When the optimal time for measuring a peak concentration would be.
- Whether the peak levels attained would be in the therapeutic range.
- What the optimal dose is for premature neonates in order to achieve therapeutic peak concentrations.
- The ideal method of administering gentamicin so that retrograde flow does not occur at slow carrier flow rates.

5.8 Implications for further research
It was beyond the scope of this research project to develop a model to predict the appropriate time for measuring peak serum concentrations of gentamicin in premature babies. The hope is that the data collected here will be able to be pooled with previous studies in order to create this model and ultimately to complete these simulations. These data may be able to support recommendations for the flush volume to be administered (peripherally and centrally) based on the recovery of gentamicin observed. Furthermore, as this was a laboratory-based project there are numerous clinical factors which have not yet been considered. Physiologically, the volume of fluid the baby receives is an
important consideration as is the volume of blood required for testing of serum
gentamicin levels and the clinical condition of the baby. These factors and their
relationship to serum gentamicin levels should be investigated with future studies.
6 Conclusion

Variability in neonatal gentamicin infusions is illustrated in a number of different ways in these experiments. This has added to current understanding about the influence of background infusion rate and flush volume on recovery of gentamicin when prescribed in the NICU setting.

Our survey of current neonatal nursing practice for administration of gentamicin in the 48 hours immediately after birth showed variability in the site of drug administration and the flush volume that would be used. Free-text responses indicated that the most common reason for administering a dose of gentamicin through a PIV line if this was available was to preserve the sterile integrity of the umbilical central line.

The survey data was used to formulate experimental studies investigating the recovery of gentamicin when administered in different venous access devices. In order to fully recover a dose of gentamicin when it is administered via a PIV line, the flush volume must be greater than the dead-space of the IV infusion components through which it is being administered. With the current set-up in Dunedin NICU used in this experiment, the smallest flush volume that allowed full dose recovery of both 2 mg and 5 mg gentamicin doses was 4 mL.

However, when administered via the UVC with a consistent slow background infusion rate of 0.5 mL/hr recovery of the intended gentamicin dose of 2 mg or 5 mg is observed when both 1 and 2 mL flush volumes used. There is considerable variability in the amount of drug recovered at 5 and 10 minutes but less inconsistency in all cases after 10 minutes have elapsed and > 90% dose recovery by 1 hour.

Variability is also noted in the volumes of drug and flush prepared for administration which could have clinically significant effects on the treatment of neonatal sepsis.
Retrograde flow of dye was noted which may explain some of the variability seen in gentamicin recovery. These findings support the clinical practice of measuring peak serum gentamicin levels 30 – 60 minutes after administration of gentamicin via UVC into a slow-flowing background infusion but this recommendation does not take into account the effect that drug distribution or clearance may have on the peak level obtained.
7 References


24 Kremer L. personal communication May 2014.


8 Appendices

8.1 Dunedin Hospital gentamicin dosing protocol

Gentamicin Sulphate - NICU Medication Manual (Otago)

Note: All medication must be checked by two registered practitioners prior to administration.

Aminoglycoside antibiotic

<table>
<thead>
<tr>
<th>Route</th>
<th>Dosage</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow IV push over 3-5 minutes [Dose volumes less than 1mL (10mg) can be given as a fast push. The first 1mL of any dose can also be given as a fast push.] IM injection is associated with variable absorption, especially in the very small infant</td>
<td>See dose chart below</td>
<td>Injection (ampoule) 10mg/1mL (solution) ▪ Refer to administration and comments below</td>
</tr>
</tbody>
</table>

DOSE:

<table>
<thead>
<tr>
<th>Gestational Age (Weeks)</th>
<th>Postnatal Age (days)</th>
<th>Dose (mg/kg)</th>
<th>Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤29*</td>
<td>0 to 7</td>
<td>5</td>
<td>Determined by level at 24 hours: See below</td>
</tr>
<tr>
<td></td>
<td>&gt;7</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>30 to 34</td>
<td>0 to 7</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>≥35</td>
<td>ALL</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Use: Treatment of infections caused by aerobic gram-negative bacilli (e.g. *Pseudomonas, Klebsiella, E.coli*). Usually used in combination with a β-lactam antibiotic (e.g. amoxicillin).

Monitoring

▪ Monitor peak and trough levels with the 1st dose and again after the 3rd dose
▪ Monitor renal function (urea and creatinine).
▪ Peak levels should be measured 1 hour after administration.
▪ Trough levels should be measured 23 hours after administration.

▪ Trough - ≤ 2 microg/mL
▪ When the interval is extended >24hrs there is no need to take another trough before giving the dose in most cases.
- Peak - 6-12 microg/mL
- If the peak is too high/low → alter the dose.
- If the trough is too high/low → alter the interval according to the table below:

<table>
<thead>
<tr>
<th>Level at 24 hours</th>
<th>Dose Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤2mcg/mL</td>
<td>24hr</td>
</tr>
<tr>
<td>2.1 - 3mcg/mL</td>
<td>36hr*</td>
</tr>
<tr>
<td>3.1 - 4mcg/mL</td>
<td>48hr*</td>
</tr>
<tr>
<td>&gt;4mcg/mL</td>
<td>Consider alternative antibiotic e.g. cefotaxime</td>
</tr>
</tbody>
</table>

* If interval >24h, discuss with paediatrician whether an alternative antibiotic is required

**Adverse Effects:** Nephrotoxicity and ototoxicity may occur with gentamicin therapy.

**Administration**
- Gentamicin should be administered as a 3-5 minute IV push.
- Follow with a 2mL saline flush over 3-5 minutes.
- Peak levels (if required) are to be taken one hour after administration

**Interactions:**
- Increased risk of nephrotoxicity with concomitant amphotericin, indomethacin (indometacin) and vancomycin.
- Increased risk of ototoxicity with concomitant furosemide.
- ß-lactam antibiotics have been associated with inactivation of gentamicin. This is probably only clinically significant when penicillin containing compounds are mixed in IV solutions or when the blood is at room temperature several hours before the assay is performed.

**Compatibility:**
- pH: 3 - 5.5
- IV Fluid: n/s, G, G10%, TPN
- For TPN & lipid compatibility, see Comments.
- **Compatible:** aciclovir, clindamycin, dopamine, fluconazole, insulin, heparin (concentrations <1u/mL), magnesium sulphate, meropenem, metronidazole, midazolam, morphine, prostaglandin E1, ranitidine
- **Incompatible:** amoxicillin, amphotericin, erythromycin, furosemide, heparin (concentrations >1u/mL), imipenem/cilastatin, indomethacin
(indometacin), penicillin G, sodium bicarbonate, ticarcillin/clavulanate.

**Storage / Stability:**

- Store at room temperature below 25ºC.

**Notes:** Ampoules are single use only. Discard unused remainder of ampoule after opening.

**Plugged Lines**

*For plugged lines* always administer via a separate line to the TPN if available. For plugged lines with no carrier fluid, give gentamicin as a 3-5 minute IV push, followed by a 4mL normal saline flush as 20 minute infusion via syringe driver. For small babies, be aware how much fluid is given with the gentamicin/flush, and discuss with medical team if it is significant.

- Gentamicin is incompatible with lipid emulsion when they are mixed together, but available evidence suggests terminal injection site compatibility.
- It is preferred that gentamicin be administered via a separate line to the TPN ± lipid. If this is not possible, administer via three way tap site closest to baby. The dose must go through a 0.22-micron filter regardless of where it is administered in the overall line set-up.
- If other infusions of drug are running e.g. morphine, make sure these are not moved through too quickly by the gentamicin/flush.

**References:**

- None

**General Notes**

**Scope of Practice:** Ensure you are fully qualified to perform the role specified in any document.

**Deviation:** If you need to deviate from any procedure, policy, or guideline, make notes and follow up.

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8.2 NICU nurses survey questions
Dear NICU nurses,

Thank you for taking the time to help me with this quality improvement exercise. The overall aim of this work is to determine the most appropriate time to monitor gentamicin drug levels, as gentamicin effectiveness is known to correlate well with serum concentrations. These levels vary depending on how and where the antibiotic is given to extremely low birth weight babies, and we are currently unsure of the best way to administer gentamicin.

I would like to gain an appreciation for how gentamicin is currently administered in our neonatal unit. This survey is completely anonymous and not meant as a test, therefore there are no right or wrong answers!

The following diagram depicts our current set-up when using a double lumen umbilical venous catheter (UVC) in a preterm baby. The letters A and B refer to ports available for drug administration in the questions on the next page.

1. If empirical antibiotics are prescribed in the first 24-48 hours of life, where would you administer the dose of gentamicin to the following babies if only the UVC depicted above was available (in the absence of other drugs or blood products being infused simultaneously)?
   a. 700g 24-weeker receiving 90ml/kg/day of fluid (2.6ml/hr)
      A   B   Other (please draw on diagram)
   b. 1800g 32-weeker receiving 90ml/kg/day of fluid (6.7ml/hr)
      A   B   Other (please draw on diagram)

2. If a peripheral line was present in addition to the UVC in the above scenarios would this change your answers above? Yes No

   How/why........................................................................................................
   ....................................................................................................................
   ....................................................................................................................
   ....................................................................................................................

3. Please fill in the table with the flush volume you would use in the following scenarios

<table>
<thead>
<tr>
<th>Case</th>
<th>Plugged peripheral Intravenous line in situ</th>
<th>Gentamicin bolus dose given at point A</th>
<th>Gentamicin bolus dose given at point B</th>
</tr>
</thead>
<tbody>
<tr>
<td>700g 24-weeker receiving 90ml/kg/day of total fluid (2.6ml/hr)</td>
<td></td>
<td>_______mL</td>
<td>_______mL</td>
</tr>
<tr>
<td>1800g 32-weeker receiving 90ml/kg/day of total fluid (6.7ml/hr)</td>
<td></td>
<td>_______mL</td>
<td>_______mL</td>
</tr>
</tbody>
</table>

   Thanks very much for your time and expertise. Additional comments are most welcome.

Anita Lala
8.3 Published journal article