Respiratory infections and immune biomarkers of infection and inflammation in cases of Sudden Unexpected Death in Infancy (SUDI) at the Tygerberg Medico-Legal Mortuary

by

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DECLARATION

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Date

SUMMARY

Sudden infant death syndrome (SIDS) is the leading cause of infant death in the post-neonatal stage in developing and developed countries. A diagnosis of SIDS is made after medico-legal investigations fail to demonstrate an adequate cause of death in sudden unexpected death in infancy (SUDI) cases and SIDS is diagnosed in over 60% of SUDI cases. The peak incidence of SUDI is observed when infants are aged between two to four months. It is also at this stage that infants lose their maternally acquired immunity against infections. During medico-legal investigations in SUDI cases, viruses and bacteria have been confirmed, although none of them was consistently and exclusively associated with SUDI to date. Furthermore, viral respiratory symptoms are reported in 44% of SUDI cases, but the exact role they play in the events leading up to the death of the infant remains unknown. Viral infections can either facilitate easier bacterial colonisation of the respiratory system or induce an unregulated immune response through the release of cytokines and chemokines (immune biomarkers) that lead to formation of immune complexes in lungs and other respiratory organs. This could result in the loss of functionality and ultimately death of the infant. The role played by viruses and the interaction of the immune system in the events leading to SIDI therefore require further investigation to get a clearer understanding.

Objectives: The first aim of this cross-sectional, descriptive study was to characterise the respiratory viruses observed in SUDI cases investigations at the Tygerberg Medico-Legal Mortuary using the Seeplex RV15 ACE detection multiplex PCR kit. The multiplex PCR viral detection results were compared to routine shell vial culture results to determine the superior viral detection method. The second aim was to assess 16 target immune biomarkers as indicators of infection or inflammation prior to or at the time of death of an infant.

Methods: Samples were collected from 183 SUDI cases admitted to the Tygerberg Medico-Legal Mortuary between July 2015 and June 2016. Swabs collected from the trachea and left and right lungs were collected for multiplex PCR detection of 15 respiratory viruses. These viral targets were human adenovirus, human bocavirus, human coronavirus 229E/NL63, OC43, human enterovirus, influenza A and B, human metapneumovirus, human parainfluenza 1-4, respiratory syncytial virus A and B and human rhinovirus A/B/C. Serum was also collected for immune biomarker testing. Tissue from both lungs were collected for shell vial culture and blood was collected for HIV 1/2 antibody testing. Microbiology routine testing included culture of heart, left and right lung swab samples.

Results: The gender distribution of infants in this study was not consistent with literature. There were 93 (50.8%) females and 90 (49.2%) males, although males have been identified as being at a greater

risk of SUDI than females. However, other socio-demographic risk factors for SUDI, such as the greatest risk of death being at age two to four months were consistent with literature. The detection of viruses by multiplex PCR proved to be superior to SVC as the former detected viruses in more cases than the latter. The most commonly detected virus by multiplex PCR was human Rhinovirus A/B/C, which was detected in 65 (35.5%) of the 183 cases tested. Adenovirus was the second most frequently detected virus as it was present in 18 (12.6%) of the cases tested. Parainfluenza 3, Enterovirus and RSV B were detected in 10 (5.5%), 9 (4.9%) and 7 (3.8%) cases respectively. Human metapneumovirus was not detected at all by either assay.

The serum concentrations of CRP and IL-6 were significantly elevated in the serum of SUDI cases where infection was the cause death compared to cases that were diagnosed as SIDS. However, levels of IL-18 were significantly reduced in the serum of SUDI cases where infection was the cause of death compared to cases with a final cause of death classification of SIDS.

Conclusion: It is possible to change the cause of death from SIDS to infection if serum immune biomarker results and multiplex PCR are used in addition to tissue histology. The current study showed that serum CRP, IL-6 and IL-18 levels can possibly be regarded as candidates for use as indicators of infectious death and these findings need to be further investigated in larger study cohorts and over longer study periods.

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DEDICATION

This thesis is dedicated to my baby brother, Keith. Although I only had three months with you on this Earth, those were the best three months of my life. You inspired me to take on the challenge of studying child and infant mortality more than two decades after I last saw you. I know you are in a better place now.

To my dearest friend Hilory Munhuweyi, I know every day that passes you look down on me from heaven and smile as I try to make you proud in every way possible. It has not been easy without you my friend, but I know you are also in a better place. I miss you.

Rest in peace.

A baby fills a place in your heart you never knew was empty

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AAP	American Academy of Pediatrics
AIDS	Acquired Immunodeficiency Syndrome
BAL	Bronchoalveolar lavage
bp	Base pair
CDC	Centre for Disease Control and Prevention
cDNA	complementary DNA
CMV	Cytomegalovirus
COD	Cause of death
CPE	Cytopathic effect
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DAMP	Danger-associated molecular pattern
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EMEM	Eagle's Minimum Essential Medium
FITC	Fluorescein isothiocyanate
GHO	Global Health Observatory
gp	Glycoprotein
H&E	Haematoxylin and Eosin
HAdV	Human adenovirus
HBoV	Human bocavirus
HCoV	Human coronavirus
HCV	Hepatitis C virus
HEV	Human enterovirus
HF	Human fibroblast
HIV	Human immunodeficiency virus
HMPV	Human metapneumovirus
HPIV	Human parainfluenza virus
HREC	Human Research Ethics Committee
HRV	Human rhinovirus
IFA	Immunofluorescent assay
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IP-10	Interferon gamma induced protein 10
LPS	Lipopolysaccharide
LRTI	Lower respiratory tract infection

MDCK	Madin-Darby Canine Kidney
MDG	Millennium Development Goal
MIP	Macrophage inflammatory protein
NAAT	Nucleic acid amplification test
NHLS	National Health Laboratory Service
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NK	Natural killer
NLR	NOD-like receptor
NPA	Nasopharyngeal aspirate
OR	Odds ratio
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PM	Post-mortem
PMI	Post-mortem interval
PRR	Pattern recognition receptor
RA	Receptor antagonist
RNA	Ribonucleic acid
RPMI	Rosswell Park Memorial Institute
RSV	Respiratory syncytial virus
SIDS	Sudden Infant Death Syndrome
Staph	*Staphylococcus*
Strep	*Streptococcus*
SUDI	Sudden Unexpected Death in Infancy
SVC	Shell vial culture
TAE	Tris acetate ethylenediaminetetraacetic acid
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumour necrosis factor
UN	United Nations
URTI	Upper respiratory tract infection
USA	United States of America
UTM	Universal transport medium
WHO	World Health Organisation

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Dwight Eisenhower, the 34th president of the United States of America (USA) once said about the loss of a child, "There is no tragedy in life like the death of a child. Things never get back to the way they were." In this statement, he summarised a whirlwind of emotions that follow the loss of a loved one in their formative years and the life-changing impact it has on those that remain behind.

Quite a number of factors can contribute towards the death of infants. The Centre for Disease Control and Prevention (CDC) identified among others, Sudden Infant Death Syndrome (SIDS) as one of the leading causes of death in infancy. Prematurity, SIDS, birth defects, complications in pregnancy and suffocation alone were responsible for more than 55% of infant deaths in the USA in 2011. In the District of Columbia and the rest of the USA, SIDS was the leading cause of infant death in the post-neonatal period (Heron, 2015), fuelling more investigative interest in SIDS and the dynamics surrounding it (Bajanowski *et al*., 2007).

Initially, SIDS was defined as the sudden death of any infant or young child, which is unexpected by history, and in which a thorough post-mortem (PM) examination fails to demonstrate an adequate cause of death (COD). This definition was widely referred to as the "Seattle Definition" of SIDS (Beckwith, 2003). Over the years, the definition of SIDS has evolved with the emergence of new information from various fields of study. Some twenty years after formulation of the original SIDS definition, the National Institute of Child Health and Human Development (NICHD) set up a panel of experts to further clarify the definition. The panel ultimately made a few changes to the original definition. The concept of SIDS was now limited to death of infants under the age of one year and investigation of the death scene formed a part of an inquest into an infant's death (Krous, 2010). The most recently documented definition of SIDS is the 2004 'San Diego Definition'. This defines SIDS as the sudden and unexpected death of an infant under one year of age, with onset of the lethal episode apparently occurring during sleep, which remains unexplained after a thorough investigation, including conducting a complete autopsy, and review of the circumstances of death and the clinical history (Krous *et al.*, 2004). Making a SIDS diagnosis is only possible when there is no evidence of an adequate COD and it is for this reason that it is referred to as a diagnosis of exclusion (Blackwell *et al.*, 2015). The terms SIDS and SUDI (Sudden Unexpected Death in infancy) are being used interchangeably in literature although they do not necessarily mean the same. It is important to note that SUDI is an all-encompassing term describing all cases of sudden and infant unexpected death before any investigation is conducted. As such, SIDS is a sub-category of SUDI.

Various theories on the possible causes of SIDS have been proposed over the past five decades, including the possible role of respiratory viruses, such as respiratory syncytial virus (RSV), human adenovirus (HAdV), influenza virus, human coronavirus (HCoV), human parainfluenza virus (HPIV) and human metapneumovirus (HMPV). Although the involvement of these viruses in SIDS has been a subject of intense investigative research, their exact role remains a mystery, as there has been no consistent and direct link between any single virus and sudden infant death (Raza & Blackwell, 1999).

1.1 Investigations into SUDI cases in South Africa

Medico-legal SUDI case investigations are conducted in most developed countries and a handful other developing countries (du Toit-Prinsloo *et al.*, 2013). However, the detail of such investigations frequently depends on the available resources at particular institutions (du Toit-Prinsloo *et al.*, 2011). According to the South African Inquests Act (Act 58 of 1959), all unnatural deaths, including SUDI, must be investigated to ascertain the COD. At Tygerberg Medico-Legal Mortuary, all SUDI cases are investigated as required by the Act. However, the way in which these investigations are conducted varies significantly among institutions. Du Toit-Prinsloo and colleagues (2011) investigated SUDI cases at the Medico-Legal Mortuaries of Tygerberg (Cape Town) and Pretoria and they identified differences in investigation protocols and ancillary investigations conducted. However, despite this, South Africa has no nationally accepted, standardised SUDI case investigation protocol and the Tygerberg Medico-Legal Mortuary utilises an institutional investigation protocol. The information generated from these investigations at different medico-legal centres is therefore difficult, if not impossible to compare. As more research emerges in the field, some of the findings might be valuable in establishing regional and national standard SUDI case investigation protocols. A standardised SUDI investigation protocol will allow for the generation of a database that can serve as a reference point to identify possible trends in SUDI cases in South Africa and may lead to steps being taken to address the problem.

In addition to a thorough autopsy and review of the medical history of the infant, investigations into SUDI cases also include an array of laboratory tests. The gold standard in these investigations is histological examination of lung and liver tissue, although histology might fail at times to determine the exact cause in sudden infant death. Ancillary investigations can include the use of viral culture techniques, such as shell vial cultures (SVC) to detect viruses in lung and liver tissue and microbiological culture of swabs taken from the lungs, liver and heart to detect bacteria and other microorganisms. However, an increased PM interval (PMI) in some cases often results in the loss of viral viability in tissue samples prepared for SVC. As such, most viral culture results are negative for any of the target respiratory viruses routinely being tested for.

1.2 Diagnostic testing of respiratory viruses

Viral detection methods have evolved over the years, as novel viruses continue to emerge due to zoonotic transmission as well as evolution and identification of new viral strains. These methods involve viral culture in specific cell lines, detection of viral components (antigens or nucleic acids), as well as detection of antiviral antibodies. However, each assay has its own shortcomings and can as such limit its usefulness under specific circumstances.

1.2.1 Viral culture

RSV, Influenza, PIV and HAdV are generally diagnosed using viral culture techniques (van de Pol *et al.*, 2007). This technique involves the inoculation of viruses into cell lines and observing cytopathic effects (CPE), such as syncytia formation, lysis of cells and cell rounding that signifies the presence of the virus in cells (Datta *et al.*, 2015). The use of immunofluorescence further assists in identifying the virus responsible for the CPE, while further confirmatory tests, such as tests for acid lability, are used to distinguish between human rhinoviruses (HRV) and human enteroviruses (HEV) (van Elden *et al.*, 2002). However, viral culture is laborious and time consuming and some viruses, such as human bocavirus (HBoV), HCoV, HRV and HMPV, can be difficult to culture (Freymuth *et al.*, 2006; Burns *et al.*, 2012). This has led to the implementation of more sensitive and rapid viral detection techniques.

1.2.2 Antigen and antibody based detection

The detection of viral antigens or antibodies specific for that virus in patient samples confirms the presence of that virus at a certain point in the patient's life. One method that utilises this principle is the enzyme linked immunosorbent assay (ELISA). Targeting of antigens is generally in the acute stage of infection, while antibody targeting is useful at later stages once seroconversion has taken place. Several respiratory viruses, such as RSV, have been detected in nasopharyngeal secretions by identifying virus-specific antigens by using virus-specific antibodies in an antigen capture enzyme immunoassay (Abels *et al.*, 2001). In a study to detect RSV antigen in nasopharyngeal aspirates (NPA) obtained from hospitalised paediatric patients with suspected respiratory viral infection, ELISA proved to be insensitive in cases where the viral load was low when compared to reverse transcriptase polymerase chain reaction (PCR) technology, which was highly sensitive (Mentel *et al.*, 2005).

1.2.3 Nucleic acid amplification

Nucleic acid amplification techniques have greatly improved the sensitivity and specificity for identifying respiratory viruses in patient samples (Abels *et al.*, 2001; Jansen *et al.*, 2011). These PCR

assays are also rapid and have a much faster turnaround time than viral culture. However, this raises the question of whether or not the mere detection of a particular virus can be regarded as clinically relevant. The conduction of PCR to detect respiratory viruses has been on symptomatic individuals rather than asymptomatic. In a case-control study on symptomatic and asymptomatic children conducted by Jansen *et al.*, (2011), it was shown that unlike HRV, which was present in both symptomatic and asymptomatic children, RSV was more often detected in symptomatic than asymptomatic children. Children with respiratory symptoms showed an association between the presence of RSV confirmed with PCR and the clinical picture observed (Jansen *et al.*, 2011).

After the conduction of medico-legal investigations, more than two thirds of the SUDI cases remain without a definite COD and are therefore classified as SIDS (Dempers *et al.*, 2016). With viral culture methods alone, it is often difficult to diagnose infection, as viruses tend to lose viability after death, rendering SVC unable to produce positive results. However, if immune biomarker testing is used in parallel with tissue histology and multiplex PCR, an infectious aetiology in SIDS can be identified more often and with greater confidence. Consequently, some of the SUDI cases in which a SIDS diagnosis is made can actually be due to infection if properly diagnosed.

This was in part the reason we hypothesised that serum levels of immune markers in SUDI cases indicate an infection or inflammatory event prior to the sudden death of an infant.

1.3 Routine investigation of SUDI cases

At the time of this research, routine SUDI case investigations at the Tygerberg Medico-Legal Mortuary included human immunodeficiency virus (HIV) 1/2 antibody testing, centrifugation-enhanced SVC of lung and liver tissue, microbiological culture of swabs taken from the left lung, heart and liver, as well as histological examination of haematoxylin and eosin (H&E) stained tissue sections.

HIV 1/2 antibody testing is conducted on serum using a rapid, point-of-care ELISA based kit. However, the main limitation of conducting antibody tests in infants under the age of one year is the high percentage of false positive results due to the presence of maternal immunoglobulin (Ig) G.

A centrifugation-enhanced SVC method is used to detect viruses like HMPV, Cytomegalovirus (CMV), Influenza A and B, HAdV, RSV and HPIV 1-3 in lung and liver tissue. Different cell lines were used to culture different viruses and a commercial respiratory virus immunofluorescence assay was used to confirm the results. However, viruses from PM samples generally lose viability during the PMI and as such rarely produced positive viral culture results.

Microbiological swabs from the left lung, heart and the liver are inoculated onto MacConkey, blood and chocolate agar culture plates to detect bacteria that might have been present in these organs prior to or at the time of death of the infant. Bacteria such as *Staphylococcus (Staph) aureus*, *Haemophilus influenzae*, *Streptococcus (Strep) pneumoniae* and *Strep pyogenes* were expected to be present due to prior detection in some sudden infant death investigations (Forsyth, 1999).

Lung tissue is collected during the autopsy and stored in 10% formalin. It is then processed, embedded in paraffin wax, cut with a microtome and fixed to microscope slides. The slides are stained with H&E and evaluated under a microscope by forensic pathologists. Pathological and morphological changes (severity of tissue inflammation) were noted, including those that might be indicative of an infection or a prior inflammatory event.

1.4 Alternative molecular testing

Nucleic acid amplification tests (NAATs), such as the Seeplex RV15 ACE Multiplex PCR, which target HAdV, HBoV, HCoV, HEV, human influenza virus, HMPV, HPIV 1-4, RSV and HRV can be used to detect viral nucleic acid in tissue. However, detection of viral nucleic acid with multiplex PCR assay alone does not indicate involvement of the virus in the events leading to the death of the infant.

1.5 Testing for immune biomarkers in SIDS

Some studies proposed an improper immune response to seemingly harmless respiratory viruses, aggravated by an uncontrolled release of cytokines and chemokines, as a possible role player in the events leading up to the sudden death of an infant. After the immune response is complete, cytokines and chemokines remain in the circulation at elevated levels for several days, such as C-reactive protein (CRP) (Uhlin-Hansen, 2001). As such, they can be a target for testing to indicate a prior immune or inflammatory event in the moments leading up to the death of an infant.

There is a paucity of data for normal serum cytokine levels in infants and although biomarker testing in serum or cerebrospinal fluid (CSF) from SUDI cases has been ongoing in developed countries around the world (Vennemann *et al.*, 2012), no such information is available for South Africa. It was therefore worthwhile to explore the levels of 16 immune biomarkers of infection and inflammation in serum from SUDI cases as a possible indicator of infection prior to or at the time of death of the infants. Correlation of these immunology results with the histological picture of the lungs should contribute towards deciding the value of immunological testing and PCR screening in identifying a possible infection or inflammatory event in SUDI cases.

Additionally, comparison of culture and molecular-based screening methods for respiratory viruses should also contribute towards the establishment of a standardised, nationally accepted SUDI case investigation protocol for South Africa concerning the selection of viruses to be screened for and the best laboratory method to be used.

1.6 South African SIDS studies

Although SIDS remains one of the leading causes of death in the post-neonatal stage in developed and developing countries (Blackwell *et al.*, 2015), literature searches on PubMed and Google Scholar for studies conducted on SIDS in South Africa did not return many articles. This is in direct contrast to countries like USA, Germany, New Zealand and the United Kingdom where a lot of research are continuously being done on SIDS (Bajanowski *et al.*, 2003; Fernandez-Rodriguez *et al.*, 2006; Alvarez-Lafuente *et al.*, 2008; Weber *et al.*, 2008). More research on sudden infant death will add to better understanding of the pathogenesis of SIDS, thus paving the way forward for better management and prevention where possible. Solid, research-based evidence will add credibility in the initiation of education programmes and policy changes.

CHAPTER 2: LITERATURE REVIEW

2.1 Child and infant mortality

In September 2000, world leaders attended a summit held at the United Nations (UN) headquarters in New York to discuss possible ways in which the UN would play a role in improving the quality of life for the less privileged in underdeveloped countries. Eight goals were set and earmarked for achievement by the year 2015. The summit gave rise to what is known today as the Millennium Development Goals (MDGs). While other MDGs targeted the promotion of gender equality and combating malaria, HIV and Acquired Immunodeficiency Syndrome (AIDS), etc., the fourth MDG (MDG4) was set to target the reduction of child mortality from 1990 to 2015 by over 65% as outlined in the 2014 United Nations MDG Report[1].

The two markers identified for child mortality were the under-five mortality rate and infant mortality rate. The World Health Organisation (WHO) defines the under-five mortality rate as the number of deaths of children under the age of five per 1 000 live births. Infant mortality rate is defined as the number of infants dying under the age of one year per 1 000 live births. Global Health Observatory (GHO) data from the WHO shows a global decline during a 13 year period (2000-2013) in the number of children that are most likely to die before the age of five per 1 000 live births (Figures 2.1 and 2.2).

Despite various efforts put into achieving MDG4, the set target is quite a long way off from achievement and child and infant mortality remain global problems in both developing and developed countries (UN Millennium Development Goals Report, 2014).

2.2 Evolution of the SIDS definition

Ever since Bruce Beckwith and his colleagues coined the term “sudden infant death syndrome” in 1969, there has been much controversy surrounding the subject (Beckwith, 1970; Bajanowski *et al*., 2006).

Since the beginning of time, the death of a child or an infant has been an ever-present reality. In the Bible, there is an account of one of the earliest cases of sudden and unexpected infant death in 1 Kings 3:19, where a mother lost her son during the night because she laid on him.

[1] http://www.un.org/millenniumgoals/2014%20MDG%20report/MDG%202014%20English%20web.pdf

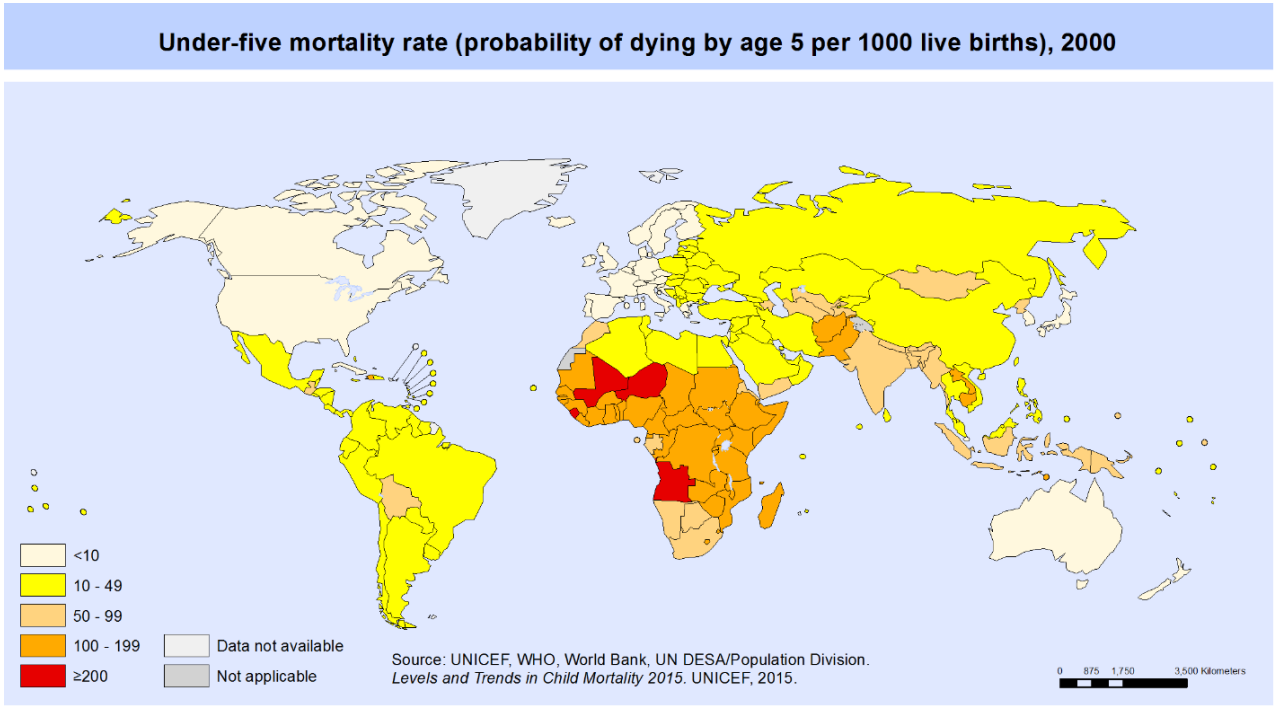

Figure 2.1 Under-five mortality rate (probability of dying by age 5 per 1000 live births), 2000[2]

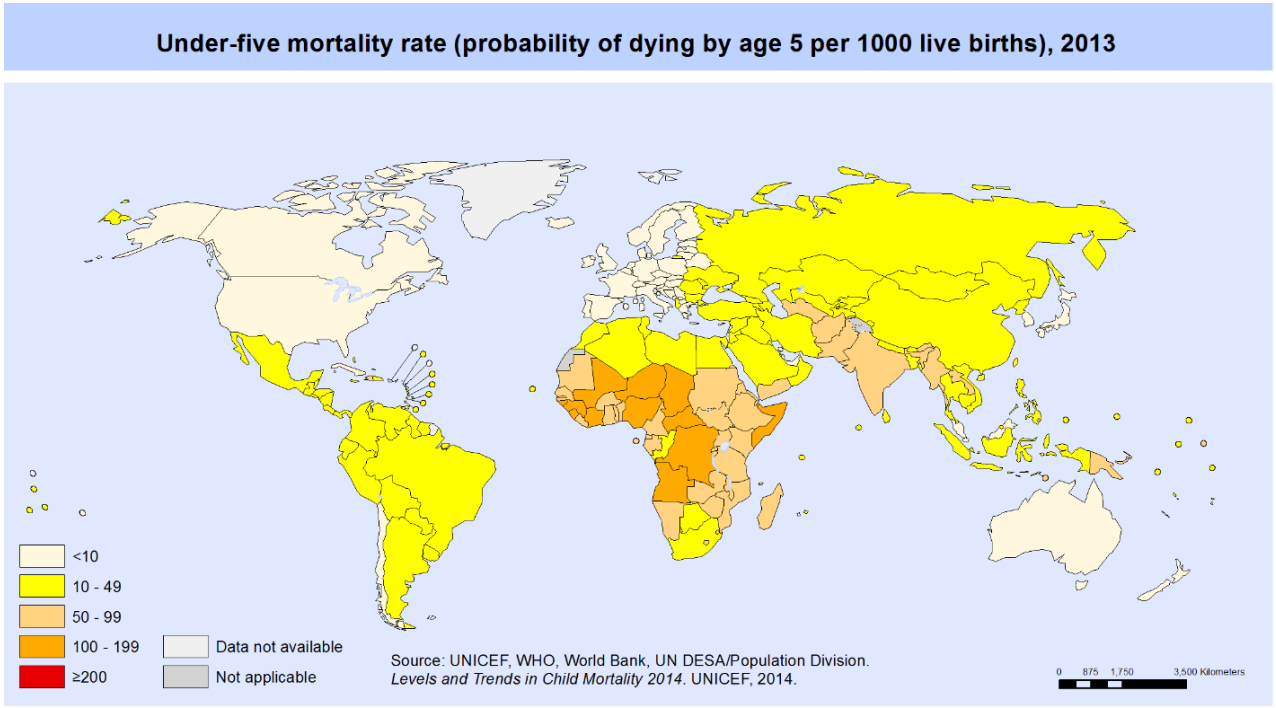

Figure 2.2 Under-five mortality rate (probability of dying by age 5 per 1000 live births), 2013[3]

[2] http://gamapserver.who.int/mapLibrary/Files/Maps/global_underfivemortality_2000.png
[3] http://gamapserver.who.int/mapLibrary/Files/Maps/global_underfivemortality_2013.png

2.2.1 The San Diego Definition and classification of death

Being the most recent definition of SIDS, the San Diego definition of 2004 is widely used to classify SIDS cases. Different sub-categories of SIDS exist and cases are classified into these sub-categories based on the investigations conducted, as well as the findings of those investigations as shown below (Bajanowski *et al.*, 2006).

Category IA SIDS

SIDS cases that meet any of the following criteria:

- Infants older than 21 days, but less than nine months old at time of death where investigation of the death scene provided no explanation of death and pathological findings observed during autopsy were not lethal.
- Infants with a normal clinical history and slept in a safe environment with autopsy showing no evidence of any unexplained trauma, abuse, neglect or unintentional injury.
- A full term infant (>37 weeks) who died in the absence of any evidence of an accident and autopsy showed no substantial thymic stress.
- An infant who grew and developed normally and toxicology, radiology, microbiology, vitreous chemistry and metabolic screening were negative at autopsy.
- An infant from a family where there was no similar death in siblings or relatives.

Category IB SIDS

SIDS cases that meet any of the following criteria:

- Full term infants older than 21 days, but less than nine months old at time of death with a normal clinical history, growth and development and from a family where there was no similar death in siblings or relatives. There was no death scene investigation and at autopsy, there was no observation of lethal pathological findings, unexplained trauma, abuse, neglect or unintentional injury, substantial thymic stress and toxicology, radiology, microbiology, vitreous chemistry and metabolic screening were negative.
- Full term infants older than 21 days, but less than nine months old at time of death with a normal clinical history, growth and development and from a family where there was no similar death in siblings or relatives. There was no death scene investigation and one or more of toxicology, radiology, microbiology, vitreous chemistry and metabolic screening were not done at autopsy.

Category II SIDS

SIDS cases that meet any of the following criteria:

- Infants whose clinical history at time of death differs from those in Category I SIDS: mechanical asphyxia or suffocation by overlaying was not determined with certainty as the circumstance of death. At autopsy, there was no observation of lethal pathological findings, unexplained trauma, abuse, neglect or unintentional injury, substantial thymic stress and toxicology, radiology, microbiology, vitreous chemistry and metabolic screening were negative or there was no conduction of one of the tests.
- Infants whose ages at the time of death ranged from 0-21 days or 270-365 days. At autopsy, abnormal growth and development were not thought to have contributed to death.
- Infants whose neonatal/perinatal conditions have resolved by the time of death and at autopsy, marked inflammatory changes or abnormalities are deemed insufficient to cause death.
- Infants from a family where there has been similar deaths in siblings or near relatives.

Unclassified Sudden Infant Death (USID)

SIDS cases that meet any of the following criteria:

- Infants whose clinical history does not meet criteria for classification into Category I and II SIDS; alternative diagnoses of natural or unnatural death are equivocal and an autopsy was not performed.

South Africa, being a developing country, does not have readily available financial resources. As such, and as per the San Diego definition of SIDS, cases diagnosed as SIDS in South Africa are classified as Category IB SIDS where death scene investigation and other supplementary tests are not conducted (du Toit-Prinsloo *et al.*, 2013).

A case in which an apparently healthy infant under the age of one year dies suddenly and unexpectedly is described as SUDI regardless of the COD being explained or not (Weber & Sebire, 2009). Various medico-legal investigations including conduction of a complete autopsy, review of infant's medical history and death scene investigation are conducted to determine the COD in SUDI cases (Figure 2.3) and where investigations fail to determine the COD, the cases are referred to as SIDS (Dempers *et al.*, 2016). It is for this reason that in reference to SIDS, the term 'diagnosis of exclusion' is sometimes used (Krous *et al.*, 2004; Blackwell *et al.*, 2015). However, despite conduction of thorough medico-legal investigations, the COD in 50-80% of SUDI cases remains

unknown (Dempers *et al.*, 2016). As such, more than half of SUDI cases have a final COD classification of SIDS.

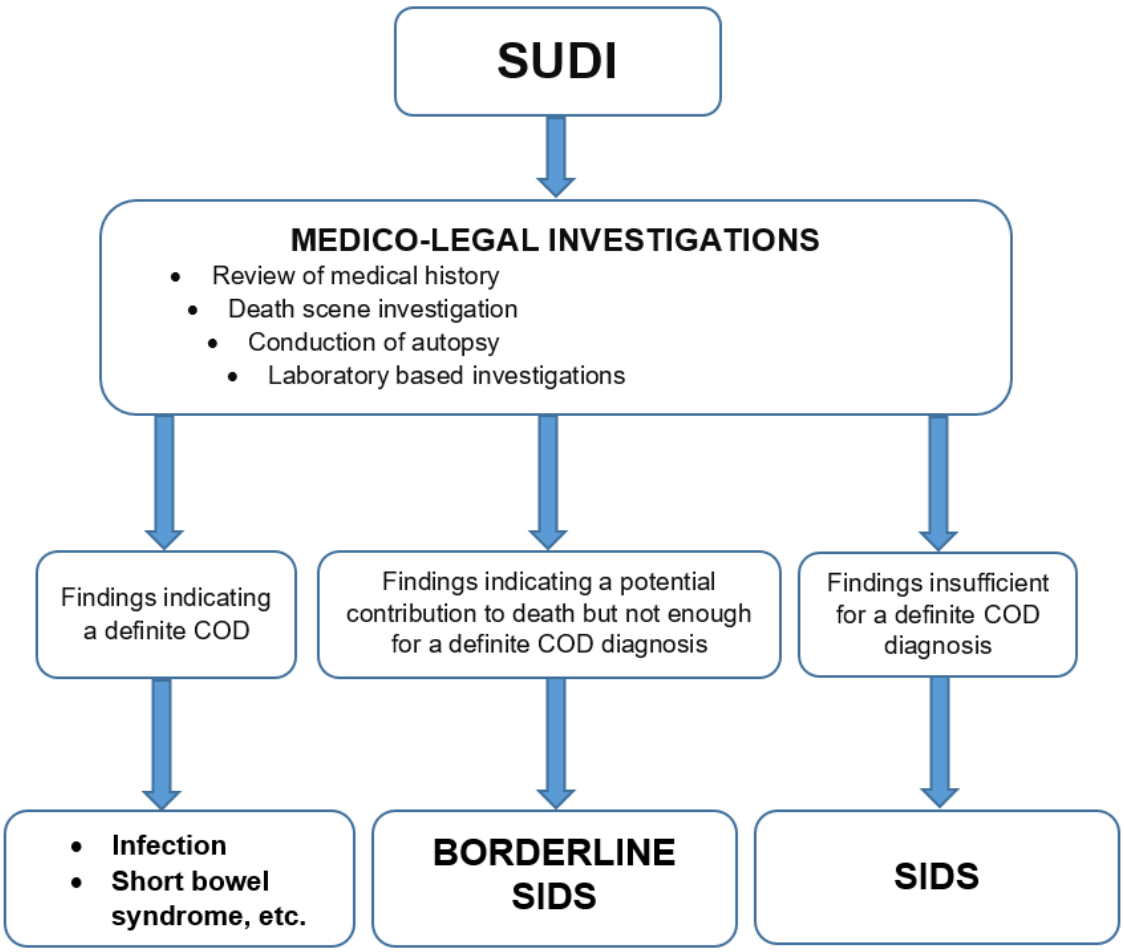

Figure 2.3 Flowchart indicating the investigation of SUDI cases to assign the COD classification to a case

2.3 SUDI and SIDS

For the last half a century or so, use of the terms SIDS and SUDI has been interchangeable. The use of either term has been largely dependent on the geographical location of the group conducting the research and reporting the results. A potential problem arising from this inconsistency in reporting results is the inability to compare data reliably between countries or institutions that reported cases differently. Differences in the definitions used to classify SIDS cases lead to misleading variations in the incidence of SIDS around the world (Jensen *et al.*, 2012). To avoid confusion around proper terminology, the term SUDI has been used increasingly as an all-encompassing term describing all cases that involve the sudden and unexpected death of an infant under the age of one year (Weber *et al.*, 2008), while SIDS has been used as a sub-classification of SUDI (Alfelali & Khandaker, 2014).

It is important to note that a diagnosis of SIDS can only be made after completion of a full medico-legal investigation that involves various inter-disciplinary components (du Toit-Prinsloo *et al.*, 2013; Hunt *et al.*, 2015).

2.4 SIDS: The Global Picture

The incidence of SIDS was remarkably high in the second half of the 20th century, specifically between 1979 and 1989, even in developed countries (Toro *et al.*, 2015). The problem has been an ever-present concern and has ultimately become a field of research in a vast number of scientific disciplines, inclusive of virology, microbiology, neuropathology, histology and genetics, among others (Blood-Siegfried, 2009). SIDS studies have mainly stemmed from the economically stable communities in Germany, New Zealand, USA and Britain and less so in developing communities in sub-Saharan Africa in an effort to better understand the underlying pathogenesis to this challenge. In 2005, the incidence of SIDS in England and Wales was 0.41 deaths per 1 000 live births (Weber, *et al.*, 2008), while an almost similar rate of 0.4 per 1 000 live births was seen in the same year in Germany (Vennemann *et al.*, 2009). In 2009, SIDS incidence in the USA was 0.57 per 1 000 live births with over 2 000 infant deaths recorded every year (Adams *et al.*, 2009). Six years later in 2015, close to 4 000 infants died suddenly and unexpectedly in USA, while 1 600 of those deaths were attributed to SIDS (Goldstein *et al.*, 2016).

The face down or prone sleeping position in infants is a modifiable risk factor for SIDS and as such was a target for intervention campaigns to address the increasing incidence of SIDS (Adams *et al.*, 2015). Through the initiation of the “Back to Sleep” campaign in the early 1990s, parents and caregivers were educated on the dangers of putting infants to sleep in the prone position. The campaign was a great success and saw a decrease of more than 50% in the incidence of SIDS (Adams *et al.*, 2015; Mitchell & Krous, 2015; Ferrante *et al.*, 2016). Despite this, SIDS is still regarded one of the leading COD in infancy in both developed and developing countries (Blackwell *et al.*, 2015).

Although the “Back to Sleep” campaign managed to reduce SIDS incidence by over 50% in the late 1990s, SIDS still ranks among the leading post-neonatal COD in the first year of life. Other risk factors for SIDS pointing towards an infectious aetiology still exist. These include a peak winter incidence, exposure to cigarette smoke, poor socio-economic background and predominance of infants aged between two to four months (Raza & Blackwell, 1999; Fleming *et al.*, 2015). The consensus in the scientific community is that SIDS is a problem that needs careful consideration and decisive action in both developed and developing communities (Blackwell *et al.*, 2015).

With the various advancements in the scientific landscape over the past century, sudden and unexpected infant deaths have been a subject of more evidence-based scrutiny. This has enabled conduction of various research projects that have served as stepping stones for better understanding the dynamics of child and infant mortality, leading to various causes of infant death being reported.

Substantial financial investments made over the years focussed on the role of respiratory viruses in SIDS as identified by different assays. Viral culture, immunofluorescence and PCR assays have detected HAdV, HRV, influenza, RSV and other viruses during SUDI case investigations (Weber *et al.*, 2010), but none of them has been identified as the sole causative agent in sudden infant death. Respiratory viral infections are often acute and self-limiting and might therefore be less likely to directly lead to the death of an infant (Ghani *et al.*, 2012).

2.5 The South African Picture

There is not nearly enough studies in South Africa on SUDI case investigations (du Toit-Prinsloo *et al.*, 2013). As such, it is very difficult to predict the current incidence of SIDS in South Africa accurately. In Cape Town, South Africa, the estimated incidence of SIDS ranks among the highest in the world, being 3.41 per 1 000 live births (Dempers *et al.*, 2016). Although there are seven academic medico-legal centres across South Africa, only two of these (Tygerberg and Pretoria) have been relatively active in SIDS research. Furthermore, there is no inter-institutional standardisation in the conduction of SUDI case investigations between these medico-legal institutions and this is as a major drawback to the establishment of a comprehensive source of SIDS incidence data (du Toit-Prinsloo *et al.*, 2011).

2.6 Risk factors for SIDS

One of the most important questions that needed to be answered was whether all infants were equally vulnerable to succumbing to sudden death and if not, why not? Epidemiological studies and death scene investigations all over the world have identified various risk factors for sudden and unexpected death in infants. These risk factors are loosely categorised into namely socio-demographic, environmental, pregnancy, genetic and infection-related risks (du Toit-Prinsloo *et al.*, 2011).

2.6.1 Socio-demographic risks

The socio-demographic characteristics of an individual are generally described by their age, sex, education and income. Infants aged between two and four months are at a greater risk of succumbing to SIDS. It is at this age that passively acquired maternal immunity wanes and the infant's own immune system, although not fully mature yet, needs to combat any foreign pathogens (Moscovis *et*

al., 2014). As such, infants are most vulnerable during this time and a peak SIDS incidence at this age is not a coincidence.

In addition, male infants are more likely to die suddenly and unexpectedly as compared to their female counterparts. An *in vitro* study to assess the cytokine responses of male and female peripheral blood mononuclear cells (PBMC) to a bacterial antigen challenge showed a lower production of pro-inflammatory cytokines by males than females. This inability to produce sufficient cytokines against a bacterial antigen suggests possible vulnerability to infection (Moscovis *et al.*, 2014).

2.6.2 Sleep related risks

The sleeping environment of infants is critical. The most significant sleep related SIDS risk identified over the years has been the sleeping position of the infant. An infant sleeping in a prone position was found to be at a greater risk for SIDS compared to infants who were put to sleep on their backs (supine sleeping position) (Vennemann *et al.*, 2007). Additionally, prone sleeping may render infants more susceptible to infection of the URTI by pathogenic bacteria, especially if the infant sleeps on contaminated surfaces (Highet, 2008).

The prone sleeping position was identified as an easily modifiable risk factor for SIDS and was therefore targeted for intervention. As mentioned previously, the American Academy of Pediatrics (AAP) and National Institutes of Health (NIH) came together in 1994 to implement the “Back to Sleep” campaign aimed at educating mothers and caregivers on the dangers of prone sleeping (Adams *et al.*, 2009). Despite a 50% reduction in SIDS incidence rates achieved by campaigns such as this (Mitchell & Krous, 2015), SIDS is still regarded as a leading COD in infants under one year in developed countries (Blackwell *et al.*, 2005).

Co-sharing of a bed between an infant and a parent(s) or another individual(s) was also confirmed as a risk factor for sudden and unexpected death. The risk was significantly amplified in cases of low birthweight infants (Adams *et al.*, 2009), direct exposure to cigarette smoke from parents or caregivers and the use of alcohol and drugs by parents or caregivers, both before and after birth (Gunn *et al.*, 2000; Mitchell & Krous 2015).

2.6.3 Pregnancy-related risks

Infants born to young mothers were seen to be at an increased risk of dying suddenly and unexpectedly (Fleming *et al.*, 2003), while smoking during the pregnancy exacerbated the risk. Evidence that premature birth increases the possibility of succumbing to SIDS also exists. Infants depend on passively acquired maternal antibodies for protection against infections. However, only a

limited number of antibodies are passed on to the infant during the first and second trimester of pregnancy. It is only after 32 weeks gestation that the infant starts acquiring maternal IgG (Crowe & Williams, 2003). As such, preterm infants are considered to be at a greater risk of developing more severe symptoms of RSV infection with possible subsequent SIDS compared to term infants (Horn & Smout, 2003; Mitchell & Krous, 2015).

2.6.4 Genetic risks

Over the years, research to try to elucidate the possible links between genetic factors and sudden infant death has delivered inconclusive findings. In 2004, Opdal and Rognum postulated the existence of a SIDS gene or genes and proposed two possible mechanisms in which genetic factors could play a role in sudden and unexpected death. The first was that specific gene mutations could give rise to metabolic disorders that can directly result in the death of an infant. Alternatively, a change in the morphology of a gene could predispose infants who found themselves in a vulnerable position to sudden infant death (Opdal & Rognum, 2004). However, the involvement of genes in the pathogenesis of SIDS is still inconclusive and mostly a matter of differing opinion.

2.6.5 Infection related risks

Evidence of infection has been well documented in SIDS cases over the years and other risk factors, such as the peak age range and high winter incidence, agree with increased vulnerability to URTI and LRTI (Raza & Blackwell, 1999). As early as the 1960s, SIDS investigations demonstrated viral presence in autopsies of 12 of the 48 infants included in the study (Alfelali & Khandaker, 2014). The peak incidence during winter months further supports the possible involvement of viral infections in the pathogenesis of SIDS (Morris, 1999; Alfelali & Khandaker, 2014), although viral infections are common in young, living children as well (Samuels, 2003). These infections are rarely severe and cause acute URTI and LRTI that often resolve without any major complications (Ghani *et al.*, 2012). As such, it is possible that the existence of some other factors rather than infection alone, predispose an infant to sudden and unexpected death. Infants may have an inherited condition or conditions rendering them susceptible to viral infections and more at risk of SIDS than the mere presence of the virus itself. Environments that expose infants to tobacco smoke also enhance the susceptibility of an infant to a viral infection that otherwise would not have been fatal in the first place (Samuels, 2003). The inflammatory response to infection has been identified as an important potential role player in the sequence of events that lead to infant death (Blackwell *et al.*, 2002).

Although bacteria, such as *Staph aureus, Haemophilus influenzae, Strep pyogenes* and *Strep pneumoniae* have been identified in culture during SUDI medico-legal investigations, none have been

consistently associated with sudden and unexpected infant death (Forsyth, 1999), with some being dismissed as PM contamination (Rambaud *et al.*, 1999).

2.7 Mechanisms of death

The *Triple Risk Hypothesis* was first proposed by Rognum and Saugstad (1993) and subsequently described by Filiano and Kinney (1994). It emphasises the multifactorial nature of SIDS (Figure 2.4), where risk factors are not the sole cause of sudden infant death, but merely increase the infant's susceptibility which may lead to death (Blood-Siegfried, 2009).

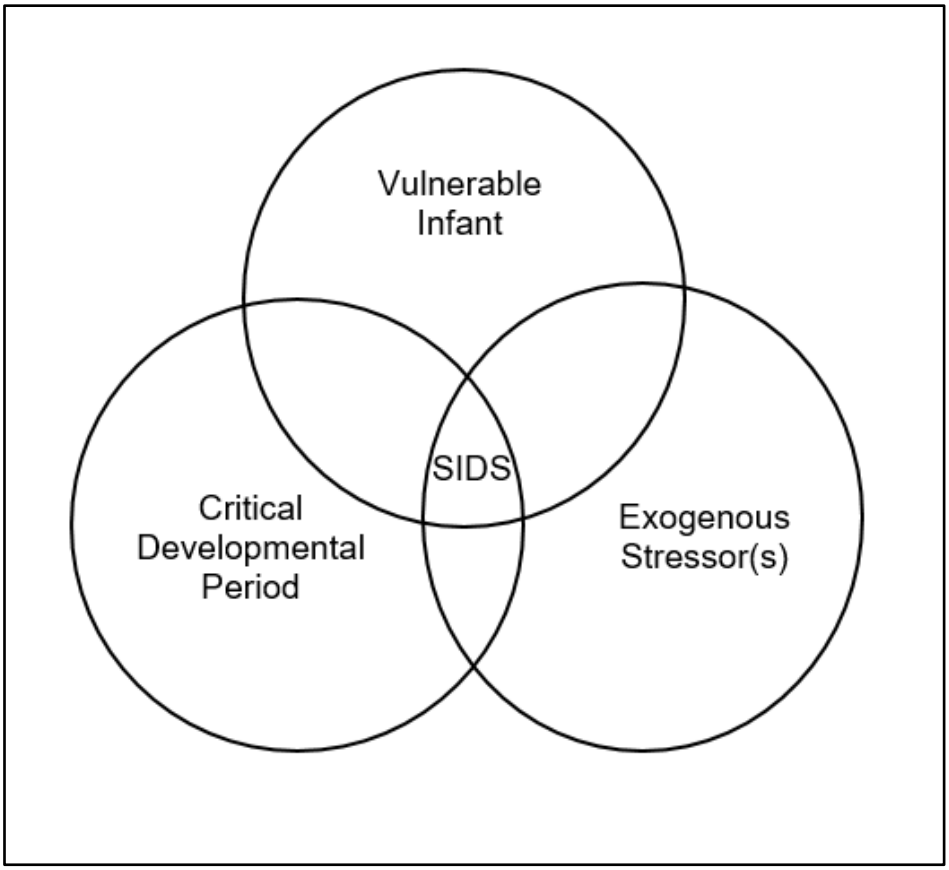

Figure 2.4 The Triple Risk Hypothesis (adapted from Filliano & Kinney, 1994)

The sequence of events that ultimately lead to the death of a seemingly healthy infant is still not fully understood. Accidental suffocation, metabolic disease and sudden hypoxaemia are some of the physiological mechanisms that can contribute to infant death. Hypoxaemia can be a result of respiratory infections in some instances (Samuels, 2003). Although several hypotheses about SIDS have been proposed, it is generally agreed that the sudden death of an infant results when various circumstances or events trigger a reaction at a specific, opportune time of vulnerability. Therefore, the Triple Risk Hypothesis suggests that sudden death in infants is most likely to occur when there is an overlap of external stressors (viral infection or bed-sharing) in a vulnerable infant (one with a

prenatal brainstem injury or other predisposing genetic factor) at a critical developmental stage in the life of the infant (two to four months of age) (Guntheroth & Spiers, 2002).

2.8 Respiratory viruses in infancy and SIDS

Viral respiratory infections play a role as potential triggers in SIDS (Samuels, 2003). Infancy is a very early stage of growth and development and as such represents the most vulnerable period of life. At this stage in life, an infant depends on short-lived maternal immunity passed on from their mothers during the pre- and post-natal period for protection against invading pathogens. The infant's immune system at this point is not yet fully developed and capable to combat the various immune challenges that may be presented to it on its own. It is also at this time when infections with various bacteria and viruses are most likely to happen (Goenka & Kollmann, 2015). Viral LRTI can lead to an influx of inflammatory cells into the lungs, causing damage to the lung tissue as seen with RSV infections. As such, the infiltration of these cells into the tissue has to be well regulated to prevent damage to the lungs themselves while fighting off the pathogen (Loebbermann, *et al.*, 2012).

Viral infections are often implicated in infant morbidity and mortality (Ghani *et al.*, 2012). Additionally, acute respiratory infections are some of the leading causes of morbidity and mortality in infants and young children (Williams *et al.*, 2002). During winter, children worldwide are most frequently hospitalised because of viral respiratory infections (Melendi *et al.*, 2007).

Dagan *et al*. (1989) conducted a study in the USA on previously healthy infants less than three months of age to determine the frequency of viral infections in infants brought to the hospital with a suspicion of bacterial infection and found that these two types of infection (bacterial and viral) occurred in tandem in their study participants. The most isolated viruses in this study were RSV, influenza A and HEV, while RSV and influenza A were not detected in their control group. Viruses commonly associated with acute LRTI in infants include HPIV, influenza, RSV and HAdV (Choi *et al.*, 2006). Viral respiratory infections in the first year of life occur frequently and more than 50% of infants have a LRTI during this period (Ghani *et al.*, 2012). Although it causes severe upper and lower respiratory symptoms in some cases, the symptoms in a majority of cases are not life threatening and while some infants are hospitalised; only a few need paediatric intensive care (Samuels, 2003; Ghani *et al.*, 2012). In addition to the seemingly innocuous nature of the respiratory virus infections, pre-existing comorbidities like prematurity, malnutrition and cardiac abnormalities may predispose an infant to a more severe viral infection (Ghani *et al.*, 2012). This supports the hypothesis that more than a single infection is necessary to predisposes an infant to sudden and unexpected death. Infants that have inherited conditions or are in environments that expose them to tobacco smoke are more susceptible to viral infections that would otherwise not have been fatal. As stated before, the

inflammatory processes that take place in response to infections have been confirmed as potential role players in the sequence of events that lead to infant death (Blackwell *et al.*, 2002).

Although quite a number of viruses have been associated with sudden death of infants, none of them has been independently implicated in this regard. Some of the viruses detected during SUDI case investigations are shown in Table 2.1. Quite a few bacterial species and their toxins have also consistently been isolated from PM SUDI samples. These include *Staph aureus*, *Escherichia coli*, *Clostridium perfringens* and *Clostridium botulinum*, amongst others. These bacterial pathogens induce their effect through the release of endotoxins that have various effects on the body's physiology (Blackwell *et al.*, 2002; Highet, 2008).

Table 2.1 Viruses identified in SUDI investigations

Identified Viruses	References
Adenovirus	Williams *et al.*, 1984; Weber *et al.*, 2010
Cytomegalovirus	Fernandez-Rodriguez *et al.*, 2006; Desmons *et al.*, 2013
Enterovirus	Weber *et al.*, 2010
Influenza A and B	Williams *et al.*, 1984; Weber *et al.*, 2010
Parainfluenza	Williams *et al.*, 1984
RSV	Williams *et al.*, 1984; Weber *et al.*, 2010
Varicella zoster virus	Desmons *et al.*, 2013

2.8.1 HAdV

HAdV is a double-stranded, deoxyribonucleic acid (DNA) virus, belonging to the family *Adenoviridae* (Mahony, 2008). Ever since it was first isolated in 1953, more than 60 HAdV types have been described. Of these, respiratory tract infections are caused by HAdV subtypes B, C and E (Liu *et al.*, 2015).

2.8.2 HBoV

HBoV was first discovered in 2005 in NPAs at Karolinska University in Sweden (Allander, 2008). The NPAs had been obtained from patients suspected of having a respiratory tract infection. It is a single-stranded DNA virus within the family *Parvoviridae*, subfamily *Parvovirinae*, and genus *Bocavirus* (Mahony, 2008).

2.8.3 Human Influenza virus

This is a negative-sense single-stranded ribonucleic acid (RNA) virus, belonging to the *Orthomyxoviridae* family. Influenza virus is responsible for annual epidemics in temperate regions and causes illness consistent with an URTI (Mahony, 2008).

2.8.4 HMPV

Discovered in 2001 in Netherlands, HMPV is a negative-sense, single-stranded RNA virus that belongs to the *Paramyxoviridae* family, subfamily *Pneumovirinae* and genus *Pneumovirus* (Mahony, 2008). It causes pneumonia, URTIs and bronchiolitis of lower severity than RSV (Guerrero-Plata *et al*., 2005).

2.8.5 HPIV

The HPIV is a single-stranded, negative-sense RNA virus belonging to the *Paramyxoviridae* family. Four serotypes in this family (PIV 1-4) are infectious to humans but PIV 3 is the major problem in infants especially in the cold months as it causes severe LRTI leading to bronchitis and pneumonia (Mahony, 2008).

2.8.6 RSV

RSV is a single-stranded, enveloped RNA virus first isolated in 1956 from a chimpanzee suffering from respiratory problems. It belongs to the genus *Parapneumovirus* within the subfamily *Pneumovirinae* and the family *Paramyxoviridae*. This is the same family that HMPV belongs to (Mahony, 2008). Two antigenic subtypes of RSV exist, namely RSV A and RSV B; the former causing more severe disease ranging from mild symptoms to severe and potentially life threatening, while RSV B infection is mostly asymptomatic. RSV is a leading cause of LRTI in infants, causing pneumonia and bronchiolitis. In the USA, 60% of infants are infected with RSV by age one, with the figure rising to 90% by the age of two. Prematurity, congenital heart disease and a deficient T-cell response predispose infants to more severe forms of RSV infection (Welliver, 2003).

2.8.7 HRV

These are positive-sense, single-stranded RNA viruses from the *Picornaviridae* family (Mahony, 2008). Like anywhere in the world, HRV infections are common throughout the year in South Africa, peaking in autumn and spring (Smuts *et al.*, 2011). Respiratory tract infections usually involve HRVs and they are an important cause of the common cold. They cause both URTI and LRTI in infants, manifesting as bronchiolitis and exacerbation of asthma often resulting in hospitalisation (Busse *et al.*, 2010; Piper *et al.*, 2013).

A virus can potentially contribute to SUDI in various ways. According to Vege and Rognum (1999), an improper or uncontrolled immune reaction to a respiratory viral infection exacerbates a seemingly harmless viral infection into a fatal one (Vege & Rognum, 1999). However, Forsyth is of the opinion that an uncontrolled immune reaction to viral and bacterial infections triggers a sequence of events that ultimately lead to the eventual death of an infant (Forsyth, 1999; Weber *et al.*, 2010).

2.9 The immune system and response to viral infections

As the maternally derived IgG antibodies begin to wane off, the infant's immune system takes over the burden of full time defence against pathogens. It starts producing cell signalling and regulatory molecules, such as cytokines, chemokines and other immune biomarkers in response to invading organisms. In this way, a specific, co-ordinated and controlled inflammatory response is produced to contain and clear the pathogens. Immune system molecules produced in response to viruses include interleukin (IL)-1α, IL-1β, IL-6, interferon (IFN)-γ and tumour necrosis factor (TNF)-α. CRP, an acute phase protein, is another molecule produced by the liver and released into circulation soon after infection occurs. Levels start increasing within 6 hours and reaches a peak around 48 hours (Fujita *et al.*, 2002), but the absence of elevated CRP does not exclude the possibility of an infection or inflammation prior to death.

When a competent immune system encounters foreign particles, such as bacteria and viruses, it reacts to minimise the potential damage that the microorganism might cause and eventually rid the body of the invading pathogen. The first line of defence against viruses is the skin and other components of the innate immune response. The innate immune system makes use of pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) to confirm the presence of the virus. These receptors identify pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs), which are associated with viruses and other pathogens. This initial recognition leads to the activation and recruitment of immune cells via the upregulation of cell adhesion molecules and secretion of various specific and non-specific immune molecules, such as cytokines, chemokines and other acute phase proteins into the bloodstream to induce an inflammatory reaction (Pryce *et al.*, 2014; Donovan *et al.*, 2016). Over the years, viruses have devised mechanisms to evade the immune system. Once the virus evades the innate immune response, cells of the adaptive immune system respond to limit the damage caused by the virus by continuing the release of immune biomarkers, like cytokines, chemokines and other mediators of inflammation. This leads to neutralisation of the effects of viral entry and replication in cells and eventual virus removal from the body. Although the ultimate aim of the immune system is to clear the virus from the body, it does so in differing ways. Different immune responses are elicited by different viruses and this was shown in

in RSV-infected infants, where there was a marked increase in serum IL-4 and IL-5 (T-helper 2 cell cytokines), while in influenza A-infected infants responded with a predominance of TNF-α, which is a T-helper 1 cell cytokine (Sung *et al.*, 2001). Once the inflammatory response is complete, the immune biomarkers persist in circulation for a while, depending on their half-life. This property makes them an ideal target for identifying a prior inflammatory event.

2.10 Inflammation and SIDS

Viral respiratory symptoms are observed two weeks prior to sudden and unexpected death in 44% of SIDS cases and these symptoms have been linked to evidence of inflammatory processes (changes in respiratory epithelium) seen in SUDI case investigations (Goldwater, 2004). This may play a significant contributory role towards SUDI. Mistchenko *et al.* (1994) conducted a study on HAdV infections in infants and showed the involvement of various cytokines in the inflammatory processes elicited by severe HAdV infections. They also suggested that circulating immune complexes might have been deposited on tissue and ultimately led to the eventual tissue damage observed.

Observation of significant inflammatory changes in tissue can be used as an indicator of a prior pathological event (infection) and in forensic pathology, tissue histology is regarded as the gold standard in determining the ultimate COD when performing autopsies (Uhlin-Hansen, 2001; Blackwell *et al.*, 2015). However, histology results alone at times may not be sufficient to determine the COD. As such, some studies in Europe and the USA included biomarkers of inflammation by measuring serum cytokines as an indicator of infection or an inflammatory process prior to the death of an infant (Vennemann *et al.*, 2012). However, there is a paucity of this kind of information or such studies being conducted in South Africa.

A history of a mild viral infection and immune stimulation of the URTI prior to death is often reported in SIDS cases (Raza & Blackwell, 1999; Vege & Rognum, 1999; Blackwell *et al.*, 2015). Studies on the possible roles played by cytokines and chemokines in sudden infant death have produced various results. While some studies failed to identify a significant difference in levels of serum and CSF cytokines (IL-1β, IL-6 and TNF-α) observed in SIDS compared to non-SIDS cases (Vennemann *et al.*, 2012), some have shown increased CSF IL-6 levels in SIDS cases compared to non-SIDS cases (Vege *et al.*, 1995). Some researchers have hypothesised that an inflammatory response to an infection precipitates a sequence of events that culminate in the death of the infant. Blackwell *et al.* (2015) showed just how detrimental to survival an incompetent inflammatory response is to the infant's chances of survival. It is possible that sudden infant death is partly a result of failure of the infant's immune system to regulate the innate inflammatory process against a viral infection (Forsyth, 1999).

2.11 Cytokines in SUDI

Cytokines are a group of peptide, signalling molecules secreted by various cells of the immune system in response to a foreign object or invading pathogen in the body. They are involved in the mediation and regulation of the inflammatory response to ensure that it is controlled and coordinated. Cytokines can be classified as pro-inflammatory or anti-inflammatory based on their effect. Various cytokines have been studied in SUDI case investigations to analyse the role that these molecules play in the inflammatory processes that take place in sudden infant death cases.

Pro-inflammatory cytokines, such as IL-1α, IL-β, IL-6, IL-12, IFN-γ and TNF-α, induce an immune response that targets control and eventual clearance of an invading pathogen. However, when the immune response is left uncontrolled, it can spiral out of control and cause more damage to the host than what the invading pathogen itself might have done (Opal & DePalo, 2000). Anti-inflammatory cytokines, such as IL-4, IL-6, IL-10, IL-11, IL-13, transforming growth factor (TGF)-β) and IL-1 receptor antagonist (IL-1RA), are important to dampen the action of pro-inflammatory cytokines, thus regulating the duration of the inflammatory process and preventing host cell damage by the immune system.

Various viruses can infect the respiratory tract of infants, but none of them induces pathognomic symptoms. Infection by two different respiratory viruses can produce a similar clinical picture. The cytokines that are released by the immune system in response to a virus differ according to the nature of the virus. This is particularly important in cases of RSV and HMPV infections that produce clinically similar manifestations. In a study by Guerrero-Plata and colleagues (2005) investigating the immune response tot RSV and HMPV in BALB/c mice, different cytokine profiles were observed for the different infections. After 24 hours, the levels of IL-1α, IL-1β, IL-6, TNF-α in bronchoalveolar lavages (BAL) collected from mice infected with either virus were found to be significantly higher in RSV-infected mice than in HMPV-infected mice. IFN-α, a major component of the innate immune response to viruses, was significantly increased in HMPV-infected mice compared to RSV-infected mice (Guerrero-Plata *et al.*, 2005). In addition, BAL IFN-γ (a key component of antiviral activity) levels were increased in RSV-infected mice compared to HMPV-infected mice one day post-infection, but only slightly increased five days post infection. RSV infection was also able to induce production of IL-10, IL-12p70 and IL-10 whereas HMPV infection failed to induce the production of these cytokines.

Cytokines have various effects on different systems in the body depending on the physiological needs and some of their major roles in infection and inflammation are outlined below.

2.11.1 CRP

It is an acute phase protein produced in the liver and is responsible for non-specific activation of the immune system upon detection of an invading pathogen. Circulating serum CRP levels can increase as a result of an inflammatory stimuli and remain elevated for up to six days after death (Uhlin-Hansen, 2001).

2.11.2 TNF-α

$CD8^+$ effector T cells are responsible for the eradication of cytopathic viruses through the secretion of TNF-α and IFN-γ (van den Broek, *et al.*, 2000).

2.11.3 IFN-γ

IFN-γ is a type II interferon mainly produced by natural killer (NK) cells, a large proportion of which are found in the lungs. However, CD4 T-helper cells and CD8 T-cytotoxic cells can also produce IFN-γ after the development of antigen-specific immunity. Some of its major roles in the immune system include enhancing phagocytosis and directly limiting the replication of an invading virus (Newton *et al.*, 2016). Abboud *et al.* (2015) confirmed in a mouse model the essential role of IFN-γ in limiting the replication of vaccinia virus and eventual clearance of the virus with the help of CD8 T cells.

2.11.4 IFN-γ induced protein 10 (IP-10)

IP-10, also known as CXCL10, is a chemokine whose receptor, CXCR3, is found on activated T lymphocytes and NK cells. In the event of a viral infection, the chemotactic abilities exhibited by IP-10 are responsible for the recruitment of activated T lymphocytes and NK cells to the site of infection. Studies suggest that IP-10 expression can be induced by a viral infection, such as chronic hepatitis C virus (HCV) infection. HCV, present in the hepatocytes, induces IP-10, which in turn leads to the migration of inflammatory cells into the liver lobules; a vital indicator of the progression of disease. IP-10 expression has been correlated with inflammatory activity (Harvey *et al.*, 2003). In a study conducted to evaluate chemokine responses to serious acute respiratory illness in BAL samples, IP-10 levels were significantly increased in patients where a virus had been detected by multiplex PCR in comparison to those where no virus was detected (Sumino *et al.*, 2010).

2.11.5 IL-1

An agonist is a molecule which, when combined with a receptor, initiates a physiological response. The IL-1 family comprises of two such agonists, namely IL-1α and IL-1β and these are important in host defence against organisms that divide inside cells. In addition, there is also a receptor antagonist,

IL-1RA, which binds to the IL-1 cell surface receptors, thus inhibiting the activity of IL-1. In patients with an infection or inflammatory condition, IL-RA levels are increased. If there is an imbalance in the levels of IL-1 and IL-RA, inflammatory disease and structural damage can be observed in the lungs and other organs (Arend, 2002). IL-RA has strict anti-inflammatory properties. It is produced by macrophages to inhibit the activation of cells by IL-1α and IL-1β by competitive blocking of membrane bound IL-1α and IL-1β receptors. IL-RA release is elicited by the presence of bacterial lipopolysaccharide (LPS) in the circulation (Opal & DePalo, 2000).

2.11.6 IL-6

IL-6 is produced by B and T cells, as well as monocytes. Although it is often identified as both a pro- and anti-inflammatory cytokine, IL-6 is mostly involved in anti-inflammatory responses. Its main function is to inhibit the production of the pro-inflammatory cytokines IFN-γ, Macrophage Inflammatory Protein (MIP)-2), IL-1 and TNF by macrophages, while favouring the production of IL-RA.

2.11.7 IL-8

Also known as CXCL8, this chemokine is produced by macrophages as an innate immune response to infection and is mainly involved in inducing the migration of neutrophils to the site of infection.

2.11.8 IL-10

It is regarded as the most important anti-inflammatory cytokine in the human immune response due to its potency. It is mainly produced by the T helper-2 cells, monocytes and B cells. It is involved in downregulating the expression of pro-inflammatory cytokines, such as IFN-γ, IL-1β and TNF-α.

2.11.9 IL-12

IL-12 is an important cytokine responsible for inducing the development of $CD4^+$ T helper cells that are essential for viral clearance (van den Broek, *et al.*, 2000).

As stated before, detection of viral nucleic acid alone with multiplex PCR is not enough to classify the COD as infection in SUDI cases. As such, understanding the interactions of the immune system with an invading virus and the resulting inflammatory changes as observed in tissue samples may prove to be vital in shedding more light into the possible role of viral infections in the events leading up to SUDI.

CHAPTER 3: MATERIALS AND METHODS

3.1 Ethics

The Stellenbosch University Health Research Ethics Committee (HREC) first approved this project on the 7th of March 2012 (Ethics Reference Number N12/02/007). The ethics approval was renewed on an annual basis. Since the investigations conducted in this study did not deviate from the routine laboratory investigation protocol for respiratory pathogens in SUDI cases, a waiver of consent was granted, meaning that the deceased infants' parents did not have to give additional permission for the collection of samples to be used in the study.

3.2 Study design

The study was a cross-sectional, analytical, descriptive study, divided into two parts. The first part of the study was virological testing of swabs collected from the left and right lung well as the trachea of all SUDI cases admitted to the Tygerberg Medico-Legal Mortuary between July 2015 and June 2016.

The second part of the study was testing for 16 immune biomarkers in serum samples collected from all SUDI cases admitted to the Tygerberg Medico-Legal Mortuary between July 2015 and June 2016.

3.2.1 Inclusion criteria

Death must have been sudden and/or unexpected and the circumstances must have been unexplained. The case must have presented as SUDI in terms of the definitions of the National Health Act, (No 61 of 2003) and the subsequently formulated Forensic Pathology Guidelines on unnatural deaths. The age of the infant should also have been between one day and one year at the time of death.

3.2.2 Exclusion criteria

Infants who died of unnatural causes and/or were above the age of one year at the time of death were excluded from the study.

3.2.3 Data collection method

Socio-demographic information from SUDI cases was collected from parents or caregivers by the Forensic Pathology Officers at the scene of death and at the medico-legal mortuary by completing a standard questionnaire. From these questionnaires, socio-demographic information relevant to the study (age, sex, PMI, season, sleeping position, medical history prior to death) was then collected and entered into a dedicated database. The questionnaire is attached as Addendum A1.

3.3 Aims and Objectives

The main aims of the study were:

- To profile respiratory viruses observed in lungs and trachea in SUDI case investigations conducted at the Tygerberg Medico-Legal Mortuary. This objective was carried out by conducting multiplex PCR on nucleic acid extracts from lung and tracheal swabs using the Seeplex RV15 ACE detection kit.
- To measure the levels of immune biomarkers (cytokines, chemokines and an acute phase protein) in serum from all SUDI cases admitted to the Tygerberg Medico-Legal Mortuary between July 2015 and June 2016 and assessing their usefulness as indicators of infection or inflammation prior to or at the time of death of the infant.

Different specific objectives were defined and various methods utilised to achieve them as shown below:

3.4 OBJECTIVE 1

a) To prospectively collect the lower lobes of the left and right lungs from SUDI cases for histological examination by forensic pathologists at the Forensic Pathology Division;

b) To prospectively collect heart, left and right lung swabs for routine culture testing by the NHLS Microbiology laboratory;

c) To prospectively collect blood, as well as lung and liver tissue for routine testing by the NHLS Virology laboratory.

3.4.1 Sample collection

As part of the standard, institutional investigation protocol for SUDI cases at the Tygerberg Medico-Legal Mortuary, samples were collected for routine virology, microbiology and histological examination.

3.4.2 Routine histology samples

The lower lobes of both the left and right lung tissue were collected for the purposes of histological examination by forensic pathologists. Prior to sample collection, the forensic pathologist conducting the PM inspected the lungs for any macroscopic abnormalities, such as petechial haemorrhages. Afterwards, sterile blades and scalpel handles were used to excise the entire lower lobes of both lungs. The tissue samples were then placed in pre-labelled, 90 ml polypropylene tubes (CJ Labs, Cape Town) and transported within 10 minutes to the Forensic Pathology Division where 10% formalin

was added. The time interval between tissue sample collection and addition of formalin was not prolonged and as such prevented autolysis of tissue.

Histological preparation of tissue

To prepare slides for histological examination, 5 mm x 5 mm pieces of tissue from the deeper part of both lungs were cut separately using a sterile blade and placed into two different plastic cassettes labelled with the unique case number (WC/14/case_number/year). The tissue was secured in the cassette using a cassette cover and immediately placed into 10% formalin for 24 hours to facilitate tissue fixation. Afterwards, the cassette was placed in a Tissue-Tek VIP 5 Jr tissue processor where the tissue was processed as follows:

- Fixation – tissue was placed in 10% formalin (1 hour).
- Dehydration – after fixation, the tissue was immersed in *increasing* alcohol concentrations (70%, 85%, 96% and 100% alcohol) (3 hours).
- Clearing – the alcohol was then removed from tissue by placing tissue in pure xylene (3 hours).
- Impregnation – tissue was placed in paraffin wax to fill any gaps in the tissue (4 hours).

After completion of tissue processing, tissue blocks were embedded in paraffin wax; ensuring the correct orientation of the tissue to facilitate the cutting of tissue sections that include the histology being investigated. The tissue blocks were placed on an ice tray to rapidly cool down and solidify, thereby facilitating cutting of sections as soon as possible after processing. A MICROM microtome was used to cut sections of 3 µm thick. The sections were floated in a 60°C water bath to allow melting of the wax. Afterwards, the sections were picked up and placed onto an appropriately labelled glass slide. The slide was dried by placing it in a Scientific Series 9000 incubator at 77°C for 30 minutes, immediately placed into xylene to remove any remaining wax and the following steps were followed prior to staining:

- Hydration – to remove xylene from the sections by briefly placing the slides in *decreasing* concentrations of alcohol (100%, 96%, 85% and 70%).
- The sections were then rinsed in tap water and placed in haematoxylin for five minutes to stain the nucleus; followed by rinsing in tap water and placing in Scotts tap water to intensify the colour of the haematoxylin.
- Tap water was again used to rinse the sections and the sections immediately placed in eosin to stain the cytoplasm, after which they were rinsed in tap water.

- Dehydration – water was then removed by placing the slide in *increasing* concentrations of alcohol (70%, 85%, 96% and 100%).
- Clearing – alcohol was then removed by placing the slide in pure xylene and a cover slip placed on top of the section and DPX used as a mounting medium. The slide was air dried before being assessed under a light microscope by forensic pathologists.

3.4.3 Routine microbiology samples

Swabs from the left and right lungs, as well as heart, were collected for microbiological tests. For the lung swabs, a clean pair of forceps was used to lift the lower lobe of either lung, after which the lateral surface of the lung was sterilised by scorching. A sterile blade was used to make an incision on the scorched surface of the lung. A swab was inserted into the incision and rotated a few times to ensure maximum sample collection.

The lateral surface of the left ventricle of the heart was then sterilised by scorching, after which a sterile blade was used to make an incision into the left ventricle without disturbing the anatomy too much. A swab was inserted into the incision and rotated a few times. Soon after sample collection, the swabs were placed back into the original tubes and transported to the Microbiology Division for processing.

Swab processing

The swab samples were processed at the Microbiology Division of the NHLS laboratory at Tygerberg Hospital. After confirmation at the pre-analytical desk that the information on request forms and samples matched, the three swab samples were taken to the analytical desk for processing as follows:

- Three different agar plates (Chocolate, Blood and MacConkey) were appropriately labelled with the case number and sample type as it appeared on the request forms (Figure 3.1).
- Each swab sample was inoculated into each of the three different agar plates and a three-way streak conducted.
- The nine plates were incubated in a jacketed NUAIRE CO_2 incubator at 36°C and 5.5% CO_2 for 18-24 hours to allow for microbial growth.

If there was no growth evident after 18-24 hours, the plates were incubated for another 18-24 hours under the same conditions. However, if there was general growth of organisms, biochemical confirmatory tests like the Gram stain, BBL Sensi-Disc susceptibility test and Analytical Profile Index (API) were conducted. If biochemical tests could not identify the organism growing on a plate, a Biomerieux VITEK 2 XL machine was used to identify the organism.

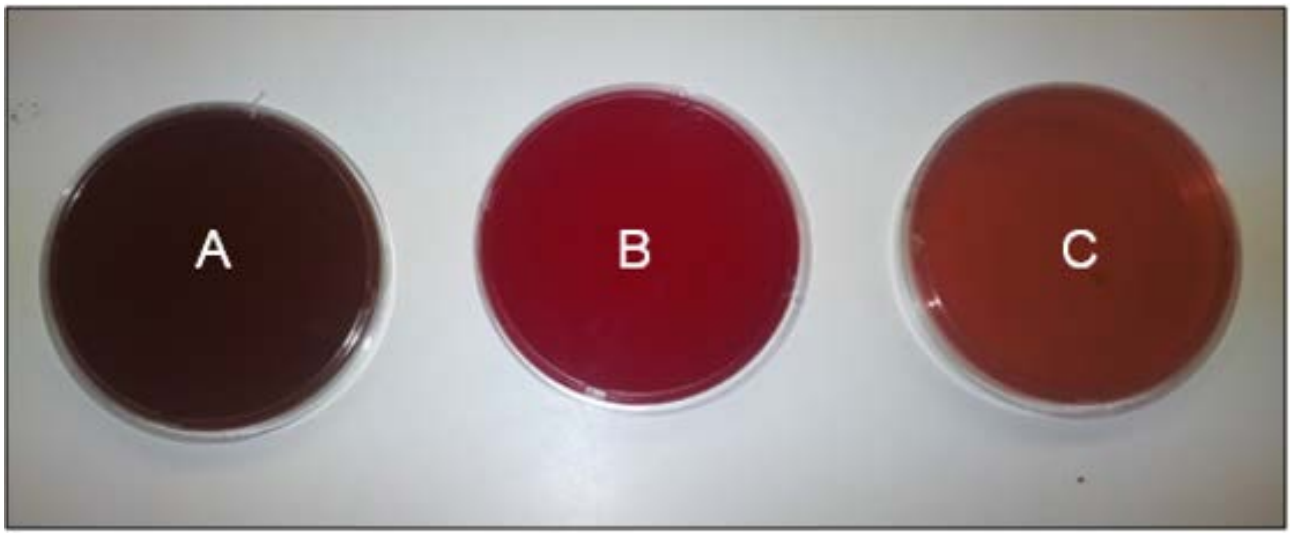

Figure 3.1 **The three agar plates, Chocolate (A), Blood (B) and MacConkey (C), used for the culture of microorganisms**

3.4.4 Routine virology samples

Three sample types were collected for routine testing at the NHLS Virology Division laboratory as part of SUDI case investigations at the Tygerberg Medico-Legal Mortuary. These include whole blood, as well as left lung and liver tissue.

Lung and liver tissue collection

Small pieces of the liver and left lung tissue were collected for routine centrifugation-enhanced SVC according to the institutional protocol. A sterile blade and scalpel handle was used to cut a 5 mm x 5 mm piece of left lung tissue, which was placed into red top glass tube. A new, sterile blade was also used to cut a 5 mm x 5 mm piece of liver tissue, which was also placed in an appropriately labelled red top glass tube. The samples were transported to the laboratories in 1.5 ml of Universal Transport Medium (UTM), aliquoted into each of the two glass tubes to preserve any viruses present.

Heart blood collection

The pericardial sac was cut open to expose the heart for macroscopic examination by a forensic pathologist prior to whole blood collection. The heart apex was then lifted using sterile forceps to allow blood to be drawn from the base of the heart using a 5 ml syringe and sterile 15-gauge needle. The collected blood was transferred to a red top glass tube. The tissue and blood specimens were transported to the Virology Division for processing.

Routine SVC

SVCs were routinely used to test for nine viruses[4] in all SUDI cases at the Tygerberg Medico-Legal Mortuary. Lung tissue was screened for all nine viruses, while the liver was only screened for HAdV and CMV. Three different cell lines stored at 37°C in an ESCO CelCulture CO_2 incubator were used for culture, depending on the target virus. Human Fibroblast (HF) cells were used to culture CMV, while a combination of Madin-Darby Canine Kidney (MDCK) and Hep 2 cells was used to culture HAdV, HMPV, Influenza A and B and HPIV 1-3.

Sample Processing for culture

After reception of samples at the Virology laboratory, lung and liver specimens were placed into separate mortars and ground with a pestle. Antibiotic cocktail (0.2 ml) was added to 6 ml viral transport medium (VTM) and the mixture was then added to the ground tissue and allowed to stand for five minutes. Thereafter, it was transferred to appropriately labelled 15 ml centrifuge tubes and centrifuged at 2 000 rpm for 10 minutes at 4°C. The specimens were then passed through a 0.2 µm disposable syringe filter attached to a 10 ml syringe (Figure 3.2) to trap any bacteria that might be present.

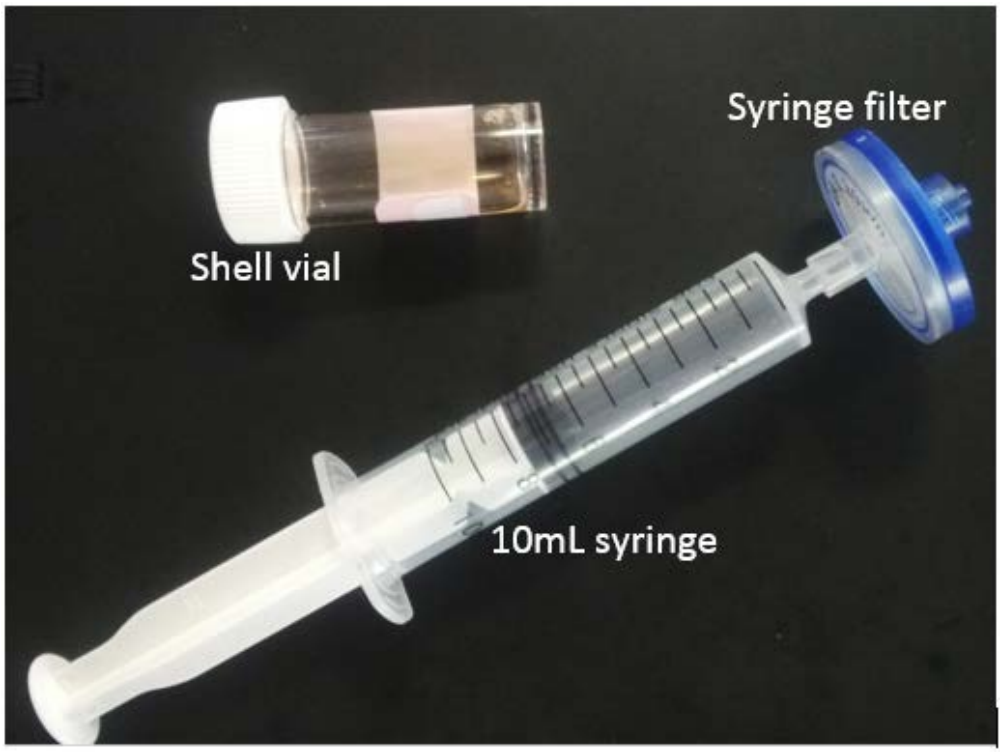

Figure 3.2 **Syringe and filter for filtering bacteria possibly present in the ground lung and liver tissue in preparation for culture in the shell vials**

[4] HAdV, CMV, Influenza A & B, HMPV, HPIV 1-3, RSV

Inoculation and incubation of prepared sample

Two vials with coverslips inside were labelled with the appropriate sample number. Each vial contained cell lines, media and antibiotics according to the target viruses as shown below:

- **HF cells:** Rosswell Park Memorial Institute (RPMI) 1640 medium, 10% foetal calf serum, penicillin and streptomycin.
- **MDCK and Hep 2 cell combination:** Eagle's Minimum Essential Medium (EMEM), 10% foetal calf serum, penicillin and streptomycin.

The prepared tissue samples (200 µl) were inoculated into the appropriate shell vials according to the target viruses. The shell vials were then centrifuged at 3 000 rpm for 45 minutes in a Heraeus Megafuge 40 centrifuge to enhance viral entry into the cells. After centrifugation, 1 ml of EMEM was added to the vial with MDCK and Hep 2 cell combination and incubated at 33°C for 48 hours. In the other vial with HF cells, 1 ml of RPMI 1640 medium was added and incubated at 37°C for 48 hours.

After incubation, the coverslips were taken out of the shell vials and fixed in 2 ml cold acetone for 10 minutes to prevent the cells from being washed off.

Determination of culture results

A Respiratory Panel Viral Screening and Identification Immunofluorescence Assay kit (Light Diagnostics, United States of America) was used to determine the culture results. Initially, the cover slips were mounted on a glass slide with Entellan. Afterwards, one drop of a cocktail of monoclonal antibodies against all target viruses was added to the appropriate coverslips and incubated at 37°C for 30 minutes. The coverslips were washed with PBS and anti-mouse IgG:Fluorescein isothiocyanate (FITC) antibodies added and the coverslips incubated at 37°C for 30 minutes. Afterwards, the coverslips were washed with phosphate-buffered saline (PBS), air dried, mounted and read under a fluorescence microscope in a dark room.

Confirmation of positive culture results

Positive screening results after culturing of tissue using a cocktail of monoclonal antibodies were confirmed and the virus identified as follows:

- The supernatant fluid from the SVC was transferred to labelled 2 ml tubes and the shell vials rinsed with PBS.

- 2 ml of PBS was poured into the shell vials and a plastic bulb used to scrape the cells off the coverslip.
- The solution was pipetted into the labelled 2 ml tubes and centrifuged briefly for 30 seconds.
- After centrifugation, supernatant fluid was discarded and 150 µl of PBS added to the cell pellet.
- The cell pellet and PBS were then thoroughly mixed, after which 15 µl was pipetted into each of the nine wells on a multi-well Teflon slide and allowed to air dry for 30 minutes.
- The slide was then fixed in cold acetone for 10 minutes.
- After fixation, an immunofluorescence test was conducted by adding antisera specific to each target viruses to the wells on the Teflon slide to determine which specific virus was responsible for the initial positive immunofluorescent assay (IFA) screening result.

IFA Interpretation

Positive result – apple green fluorescence in nucleus and/or cytoplasm (Figures 3.3 and 3.4).
Negative result – dull red staining of cells (Figure 3.5).

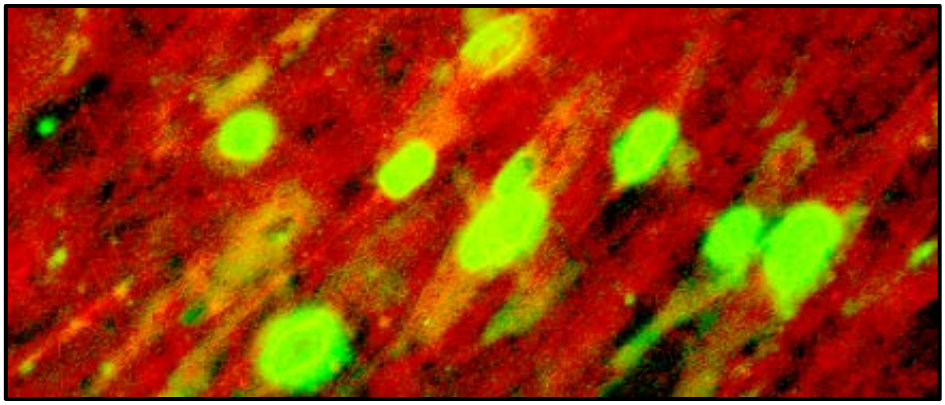

Figure 3.3 **CMV positive result on HF cells characterised by apple green fluorescence under a fluorescence microscope**

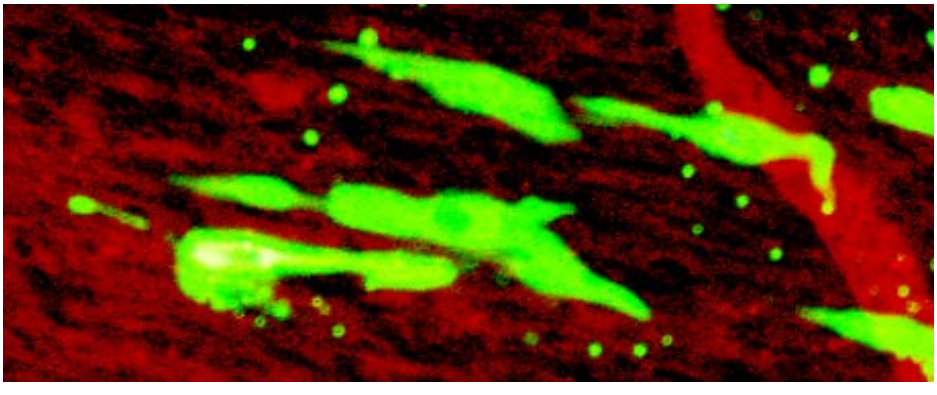

Figure 3.4 **HAdV positive result on HF cells characterised by apple green fluorescence under a fluorescence microscope**

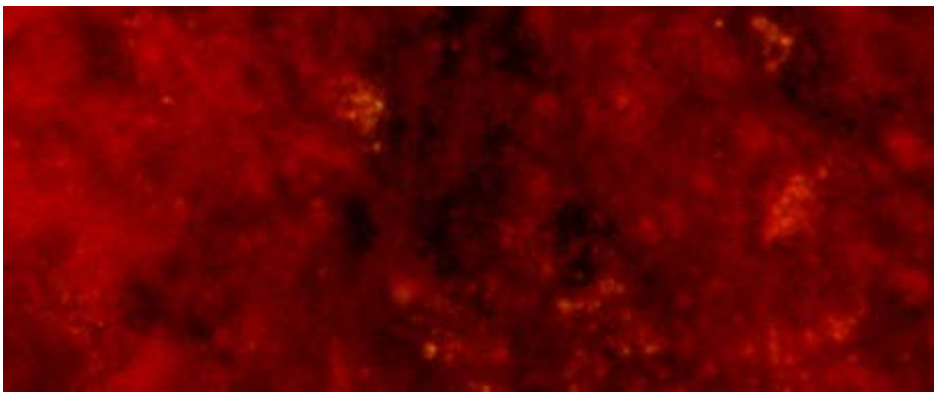

Figure 3.5 CMV negative result on HF cells. Note the absence of apple green fluorescence

3.4.5 Routine HIV 1/2 Antibody testing

Detection of antibodies against HIV 1/2 proteins, such as glycoprotein (gp)36, gp41 and p24, indicates either exposure to HIV or the possibility of maternally acquired antibody in infants under the age of 18 months. As part of the routine SUDI investigation protocol at Tygerberg Medico-Legal Mortuary, HIV 1/2 antibodies were tested for in serum from SUDI cases using a rapid VISITECT HIV 1/2 Antibody kit (Omega Diagnostics, Randburg). The kit is a point-of-care, qualitative immunoassay for the detection of antibodies to HIV 1/2 by utilising immunochromatography. The test device comprises of a membrane containing recombinant HIV 1/2 antigens conjugated to colloidal gold on the test line R. Also on the membrane is goat anti-rabbit IgG conjugated to colloidal gold on the control line C. If the sample contains HIV 1/2 antibodies, the antibodies form a complex with the colloidal gold-recombinant HIV 1/2 antigens conjugate which moves further along the membrane until it is immobilised by specific recombinant HIV 1/2 antigens at the test region. A pink line indicates a positive HIV 1/2 antibody result in the sample. Any unreacted or unbound complexes and colloidal gold conjugated rabbit IgG move along the membrane where they are immobilised by goat anti-rabbit IgG on the control region C. This serves as a performance validation of the entire test procedure (Figure 3.6).

A test result was only valid if there was a visible line in the control region marked C in Figure 3.6. In the absence of the line, the test was deemed invalid and repeated.

Test procedure

- Whole blood was centrifuged at 3 000 rpm for 10 minutes prior to testing.
- The pouch containing the test device was taken out of the refrigerator where it was stored at 4°C.
- The test device was labelled with the last four digits of a unique identifying number allocated to the sample at the Virology laboratory pre-analytical desk.

- Four drops of serum were added to the rectangular window marked S using a bulb pipette; followed by addition of five drops of diluent buffer to the circular window marked B (Figure 3.6).
- The test device was allowed to stand for 15 minutes after which the results of the test were read.

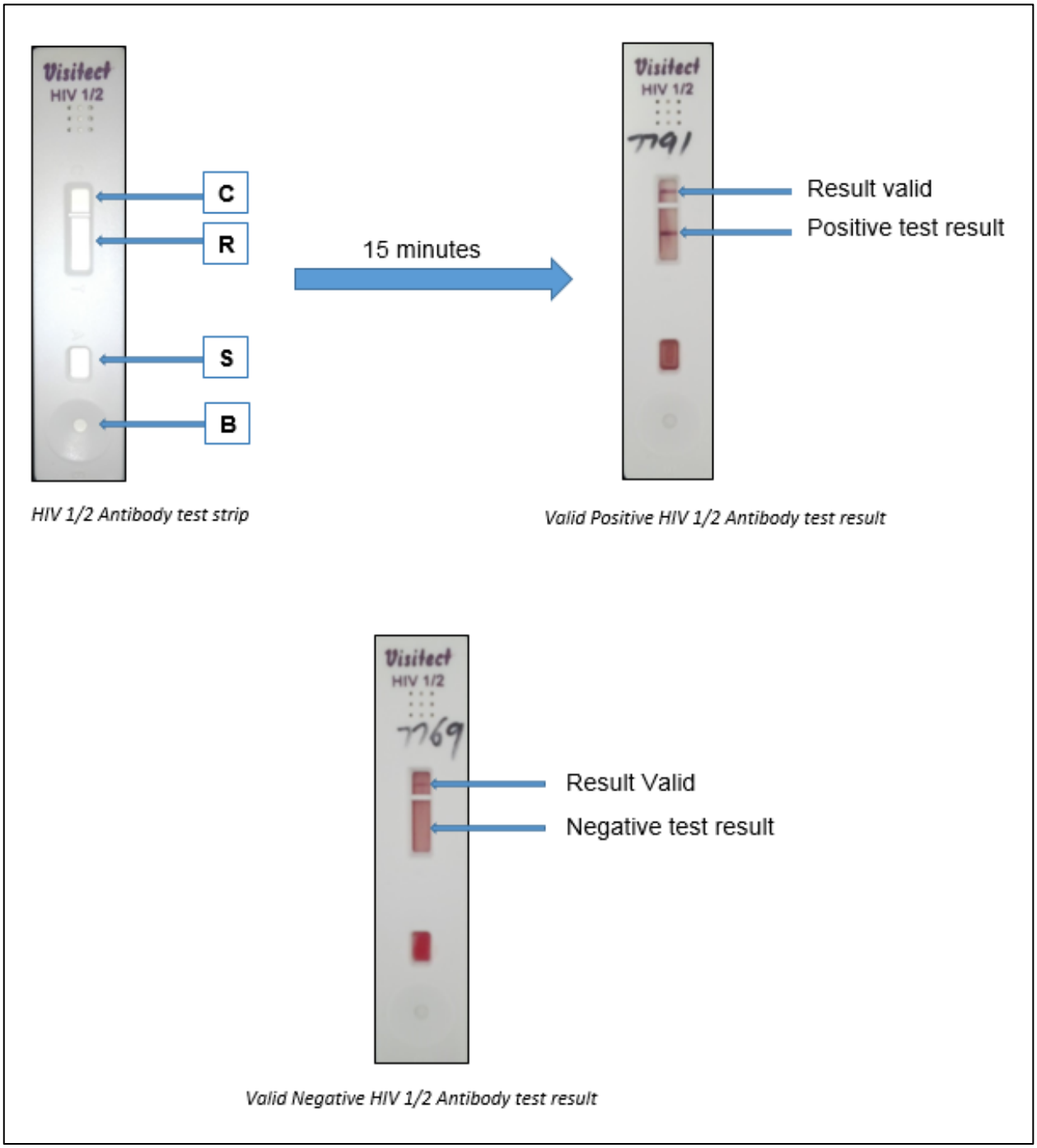

Figure 3.6 Validation and interpretation of HIV 1/2 Antibody test results on the Visitect test strip.

Result Interpretation

A positive HIV 1/2 Antibody test result was characterised by a pink line in both the control (C) and test (R) regions of the test device. If there was no pink line in the control region after 15 minutes, an

extra 15 minutes was allowed until the result was read again. If there was still no pink line in the control region after the additional 15 minutes, the test result was deemed as void and the sample was re-tested.

A negative HIV 1/2 Antibody test result was characterised by a pink line in the control region and no line in the test region. An additional 15 minutes was allowed if the pink line was not visible in the control region after the initial 15 minutes. If there was no pink line in the control region after the additional 15 minutes, the test result was deemed as void and the sample was re-tested.

3.5 OBJECTIVE 2

a) To prospectively collect swab samples from both lungs and trachea from SUDI cases through the Forensic Pathology Division at Tygerberg Hospital for virological research purposes.

b) To prospectively collect whole blood from SUDI cases for immunological research testing.

In addition to the routine samples, swabs from the lungs and trachea were collected for virological analysis. The serum that was left over after routine HIV 1/2 antibody testing was used for research immunological analysis.

3.5.1 Research virology samples

Swabs from the trachea and both lungs were collected for virological investigations. The research lung swab samples were collected from the same incision made when collecting the routine lung swab samples. A flocculated swab (Copan Diagnostics, Italy; Lasec, Cape Town) was used to collect the research lung and tracheal swab samples. The infant's neck tissue was exposed and dissected to expose the trachea. Using a sterile blade, the trachea was anteriorly excised between the sternal notch and thyroid gland. The distal tracheal end was then pulled upwards slightly to expose the lumen. The flocculated swab was then inserted into the open end and pushed downwards as far as possible. The swab was also rotated inside the trachea several times before being removed. Afterwards, these swabs were placed into a container with 3 ml UTM (Copan Diagnostics, Italy; Lasec, Cape Town) and transported to the Virology laboratory where these containers were pulse vortexed thirty times on a Heidolph vortex. The UTM containers had three glass beads at the bottom and these facilitated maximum sample recovery by constantly hitting the flocculated swabs during pulse vortexing. The recovered sample was aliquoted into two 1.5 ml microcentrifuge tubes (Whitehead Scientific, Cape Town) and stored at -80°C.

3.5.2 Research immunology samples

Whole blood was collected as described in Section 3.4.4. After centrifugation, the serum was collected and stored at -80°C for later analysis.

3.6 OBJECTIVE 3

To extract viral nucleic acid (DNA and RNA) from lung tissue and lung and tracheal swabs.

3.6.1 Sample Processing

After collection and proper storage of samples, molecular and immunology assays were used to process the samples. Firstly, viral nucleic acid was extracted from lung and tracheal swabs. After the extraction, complementary DNA (cDNA) was synthesised from the nucleic acid extracts to stabilise the extracted RNA for long time storage. A multiplex PCR was conducted on the cDNA; followed by detection of amplified nucleic acid from target respiratory viruses by agarose gel electrophoresis.

3.6.2 Viral nucleic acid extraction

Lung and tracheal swab samples previously stored at -80°C were completely thawed at room temperature (20-25°C). After thawing, the samples were pulse vortexed 10 times on a Heidolph vortex. The extraction of viral nucleic acid (DNA and RNA) was conducted using the Qiagen Cador Pathogen Mini Kit (Whitehead Scientific, Cape Town) in accordance with the manufacturer's instructions. In summary, 1.5 ml microcentrifuge tubes were labelled appropriately and 20 µl of proteinase K added. Afterwards, 200 µl of sample and 100 µl of lysis buffer VXL were added and the contents mixed thoroughly by pulse vortexing twenty times on a Heidolph vortex. After 15 minute incubation at room temperature, the tubes were centrifuged briefly and 350 µl of binding buffer ACB was added to the sample. The tubes were again pulse vortexed twenty times and then centrifuged briefly. The lysate was transferred to a QIAamp mini column placed in a 2 ml collection tube and centrifuged at 8 000 rpm for 1 minute. Thereafter, 600 µl of wash buffer AW1 was added to the mini column that had been placed in a new 2 ml collection tube. The mini column was centrifuged at 8 000 rpm for 1 minute and the collection tube with flowthrough discarded. Afterwards, the mini column was placed in a new 2 ml collection tube and 600 µl of wash buffer AW2 added into the mini column, which was then centrifuged at 8 000 rpm for 1 minute. The column was then placed in a new 2 ml collection tube and centrifuged again at 13 200 rpm for two and a half minutes to completely rid the column of residual ethanol. The mini column was then placed in a clean 1.5 ml microcentrifuge tube; 40 µl of elution buffer AVE added to the column and incubated at room temperature for one minute. The column was then centrifuged at 13 200 rpm for 90 seconds.

After the elution step, the yield and purity of the extracted nucleic acid were determined using the Nanodrop ND1000 instrument. The 260/280 values were used as a measure of purity of the extracts. For DNA, a 260/280 ratio close to 1.8 indicates high purity, while for RNA, a 260/280 ratio close to 2.0 showed a highly pure extract. As such, extracts with 260/280 ratios within a range of 1.8 to 2.2 were deemed suitably pure for downstream applications, while those out of range were re-extracted.

After optimisation of the manufacturers' extraction protocol, the following modifications were made:

- Centrifugation to completely dry the mini column was conducted at 13 200 rpm for two and a half minutes as opposed to 14 000 rpm for two minutes.
- The viral nucleic acid on the mini column was eluted in 40 µl of elution buffer as opposed to 50-150 µl and centrifugation was conducted at 13 200 rpm for one and a half minutes as opposed to 14 000 rpm for one minute.

Optimisation

In order to evaluate the success of the extraction method, a positive control of known identity was obtained from the NHLS Virology laboratory and extracted alongside the study samples. Multiplex PCR was then used to determine if it had been successfully extracted as shown in Figure 3.7. PBS was used as a negative control during extractions to identify possible cross contaminations.

Result Validation

A successful viral nucleic acid extraction procedure was characterised by the following criteria:

- Positive control with a nucleic acid yield of ≥ 50 ng/µl and a 260/280 ratio between 1.8 and 2.2.
- Correct identification of the known positive control by multiplex PCR as observed in the band circled in yellow (Figure 3.7, Panel MB, Lane 7).
- The absence of a band in the negative control lane after multiplex PCR as shown in the band circled in red (Figure 3.7, Panel MB, Lane 10).

In the event that any of the criteria above was not met, the results of the entire nucleic acid extraction procedure were disregarded and viral nucleic acid re-extracted from the same samples.

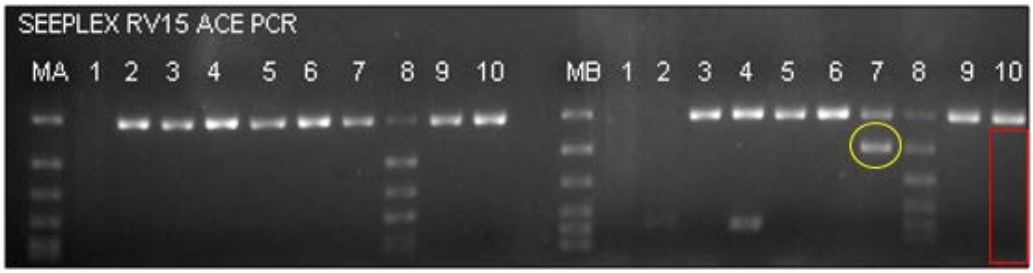

Figure 3.7 **Validation of extraction and visualisation of multiplex PCR results using agarose gel electrophoresis. The yellow circle indicates a positive PCR internal control result and the red rectangle indicates a negative result in the negative control lane.**

3.7 OBJECTIVE 4

To use the RevertAid first strand cDNA synthesis and Seeplex RV15 ACE detection kits to detect the presence of 15 respiratory viral targets in lung and tracheal swabs collected from SUDI cases.

3.7.1 cDNA synthesis

Nucleic acid, RNA in particular, degrades readily when stored for lengthy periods. Of the 15 respiratory viruses targeted in this study, 13 were RNA viruses[5]. It was therefore necessary to convert extracted viral RNA to cDNA that is more stable when stored for extended periods. Prior to the cDNA synthesis, the extracted viral nucleic acid stored at -80°C was thawed at room temperature. After the nucleic acid had completely thawed, the RevertAid First Strand cDNA Synthesis Kit (Inqaba Biotec, Pretoria) was used to make the cDNA as per manufacturer's instructions below. A cDNA synthesis mastermix was first prepared as indicated in Table 4.1 and stored at 4°C for later use.

Table 3.1 **cDNA synthesis mastermix**

REAGENT	VOLUME
5X RT buffer	4 µl
10mM dNTP	2 µl
RNase inhibitor	1 µl
Reverse transcriptase	1 µl
TOTAL VOLUME	**8 µl**

[5] Enterovirus, HCoV OC43, 229E/NL63, Influenza A and B, MPV, PIV 1 - 4, Rhinovirus, RSV A and B

- 1 µl of random hexamer and 3 µl of DEPC-treated water was added to a 0.2 ml thin walled PCR tube (Whitehead Scientific, Cape Town) on ice.
- 8 µl of extracted viral RNA was added last to the PCR tube, after which it was incubated for three minutes at 80°C on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA).
- The PCR tube was then immediately chilled on ice for 2 minutes, after which the contents of the tube were briefly centrifuged on a Labnet minifuge (Whitehead Scientific, Cape Town).
- The stored cDNA synthesis mastermix was taken out of temporary storage and 8 µl of it added to the PCR tube that had been placed on ice.
- The contents of the tube were mixed by inverting the tube five times and then briefly centrifuged.
- The tube was then incubated under the conditions shown in Figure 3.8.

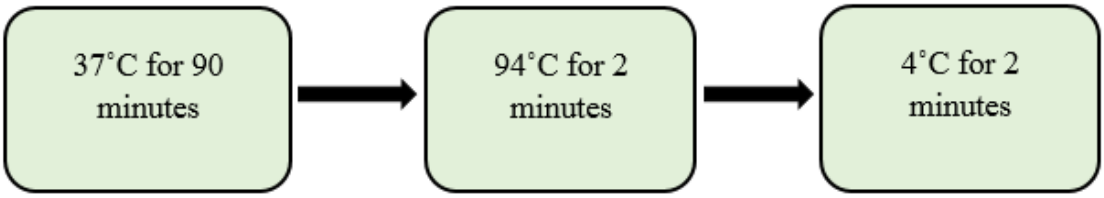

Figure 3.8 cDNA synthesis conditions

The synthesised cDNA was stored at -20°C for later analysis. To ensure that no variability in results was introduced due to the usage of different thermocyclers, the same thermocycler was used for all cDNA synthesis steps.

3.7.2 Seeplex RV15 ACE Multiplex detection PCR

The detection of 15 target viruses[6] in the extracted samples was done on cDNA using the Seeplex RV15 ACE detection kit (Seegene, Germany; Inqaba Biotec, Pretoria). The kit has three different panels; each panel composed of different viruses as shown in Table 3.2.

Viruses can be detected in respiratory samples using various techniques that target different aspects of the virus. Some immunology-based assays are directed against viral particles or antibodies to that virus, while cell culture assays utilise the ability of viruses to grow in certain cell lines to produce characteristic morphology or CPE in the cells. The Seeplex RV15 ACE detection PCR kit enables the simultaneous amplification and detection of 15 viral targets in one sample. In order to conduct the multiplex detection PCR, the reagents were removed from -20°C storage and thawed on ice. The cDNA was also removed from -20°C storage and thawed at 4°C. Each sample was amplified in three

[6] Adenovirus, Bocavirus, Enterovirus, HCoV OC43, 229E/NL63, Influenza A and B, MPV, PIV 1 - 4, Rhinovirus, RSV A and B

separate reactions simultaneously and as such, three 0.2 ml PCR tubes (Whitehead Scientific, Cape Town) were labelled for each panel and placed on a cold block. The thawed reagents were pulse vortexed and briefly centrifuged and the PCR mastermix prepared as indicated in Table 3.3.

Table 3.2 Seeplex RV15 ACE viral target panels

PANEL A	PANEL B	PANEL C
HAdV	HCoV OC43	HBoV 1/2/3/4
HCoV 229E/NL63	HRV A/B/C	Influenza B virus
HPIV 2	RSV A	HMPV
HPIV 3	Influenza A virus	HPIV 4
HPIV 1	RSV B	HEV

Table 3.3 Seeplex RV15 ACE multiplex detection PCR

REAGENT	VOLUME
5X RV15 ACE Primer Mix A/B/C	4 µl
2X Multiplex Master Mix	10 µl
8-MOP Solution	3 µl
TOTAL VOLUME	**17 µl**

The prepared mastermix was briefly vortexed and centrifuged and 17 µl pipetted into each of the 0.2 ml PCR tubes. Afterwards, 3 µl of the thawed cDNA was added and pipetted up and down five times to ensure that the contents of the tubes were completely mixed. The 0.2 ml PCR tubes were then briefly centrifuged on a minifuge and cDNA amplified by placing the 0.2 ml PCR tubes in a GeneAmp PCR System 9700 thermocycler under the cycling conditions in Table 3.4:

A positive control of known identity was supplied in the multiplex PCR kit and was included in every multiplex PCR run to serve as a validation of the amplification process. Nuclease free water was used as the template in the negative control tube, which was also included in every run to serve as an indicator of any possible contaminations in a particular multiplex PCR run. After completion of the Seegene Seeplex Multiplex Detection PCR, the PCR products were temporarily stored at 4°C.

Table 3.4 Seeplex RV15 ACE Multiplex Detection PCR cycling conditions

SEGMENT	NUMBER OF CYCLES	TEMPERATURE	DURATION
1	1	94°C	15 min
	40	94°C	0.5 min
2		60°C	1.5 min
		72°C	1.5 min
3	1	72°C	10 min

Detection of amplified viral targets

The amplified viruses in the PCR product were identified using agarose gel electrophoresis. The method utilises an electrical current to migrate negatively charged nucleic acid fragments from the negative electrode to the positive electrode using a buffer solution as a conductor of electricity. Nucleic acid fragments from different viruses are of a different base pair (bp) size and hence migrate at different rates on the agarose gel. After a while, these different sized fragments separate and are identified by comparison to molecular weight markers which are reference fragments of known size and identity. Agarose gel electrophoresis was conducted on a 2% agarose gel as follows:

- 2 g of agarose powder (Whitehead Scientific, Cape Town) were weighed and placed into a 500 ml glass container.
- The agarose powder was then dissolved in 100 ml of 1X Tris acetate EDTA (TAE) buffer by heating the mixture in a microwave for two minutes.
- The agarose was sufficiently cooled down under running tap water and poured into a moulding tray where it solidified over 25 minutes.
- 1X TAE buffer was then poured onto the solidified 2% agarose gel.
- 5 µl of the three different molecular weight markers (one for each panel of viruses) from the Seeplex RV15 ACE detection kit were mixed with a drop of novel juice (Whitehead Scientific, Cape Town) by pipetting up and down five times before being loaded onto the agarose gel.
- 5 µl of the PCR products were also loaded with a drop of novel juice.
- The agarose gel was covered with a lid and connected to a power supply where the gel was run at 65V for 56 minutes.
- Afterwards, the fluorescence on the agarose gel produced by PCR products bound to novel juice was visualised using ultraviolet light on a UVITEC Cambridge gel dock system.

Validity of results

Seeplex RV15 ACE multiplex detection PCR results were only deemed valid if:

- The internal control band was visualised in the positive and negative control lanes (18 and 19 respectively on Figure 3.9).
- The positive control lane had five other fluorescing bands besides the internal control.
- The negative control lane had no other fluorescing band except the internal control.

Figure 3.9 Internal control band for PCR validation

In the event that any of the criteria above was not met, the PCR results were deemed as void and the multiplex PCR was repeated from the start.

Result interpretation

The gel image obtained from the UVITEC Cambridge gel dock system is analysed by comparing the amplicon sizes seen on the gel image (Figure 3.10) with target virus amplicon sizes in Figure 3.11.

Figure 3.10 Interpretation of gel image results

The viral screening results as seen on Figure 3.10 would be as follows:

- Sample 1 – Negative for all viruses in Panel A and B.
- Sample 2 – Negative for all viruses in Panel A and positive for Human Rhinovirus A/B/C.
- Sample 7 – Negative for all viruses in Panel A and positive for Human Rhinovirus A/B/C.

- Sample 9 – Positive for HAdV and Human Rhinovirus A/B/C.
- Sample 13 – It is the positive control and bands amplified, hence PCR reaction worked.
- Sample 14 – It is the negative control and only the internal control band was seen as expected.

PANEL A	Size on agarose gel (bp)
Internal Control	850
Human Adenovirus	534
Human CoV 229E/NL63	375
Human Parainfluenza virus 2	264
Human Parainfluenza virus 3	189
Human Parainfluenza virus 1	153

PANEL B	Size on agarose gel (bp)
Internal Control	850
Human CoV OC43	578
Human Rhinovirus A/B/C	394
Human RSV A	269
Influenza A virus	206
Human RSV B	155

PANEL C	Size on agarose gel (bp)
Internal Control	850
Human Bocavirus 1/2/3/4	579
Influenza B virus	455
Human Metapneumovirus	351
Human Parainfluenza virus 4	249
Human Enterovirus	194

Figure 3.11 Target virus amplicon sizes

3.8 OBJECTIVE 5

To measure CRP levels in serum from all SUDI cases prospectively collected between July 2015 and June 2016 as an indicator of infection and inflammation prior to or at the time of death of the infant.

3.8.1 Serum CRP quantification

Serum CRP was quantified using a DRG high sensitivity CRP ELISA (Biocom Biotech, Cape Town). Prior to use, the kit was removed from 4°C storage and allowed to reach room temperature. Serum samples previously stored in 1.5 ml microcentrifuge tubes were also removed from -80°C storage and allowed to completely thaw at room temperature. Afterwards, the serum was pulse vortexed five times on a Heidolph vortex and then centrifuged in a 5415D Eppendorf centrifuge at 3 000 rpm for 1 minute to move any serum on the lid to the bottom of the tube.

The quantification of CRP was then conducted according to the manufacturer's instructions as follows:

- Serum was diluted 100 fold in a 1.5 ml microcentrifuge tube by mixing 5 µl of serum with 495 µl sample diluent provided in the kit, pulse vortexed five times and then briefly centrifuged.
- The desired number of mouse monoclonal anti-CRP antibody coated wells were secured in the holder.
- 10 µl of the each of the six CRP standards provided in the kit was dispensed in duplicate into the appropriate wells.
- 10 µl of diluted specimens and controls were then dispensed into the appropriate wells.
- 100 µl of CRP Enzyme Conjugate Reagent were then dispensed into each of the wells, after which the contents of the wells were thoroughly mixed by gentle shaking for 30 seconds.
- The reaction mixture was then incubated at 20°C for 45 minutes.
- Afterwards, the incubation mixture was removed by flicking the plate contents into a waste container. The plate was then washed five times with distilled water on an automated Tricontinent Multiwash II plate washer.
- The wells were also struck sharply onto absorbent material to ensure complete removal of residual water droplets.
- 100 µl of TMB solution (a chromogenic visualising agent) were dispensed into all the wells and the well contents mixed for five seconds by gently shaking the plate.
- The reaction was then incubated at 20°C for 20 minutes.
- After incubation, the reaction was stopped by adding 100 µl of Stop Solution to each well and the plate gently shaken for 30 seconds until all blue colour turned completely yellow.
- The absorbance of the samples was read on a Worldwide Diagnostics microplate reader at 450nm within 15 minutes and the concentration of CRP in the samples automatically determined from the standard curve.

3.9 OBJECTIVE 6

To measure levels of 15 cytokines and chemokines in serum samples collected from all SUDI cases admitted to the Tygerberg Medico-Legal Mortuary between July 2015 and June 2016 as an indicator of infection and inflammation prior to or at the time of death of the infant.

3.9.1 Serum immune biomarkers screening

A customised, magnetic Luminex® screening assay (R&D Biosystems, USA; Whitehead Scientific, Cape Town) was used to screen for the 15 cytokines and chemokines in Table 3.5.

Table 3.5 Analytes included in a customised, magnetic Luminex screening assay

TARGET ANALYTES		
IL-1 alpha	IL-6	IL-23
IL-1 beta	IL-8	IFN-gamma
IL-2	IL-10	IL-1RA
IL-4	IL-12	IP-10
IL-5	IL-18	TNF-alpha

The serum was taken from storage at -80°C and allowed to thaw completely at room temperature. The kit was also taken from storage at 4°C and brought to room temperature prior to use. Once the kit components had reached room temperature, reagents were prepared as follows:

Assay Standards

- Each of the three standard cocktails provided in the kit was reconstituted by adding 100 ml of Calibrator Diluent RD6-52 and allowed to stand for 15 minutes at room temperature with gentle agitation by finger tapping of the bottom of the container.
- 100 µl from each of the three reconstituted standard cocktails were pipetted into a single 1.5 ml microcentrifuge tube (Whitehead Scientific, Cape Town). 700 µl of Calibrator Diluent RD6-52 was added to make a final volume of 1 ml for Standard 1.
- 200 µl of Calibrator Diluent was added to each of the 1.5 ml microcentrifuge tubes labelled Standard 2-6.
- Standard 1 was then used to make a 3-fold dilution series by pipetting 100 µl of Standard 1 into the tube labelled Standard 2. The solution was mixed by pipetting up and down five times and 100 µl pipetted into the tube labelled Standard 3 from the Standard 2 tube. This process was repeated up to the tube labelled Standard 6 as shown in Figure 3.12[7].

[7] https://resources.rndsystems.com/pdfs/datasheets/lxsahm.pdf

Diluted Microparticle Cocktail

- The microparticle cocktail vial was centrifuged in a microcentrifuge for 30 seconds at 1 000 g and then gently pulse vortexed to resuspend the microparticles.
- The microparticle cocktail was then diluted in a mixing bottle provided by adding 2.5 ml of Diluent RD2-1 to 250 µl of the microparticle cocktail. This was prepared 30 minutes before use.

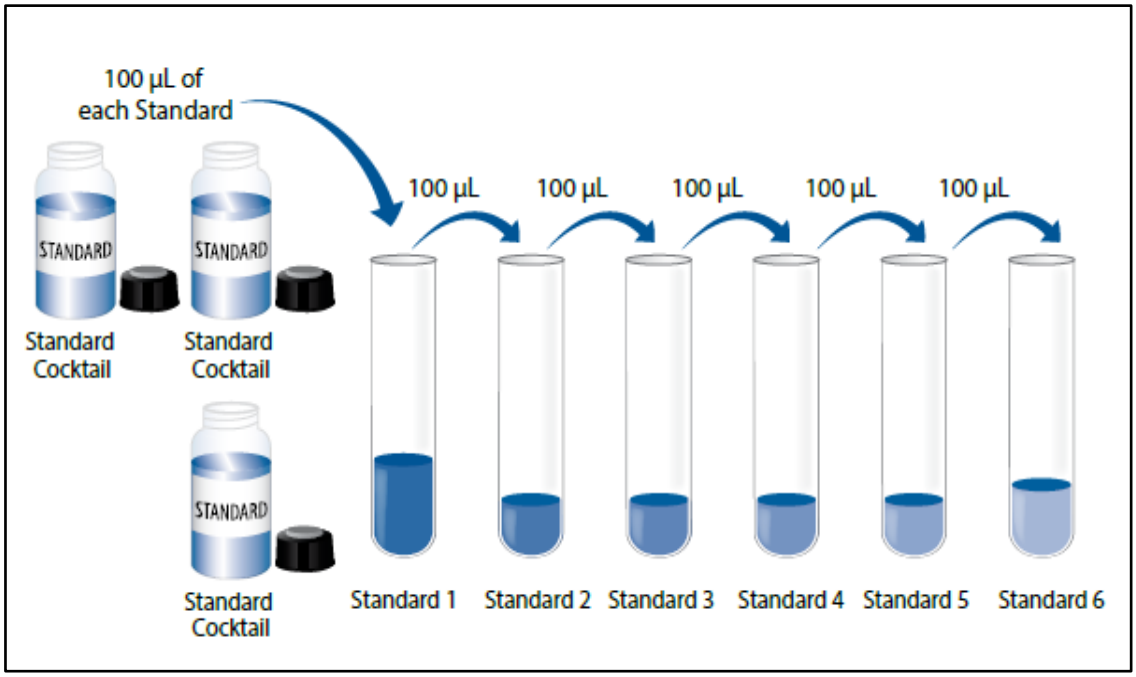

Figure 3.12 Dilution series for standard preparation

Biotin Antibody Cocktail

- The biotin antibody cocktail vial was centrifuged in a microcentrifuge for 30 seconds at 1 000 g and gently vortexed.
- The cocktail was then diluted by adding 2.5 ml of Diluent RD2-1 to 250 µl of the biotin antibody cocktail and gently tapping the bottom of the tube to mix.

Streptavidin-PE

- The Streptavidin-PE vial was centrifuged for 30 seconds at 1 000 g and gently vortexed, followed by dilution to a 1X concentrate by adding 2.68 ml of Wash Buffer to 110 µl of Streptavidin-PE concentrate.
- The dilution was conducted in a polypropylene test tube wrapped with aluminium foil.

Wash buffer

- 20 ml of Wash Buffer concentrate were added to 480 ml of distilled water in a sterile glass container and thoroughly mixed by shaking.

Assay Procedure

- The diluted microparticle particles were resuspended by pulse vortexing three times and then 50 µl pipetted into each of the wells on the flat bottom, 96 well plate (Bio-Rad, Johannesburg).
- 50 µl of the Standards were added to the appropriate wells in duplicate, starting from Standard 1 up to Standard 6 with Calibrator Diluent RD6-52 acting as the blank.
- Completely thawed, undiluted serum samples were pulse vortexed, microcentrifuged for 1 minute at 2 000 rpm and 50 µl of the serum pipetted into the appropriate wells.
- The plate was then securely covered with foil plate sealer provided and incubated for two hours at room temperature on a horizontal orbital microplate shaker set at 800 rpm.
- After incubation, the plate was washed on a magnetic plate washer by filling each well with 100 µl wash buffer and completely removing the liquid from the well. This was repeated three times.
- 50 µl of the diluted biotin antibody cocktail was added to each well and the plate securely covered with a foil plate sealer. The plate was incubated for one hour at room temperature on a shaker set at 800 rpm.
- After incubation, the plate was washed as before and 50 µl of the diluted Streptavidin-PE added to each well. The plate was again securely covered with a foil plate sealer and incubated for 30 minutes at room temperature while shaking at 800 rpm on a plate shaker.
- Washing was then conducted as before and the microparticles resuspended by adding 100 µl of wash buffer to each well. The plate was incubated again for 2 minutes at room temperature while shaking at 800 rpm.
- Finally, the plate was read on a Bio-Rad Bio-Plex analyser and the concentrations of each of the 15 analytes automatically determined by the instrument.

3.10 Data Stratification

All data was captured in Microsoft Excel®. The entire dataset was statistically analysed and measures of occurrence are reported as frequencies, proportions and ratios. Numerical data is reported as Mean ± SD, range and Median where applicable.

The dataset was stratified according to known risk factors and compared according to gender, age (< 8 weeks, 9-16 weeks, > 16 weeks), season, housing, sleeping position, as well as COD classification.

3.11 Statistical Analyses

The chi-square test was used to compare observations between the two COD classifications (SIDS and infection). When the criteria of this test were not met (observations less than 5 in one or more groups), the Fischer test was used.

To compare medians between the two cause of death groups, the non-parametric test, Mann Whitney was used. A simple logistic regression was used to measure the strength of the association between independent and dependent variables.

All variables displaying a p-value less than 0.1 in bivariate analyses were used to build a multivariate logistic model predicting the likelihood of SUDI being diagnosed as infection. Results of this model were reported as adjusted odds ratio (aOR) and corresponding 95% CI. The p-value cut-off point was 0.05.

All statistical analyses were performed with IBM SPSS statistical software with the assistance of the Centre for Statistical Consultation at the Tygerberg Campus.

The raw data is attached as Addendum A2. All non-numeric fields were coded to facilitate comparison between pre-defined groups.

CHAPTER 4: RESULTS

Different types of samples were collected from 183 SUDI cases admitted to the Tygerberg Medico-Legal Mortuary between July 2015 and June 2016 for the purposes of this study. This included the left and right lung and trachea swabs for virological analysis by targeting 15 respiratory viruses with the Seeplex RV15 ACE multiplex PCR detection kit. Additionally, serum was collected to determine the PM levels of 16 pro- and anti-inflammatory immune biomarkers of infection. The sample collection was done over a 12 month period to include all seasons, due to the seasonal variations observed in SUDI cases. For routine laboratory analysis of specimens by the NHLS diagnostic laboratory, lung and liver tissue were collected for centrifugation-enhanced SVC, while left and right lung and heart swabs were collected for microbiological culture and detection of microorganisms. Two more small pieces of the right and left lungs were used for histological examination of tissue for signs of infection. Serum was used for the detection of HIV 1/2 antibodies.

4.1 Study population characteristics

A total of 183 SUDI cases admitted to the Tygerberg Medico-Legal Mortuary were included in the study. The study group comprised 90 (49.1%) males and 93 (50.9%) females with a male : female ratio of 1:1.03. Not all the information was always captured on the questionnaires, but we will report on the number of cases that were available. There were several reasons for incomplete capture of information. Some parents or caregivers were too traumatised to answer questions, while others were not available (for reasons unknown to the research team). Furthermore, different forensic pathology officers were conducting the interviews according to their shifts, resulting in non-standardised replies.

The age was available for 170 of the cases and the average age was 13.5 ± of 12.8 weeks. The median age was 9 weeks with a range between 0.3 and 52 weeks. Quite a high percentage of the infants (39%) weighed less than 2 500 g at birth, which was regarded as low birthweight, with 22% having extreme low birthweight (< 2 000 g at birth). We did not collect any information about the gestational age of the infants, and can therefore not conclude if any of these low birthweight infants were premature as well.

The period between June and November was regarded as the cold season, while December to May was regarded as the warm season. A total of 123 cases occurred in the cold season, compared to 60 in the warm season. Interestingly, more female infants than males died in the colder months, while more male infants than females died in the warmer months as shown in Figure 4.1.

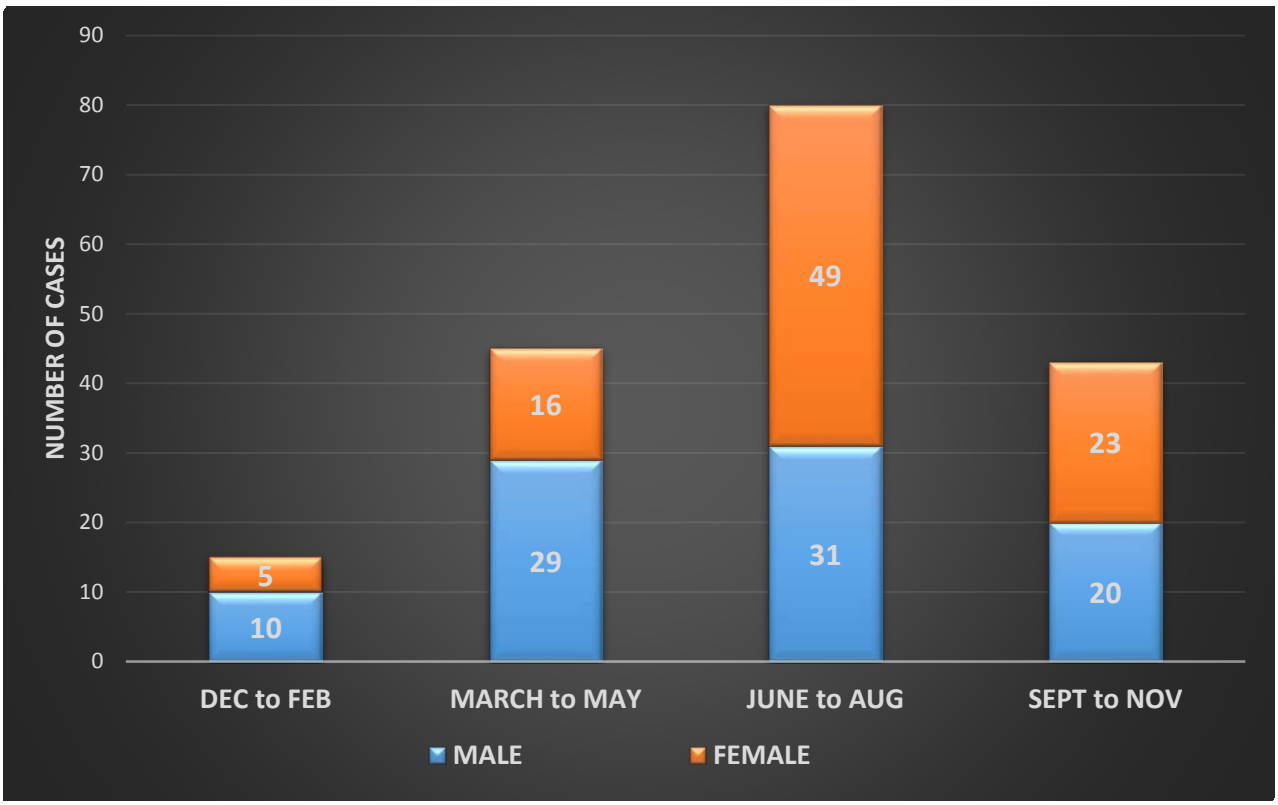

Figure 4.1 Distribution of SUDI cases by gender and season

The racial distribution (Figure 4.2) showed a majority of black infants (109, 60%), compared to 61 (33%) coloured infants. In the remaining 13 (7%) cases, no race was specified in the case files. No infants from other racial groups were admitted to the Tygerberg Medio-Legal Mortuary during the study.

The average PMI in this study was 4.6 ± 2.6 days (range 0 – 19 days). There were only a few instances where the PM was conducted after 14, 16, 17 and 19 days, which could have been as a result of bodies not being promptly identified and claimed by the infants' relatives. However, these outliers were the exception, because a Median of 4 days was reported.

Other sociodemographic information of note was bed-sharing which was reported in 96/101 cases (95%), while 64/114 (56%) reportedly lived in informal housing. Exposure to cigarette smoke from parents was less common than expected in only 35/113 (31%) and the use of alcohol was only reported in 22/113 (20%). In 55/183 (30%) of the cases, clinical symptoms were reported to be present immediately prior to their demise. The majority of these reported symptoms included flu-like or respiratory symptoms. However, these were not confirmed by a medical professional and were not included in the statistical analyses of the data.

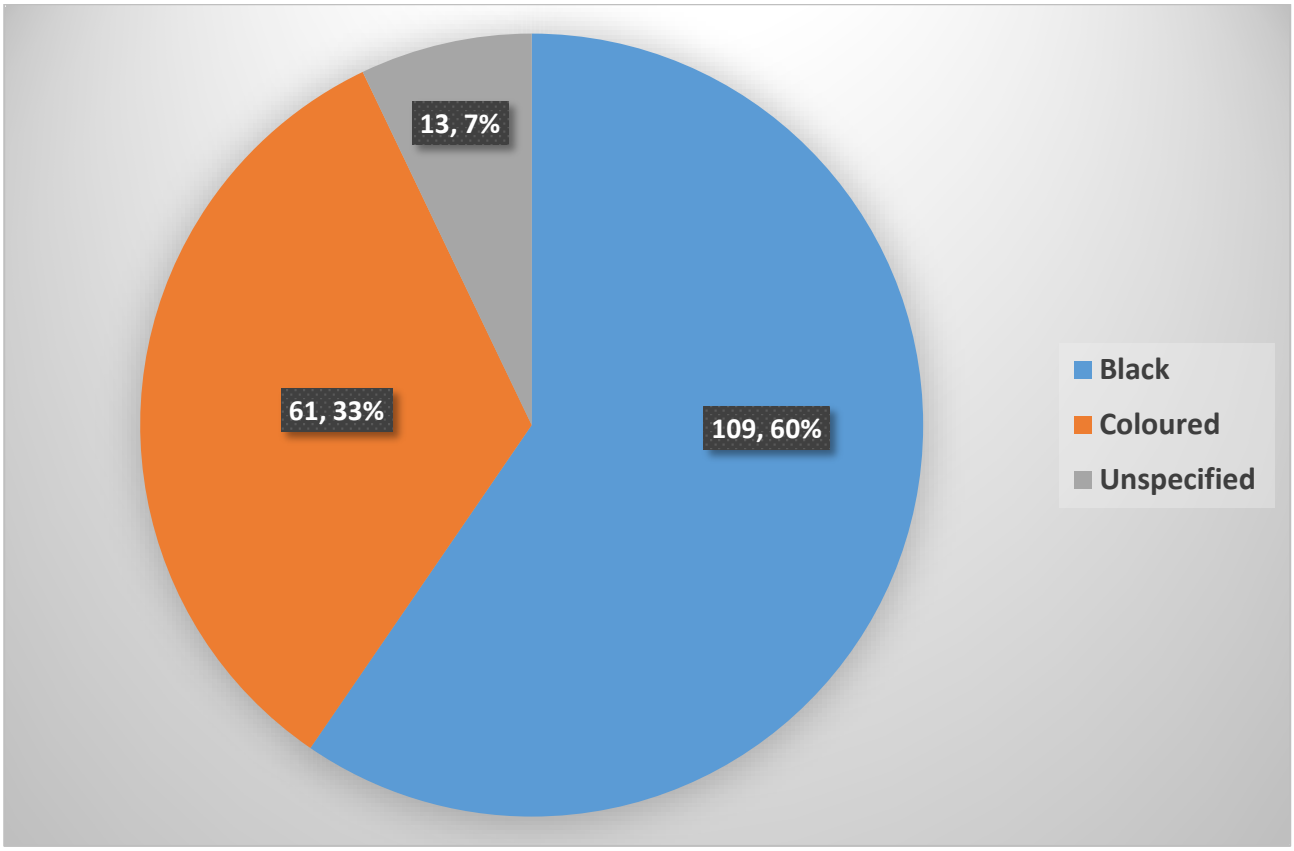

Figure 4.2 Racial distribution of cases included in the study

The sleeping position of infants received much attention in the literature and our results showed that 37/101 (37%) of infants were put to sleep on their stomach, 54/101 (53%) on their side and 10/101 (10%) on their backs, while a similar trend was evident for the position in which the infants were found, i.e. 38/137(28%), 63/137 (46%) and 34/137 (25%) on their stomach, side and back respectively. No data was available for 2 of the cases.

It is important to note that this data is based on the information that the parents or individual(s) give when being interviewed at the time when the infants are admitted to the Tygerberg Medico-Legal Mortuary and is very likely to be biased.

4.2 Routine microbiology results

Microbiological culture was conducted on the heart and left and right lung swabs to detect microorganisms, such as bacteria that might have been present prior to the death of the infant. Culture results were only available for 161/183 (88%) cases. The most commonly detected microorganisms were *Escherichia coli* and *Klebsiella pneumoniae*, followed by *Haemophilus influenzae, Enterococcus faecalis, Strep pneumoniae* and *Raoultella planticola* (Table 4.1). The majority of other bacterial species were only detected in a single case.

Table 4.1 The prevalence of different bacterial species in routine microbiological culture

Identity of bacteria	Number of positive cases (n=161)
Candida parapsilosis	1 (0.6%)
Enterococcus avium	1 (0.6%)
Escherichia coli	20 (12.4%)
Enterococcus faecalis	10 (6.2%)
Enterococcus faecium	1 (0.6%)
Haemophilus haemolyticus	1 (0.6%)
Haemophilus influenzae	10 (6.2%)
Klebsiella oxytoca	5 (3.1%)
Klebsiella pneumoniae	20 (12.4%)
Lactococcus gaviae	1 (0.6%)
Lactobacillus spp	1 (0.6%)
Moraxella group	1 (0.6%)
Proteus mirabilis	1 (0.6%)
Raoultella planticola	7 (4.3%)
Streptococcus agalactiae	2 (1.2%)
Staphylococcus aureus	6 (3.7%)
Streptococcus gallolyticus	1 (0.6%)
Streptococcus mitis/oralis	2 (1.2%)
Skrjabinema ovis	1 (0.6%)
Streptococcus pneumoniae	8 (5%)
Streptococcus pyogenes	2 (1.2%)
Streptococcus viridans	1 (0.6%)
βeta-haemolytic streptococcus	3 (1.9%)

Of the 161 heart swab samples that were cultured, 106 (66%) were negative. This was in contrast to the left and right lung swabs where negative results were found in 68 (42%) and 66 (41%) cases respectively. No bacteria could be detected in any of the three swabs in 48 (29%) of cases. However, some microorganism growth that was observed in culture, was characterised as PM flora in 32 (20%) cases.

4.3 COD classification

At the time of final data analyses, only 118 (64%) cases were signed out with a final COD by the forensic pathologists. Investigations to determine the COD were ongoing for the other 65 (36%) cases, which were still under investigation at the time of statistical analysis and write-up of this thesis. In defining or classifying the COD in these cases, pathologists only considered results from the routine SVC and microbiological culture performed by the NHLS diagnostic laboratories, as well as histological analysis of lung tissue for signs of infection.

The COD classification in this study was divided into three categories, i.e. *Infection*, *SIDS* and *Other* (Figure 4.3). Thirty-four (29%) deaths were as a result of *infection*, while *SIDS* was assigned the final COD in 57 (48%) and 27 (23%) of the deaths were classified as *other*. The *other* category included death was confirmed to be a result of aspiration of gastric contents, asphyxia or atrial septal defects.

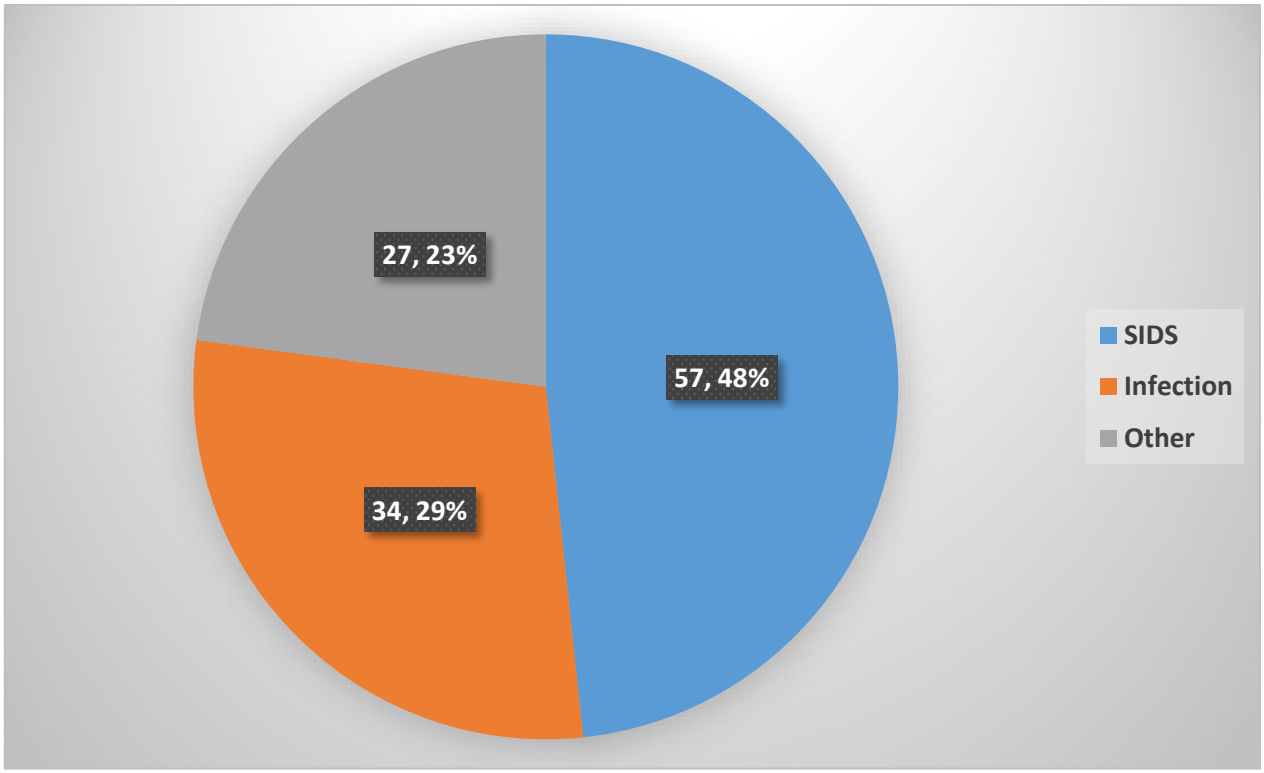

Figure 4.3 Final COD distribution in 118 cases as determined by forensic pathologists

4.4 Comparison of routine SVC with Multiplex PCR results

As illustrated in Table 4.2, multiplex PCR was able to detect a virus in considerably more cases compared to SVC. In addition, multiplex PCR was able to detect all viral targets except HMPV in at least one case, while SVC could only detect one of its nine viral targets, i.e. CMV.

Table 4.2 Comparison of the number of cases in which a viral target was detected by multiplex PCR or shell vial culture

Viral Target	Multiplex PCR	Shell Vial Culture
Adenovirus	18	0
Bocavirus 1/2/3	3	Not included in panel
Cytomegalovirus	Not included in panel	13
Coronavirus 229E/NL63	3	Not included in panel
Coronavirus OC43	3	Not included in panel
Enterovirus	9	Not included in panel
Influenza A	4	0
Influenza B	1	0
Metapneumovirus	0	0
Parainfluenza virus 1	1	0
Parainfluenza virus 2	1	0
Parainfluenza virus 3	10	0
Parainfluenza virus 4	1	Not included in panel
Rhinovirus A/B/C	65	Not included in panel
Respiratory Syncytial Virus A	2	0
Respiratory Syncytial Virus B	7	0

4.5 Multiplex PCR result breakdown

Respiratory virus detection in this study utilised the Seeplex RV15 ACE multiplex PCR detection kit. According to Table 4.1, the most commonly detected virus was HRV A/B/C, which was detected in 65 (36%) of the 183 cases tested. HAdV was the second most frequently detected virus in 18 (10%) of the cases tested. PIV 3, HEV and RSV B were detected in 10 (6%), 9 (5%) and 7 (4%) cases respectively. HMPV was the only virus that was not detected in any of the 183 cases. It was interesting to note the majority of virus positive samples were swabs collected from the trachea and not from the

lung swab samples. It is only in a few cases where the lung swabs were positive for a virus that was not detected in the tracheal swab of the same case. The reason for this is currently not known.

4.6 Serum immune biomarker testing results

Epidemiological data was not always available from the SUDI case files. The same was true for serum samples for different reasons. At times, there was not enough serum left after conduction of routine HIV 1/2 antibody testing by the NHLS Virology laboratory. In other instances during the autopsy, the forensic officers were unable to extract blood from the infant's heart.

Quantification of immune biomarkers was only performed in cases where sufficient serum was available and the descriptive statistical analyses are summarised in Table 4.3.

Table 4.3 Results of individual immune biomarker concentrations in pg/ml

Target analyte	N	Mean	SD	Median	Min	Max
IFN-γ	135	224.75	770.02	3.28	0.00	5 873.30
IL-1α	135	15.68	37.74	5.83	0.00	266.52
IL-1β	135	364.77	1 440.20	86.38	0.00	12 892.00
IL-1RA	135	11 148.00	7 581.70	9 090.00	1 328.60	46 649.00
IL-2	135	382.51	812.95	115.77	0.00	6 830.60
IL-4	135	250.45	2 044.30	32.98	0.00	23 792.00
IL-5	135	2.71	6.57	0.41	0.00	41.80
IL-6	135	591.02	2 669.80	13.31	0.00	22 733.00
IL-8	135	320.69	721.47	70.56	6.27	5 411.60
IL-10	135	3.14	9.13	0.00	0.00	72.32
IL-12p70	135	97.93	282.13	0.00	0.00	1 640.20
IL-18	135	328.78	372.87	232.50	0.00	2 255.50
IL-23	135	479.81	929.38	202.51	0.00	7 293.30
IP-10	135	93.56	195.32	46.89	0.00	2 074.90
TNF-α	135	18.25	40.16	3.46	0.00	273.32
CRP	139	3.69	4.91	1.50	0.00	18.65

4.7 Statistical analyses of serum immune biomarker data

The hypothesis tested in Table 4.4 was that socio-demographic factors, such as age, bed-sharing, maternal smoking, sleeping position, race and season play a role in the ultimate diagnosis of SUDI to be a result of infection. However, no statistically significant differences were found in the *Infection* and *SIDS/Other* groups for any of the socio-demographic factors being tested in this study. As such, none of these factors could be associated with the eventual COD being infection.

The median values of immune biomarkers in cases where the COD was infection were compared to the median values in cases where the final COD was SIDS/other using the two-sample Wilcoxon test (Table 4.5). Only two biomarkers, IL-6 (p=0.012) and IL-18 (p=0.044) were statistically significant in their usefulness as guides to the possibility of a SUDI case being diagnosed as an infection.

As summarised in Table 4.6, IL-1β (p=0.014) and IL-6 (p=0.016), there is statistical significance for the two biomarkers to be used as a predictor for SUDI being as a result of infection as opposed to SIDS/other when their concentration in serum is above the set thresholds of 80 pg/ml and 16.5 pg/ml respectively.

The hypothesis tested in Table 4.7 was also that socio-demographic factors, such as age, gender, season, sleeping position and housing play a role in the ultimate diagnosis of SUDI being as a result of infection. As summarised in Table 4.7, cold season (p=0.064) showed a trend towards statistical significance with the eventual diagnosis of a SUDI case being infection in this study and as such was a candidate for multivariate regression analysis, results of which are shown in Table 4.8.

Upon comparing the median values of biomarkers in SUDI cases where infection was the cause of death with the median values for the same biomarkers in cases with a SIDS diagnosis (Table 4.8), IL-6 (p=0.002) was statistically significant to be considered as a useful marker in diagnosing SUDI as having an infectious cause. The other biomarkers, IL-1β, IL-6, IL-8, IL-18 and CRP, by virtue of having a p-value >0.05 but less than 1 were candidates for multivariate logistic regression analysis (Table 4.8).

A multivariate logistic regression as shown in Table 4.9 was done to ascertain the effects of CRP, IL-6, IL-8, IL-1β, IL-18 and the cold season on the likelihood of SUDI being diagnosed as infection. Statistical significance was demonstrated for CRP (p=0.039) and IL-18 (p=0.011).

Table 4.4 **Socio-demographic factors associated with the cause of death in SUDI being diagnosed as infection compared to a SIDS diagnosis or other diagnoses**

	SIDS / Other	Infection	p-value	OR	95% CI
	N (%)	N (%)			
Age (weeks)					
< 22	45 (90.0)	25 (78.1)	0.137	0.34	0.08 – 1.24
≥ 22	5 (10.0)	7 (21.9)			
Alcohol Usage					
Yes	13 (19.7)	7 (18.9)	0.923	0.95	0.32 – 2.59
No	53 (80.3)	30 (81.1)			
Bed-sharing					
Yes	57 (96.6)	27 (90.0)	0.330	0.31	0.03 – 2.01
No	2 (3.4)	3 (10.0)			
Gender					
Male	57 (51.8)	23 (46.0)	0.495	0.79	0.40 – 1.54
Female	53 (48.2)	27 (54.0)			
Housing					
Informal	27 (40.9)	19 (51.4)	0.306	1.52	0.67 – 3.45
Formal	39 (59.1)	18 (48.6)			
Race					
Coloured	36 (36.0)	16 (32.7)	0.687	0.86	0.41 – 1.76
Black	64 (64.0)	33 (67.3)			
Season					
Cold	74 (67.3)	40 (80.0)	0.099	1.94	0.89 – 4.51
Warm	36 (32.7)	10 (20.0)			
Sleeping position					
Side	31 (50.8)	16 (57.1)	0.576	0.77	0.19 – 3.39
Stomach	24 (39.3)	8 (28.6)		0.50	0.11 – 2.36
Back	6 (9.8)	4 (14.3)			
Maternal smoking					
Yes	21 (31.8)	9 (24.3)	0.421	0.68	0.26 – 1.68
No	45 (68.2)	28 (75.7)			

Table 4.5 Immune biomarkers associated with a diagnosis of infection being made in SUDI cases

Target analyte	SIDS / Other (N=84)	Infection (N=34)	p-value
	Median (IQR)	Median (IQR)	
IFN-γ	6.83 (1.13 – 26.76)	2.80 (1.37 – 27.80)	0.810
IL-1α	5.86 (1.30 – 13.50)	6.17 (2.56 – 12.74)	0.725
IL-1β	78.01 (43.71 – 172.02)	109.81 (72.16 – 168.94)	0.142
IL-1RA	9 090.00 (9 033.35 – 11 161.84)	9 090.00 (9 090.00 – 12 604.25)	0.509
IL-2	106.98 (30.84 – 322.35)	91.10 (19.71 – 267.02)	0.745
IL-4	32.51 (14.30 – 90.89)	22.95 (7.32 – 63.22)	0.296
IL-5	1.28 (0.00 – 1.94)	0.20 (0.00 – 1.94)	0.386
IL-6	9.02 (2.86 – 59.25)	53.75 (10.18 – 383.54)	**0.012**
IL-8	64.01 (37.95 – 213.19)	107.76 (40.80 – 406.32)	0.139
IL-10	0.00 (0.00 – 2.82)	0.00 (0.00 – 2.51)	0.991
IL-12p70	0.00 (0.00 – 49.76)	8.71 (0.00 – 23.14)	0.503
IL-18	259.15 (113.57 – 486.02)	170.06 (20.53 – 297.98)	**0.044**
IL-23	193.08 (61.17 – 446.57)	165.53 (44.62 – 490.69)	1.000
IP 10	42.50 (0.00 – 73.52)	89.82 (3.97 – 162.86)	0.153
TNF-α	4.29 (0.00 – 14.63	3.05 (0.00 – 9.57)	0.305
CRP	1.40 (0.00 – 4.17)*	2.35 (0.10 – 9.05)**	0.193

* N=87 ** N=35

Table 4.6 **A comparison of immune biomarker levels found in the two SUDI cause of death categories**

Target analyte	SIDS / Other	Infection	p-value	OR	95% CI
	N (%)	N (%)			
CRP (mg/l)					
>0.35	53 (60.9)	25 (71.4)	0.274	1.60	0.69 – 3.88
<0.35	34 (39.1)	10 (28.6)			
IFN-γ (pg/ml)					
>15.5	64 (76.2)	26 (76.5)	0.974	1.01	0.40 – 2.71
<15.5	20 (23.8)	8 (23.5)			
IL-1β (pg/ml)					
>80	41 (48.8))	25 (73.5)	**0.014**	2.91	1.25 – 7.28
<80	43 (51.2)	9 (26.5)			
IL-6 (pg/ml)					
>16.5	34 (40.5)	22 (64.7)	**0.016**	2.69	1.19 – 6.32
<16.5	50 (59.5	12 (35.3)			
IL-10 (pg/ml)					
>5.5	9 (10.7)	3 (8.8)	1	0.80	0.17 – 2.91
<5.5	75 (89.3)	31 (91.2)			
TNF-α (pg/ml)					
>3.5	46 (54.8)	12 (35.3)	0.055	0.45	0.19 – 1.01
<3.5	38 (45.2)	22 (64.7)			

Table 4.7 **Socio-demographic factors associated with the cause of death in SUDI being diagnosed as infection compared to a diagnosis of SIDS**

	SIDS	Infection	OR	95% CI	p-value
Gender					
Male	49 (68.1)	23 (31.9)	0.852	0.430 - 1.686	0.645
Female	49 (64.5)	27 (35.5)			
Age					
< 8 weeks	21 (60.0)	14 (40.0)	0.711	0.251 -2.015	0.206
9 – 16 weeks	19 (67.9)	9 (32.1)	2.250	0.655 - 7.734	
> 16 weeks	6 (40.0)	9 (60.0)			
Season					
Cold	64 (61.5)	40 (38.5)	2.125	0.947 - 4.768	**0.064**
Warm	34 (77.3)	10 (22.7)			
Housing					
Informal	34 (65.4)	18 (34.6)	0.724	0.318 - 1.649	0.442
Formal	26 (57.8)	19 (42.2)			
Sleeping position					
Stomach	23 (74.2)	8 (25.8)	0.522	0.117 - 2.337	0.556
Side	28 (63.6)	16 (36.4)	0.857	0.210 - 3.498	
Back	6 (60)	4 (40)			

Table 4.8 **Immune biomarkers associated with a diagnosis of infection being made in SUDI compared to SIDS**

Target analyte	SIDS (N=74) Median (IQR)	Infection (N=34) Median (IQR)	p-value
IFN-γ	2.90 (0 – 24.42)	2.80 (1.37 – 27.80)	0.854
IL-1α	5.89 (1.30 – 13.36)	6.17 (2.56 – 12.74)	0.630
IL-1β	77.44 (41.23 – 161.20)	109.81 (72.16 – 168.94)	**0.095**
IL-1RA	9090 (8920.05 – 11702.01)	9090 (9090 – 12604.25)	0.689
IL-2	111.34 (33.90 – 315.46)	91.1 (19.71 – 267.02)	0.698
IL-4	32.51 (14.30 – 92.15)	22.95 (7.32 – 63.22)	0.381
IL-5	0.41 (0 – 1.94)	0.20 (0 – 1.94)	0.666
IL-6	7.94 (2.63 – 28.68)	53.75 (10.18 – 383.54)	**0.002**
IL-8	61.22 (34.33 – 102.63)	107.76 (40.80 – 406.32)	**0.061**
IL-10	0 (0 – 2.66)	0 (0 – 2.51)	0.858
IL-12p70	0 (0 – 33.23)	8.71 (0 – 23.14)	0.432
IL-18	259.15 (111.65 – 444.4)	170.06 (20.53 – 297.98)	**0.058**
IL-23	178.32 (52.4 – 419.94)	165.53 (44.62 – 490.69)	0.860
IP 10	43.04 (24.35 – 74.83)	89.82 (3.97 – 162.86)	0.178
TNF-α	3.90 (0 – 13.17)	3.05	0.476
CRP	1.00 (0 – 3.85)*	2.35 (0.1 – 9.05)**	**0.099**

* N=77 ** N=35

Table 4.9 **Multiple logistic regression to ascertain the effect of select immune biomarkers and the cold season on the likelihood of SUDI being diagnosed as infection.**

Parameter	aOR	95% CI	p-value
CRP	1.117	1.006 - 1.242	**0.039**
IL-6	1.000	0.999 -1.001	0.956
IL-8	1.000	0.999 - 1.002	0.541
IL-1β	1.000	0.998 - 1.002	0.957
IL-18	0.998	0.996 - 0.999	**0.011**
Season (cold)	2.830	0.943 - 8.492	0.064

CHAPTER 5: DISCUSSION

5.1 Overview

Close to six decades after the idea of SIDS was introduced, a lot of research has been done and continues to be done to further understand the pathogenesis of SIDS. Through epidemiological studies, a number of risk factors for SIDS were identified. These include the infant's sleeping position (prone-sleeping position), bed-sharing, poor socio-economic background, maternal smoking, colder seasons, gender and age (Morris, 1999; Raza & Blackwell, 1999; Vennemann *et al*., 2007; Fleming *et al*., 2015). An established and well-known risk factor for sudden infant death is cold weather; a time when infants are exposed to and often infected with respiratory viruses, such as HRV, which was detected in 35.5% of the cases investigated in this study. However, no association could be found between cold weather and a final COD of infection or SIDS.

Infants, especially between the ages of two to four months old, are at a stage in their life where they are particularly susceptible to infection by viruses and bacteria. It is at this stage that they start to lose the maternal antibodies that constituted their defence against these organisms up to this point in their lives. It is also at this age that the incidence of SUDI is at its highest. In this study, of the 170 infants whose age was available, about a third of cases were aged between two and four months with more males (52%) than females (48%). Although the difference was small, the observation was in agreement with published literature, which suggests that male infants are at a greater risk of succumbing to SUDI as opposed to their female counterparts (Moscovis *et al*., 2014). However, our data showed that infants who died between 8 and 16 weeks (2 to 4 months) of age did not have a statistically significant chance of dying as a result of infection when compared to infants of the same age whose deaths were classified as SIDS.

When Bruce Beckwith and his colleagues defined the term "sudden infant death syndrome" in 1969, there was so much controversy surrounding the subject (Beckwith, 1970; Bajanowski *et al*., 2006). The term SIDS described infant deaths where an adequate cause of death could not be determined after thorough investigations had been conducted (Krous *et al*., 2004). Even after conduction of thorough investigations involving death scene investigations, autopsies and review of the medical history, a larger number of sudden unexpected infant death cases remain unsolved and are finally diagnosed as SIDS. This was also the case in this study where almost half of the cases were classified as SIDS. If the cause of death in so many cases continues to elude investigators, it is reasonable to assume that the SIDS conundrum will be around for many more years.

5.3 Routine virology

In our setting, routine virology involves centrifugation-enhanced SVC of lung and liver tissue and detection of viruses using immunofluorescence. This was done for all 183 SUDI cases, but SVC was only able to detect CMV out of a possible nine viruses and only in a very few cases. This could be a result of the 4-5 day PMI or inadequate transport and storage of samples. However, all the tissue samples were placed in preservation media immediately after collection and transportation to the laboratories within 10 minutes after collection, making the second scenario highly unlikely. When there is a time lapse between death and collection and culture of the tissue, there might be a loss of virus viability and the virus might fail to grow in culture. Interestingly, all but two cases in which CMV was detected had an average PMI of three days, which was shorter that the average PMI of 4-5 days for all cases included in the study. Even more interesting was the fact that the remaining two CMV positive cases had PMIs of nine and 14 days respectively. After further investigation, the cause of death in one case was found to be CMV nephritis. The most plausible explanation for this sample being positive for CMV despite the long PMI is that there could have been a high CMV viral load in the tissue sample. Another issue to consider is the fact that CMV tends to become latent and the effect of the PMI has not been evaluated in latent CMV infection.

The second CMV positive case has a PMI of 14 days and had a diagnosis of bronchiolitis and again there might have been a high viral load that could have contributed to the infant's bronchiolitis and eventual death. This is consistent with a study on a previously healthy, 37-day old Asian male infant who died as a result of bronchiolitis obliterans, a lung disease that is a result of severe infection of the lower respiratory tract. In this specific case, CMV was the viral agent responsible for the condition that led to the infant dying (Byun *et al*., 2014).

However, the viral load was not tested quantitatively in either of the two cases and CMV is not included in the commercial Multiplex PCR assay used in the study. As such, direct comparison was not possible. This is a field of future research.

5.4 Routine microbiology

Many different species of bacteria are detected during medico-legal SUDI case investigations. Bacteria, such as *Staph aureus*, *Haemophilus influenzae*, *Strep pneumoniae* and *Strep pyogenes*, were often detected in sudden infant death investigations and reported in the literature (Forsyth, 1999). However, this was not the case in this study as the most frequently encountered bacteria were *E.coli*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Enterococcus faecalis* and *Strep pneumoniae*. The significance and interpretation of bacteria detected during SUDI case investigations are extremely

challenging, as some of these bacteria are regarded as PM contaminants. A clear histological picture showing bacterial infection is required to confirm a bacterial COD. An *in vitro* study to assess the cytokine responses of male and female PBMCs to a bacterial antigen showed a comparatively lower production of pro-inflammatory cytokines by male than female PBMCs. This inability to produce sufficient cytokines against a bacterial antigen could also partly explain why male infants are at a higher risk of death in SUDI due to vulnerability to infection (Moscovis *et al*., 2014).

5.5 Multiplex PCR

When forensic pathologists were defining the COD classifications, they were blinded to the multiplex PCR results. As a result, 20 cases had a COD classification of SIDS, although respiratory viruses were detected in these cases. Co-infections with two or three different viruses were present in these cases. This emphasises the importance of adding ancillary tests, such as multiplex PCR, in investigating SUDI cases instead of SVCs, which are almost always negative (personal observation). Had the multiplex PCR results been available to the forensic pathologists at the time of classifying the COD in these 20 cases, some might have been diagnosed as infection and not SIDS. The failure to detect hMPV in any of the SUDI cases investigated was unexpected, because hMPV is usually implicated in LRTI in infants and children. However, it is possible that none of the infants investigated in this study were in fact infected with hMPV prior to or at the time of death. The hMPV positive controls utilised in the multiplex PCR were positive on the agarose gel electrophoresis images and as such rule out any false negative findings. Co-circulation of RSV and hMPV has been reported in literature (Budge *et al*., 2014). However, despite RSV being identified as a leading cause of respiratory infections in infants worldwide, it was detected in only nine of the 183 cases analysed in this study. It is therefore postulated that the respiratory samples were collected for this study at a time when hMPV and RSV incidences were low and could actually account for the non-detection of hMPV in all 183 cases. The seasonality of RSV outbreaks has also been described in literature (Noveroske *et al*., 2016). It is critical to note that the detection of viral nucleic acid alone does not necessarily mean that the virus was responsible for the death of the infant. However, with tissue histology and addition of immune biomarkers to the investigation process, the interpretation of the multiplex PCR results would be easier and the results more valuable to SUDI case COD diagnosis.

5.6 Immune biomarkers

According to the analyte-specific standard deviations shown in Table 4.3, there was a lot of variation in the levels of the same cytokine in different SUDI cases. This observation was not unexpected, as every individual has an immunological profile unique to activities of their immune system. A range of symptoms suggestive of a viral respiratory infection, such as a running nose or cold, prior to death

in some of these infants could be the reason why there were such differences in the immunological profiles. There was however, no significant difference between serum levels of IFN-γ, IL-1α, IL-1RA, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-23, IP 10 and TNF-α in cases where infection was the COD and cases with a SIDS or non-infectious COD. There was also no statistically significant difference in the levels of serum IL-1β in infectious deaths and SIDS or other non-infectious deaths. However, when serum IL-1β levels were greater than 85 pg/ml, which is the upper reference limit for IL-1β, the difference became statistically significant in the group with a COD of infection compared to the rest. Being a pro-inflammatory cytokine, IL-1β in excessive amounts could possibly be detrimental to the infant. Inflammatory disease and lung damage are seen in situations where there is an imbalance of IL-1 and IL-1RA (Arend, 2002). This could partially be the reason why IL-1β serum levels become a possible indicator of an infectious death above the normal cut-off level of 85 pg/ml. Statistically significant observations were also made for the three immune biomarkers, CRP, IL-6 and IL-18, which are all three important biomarkers in the inflammatory process.

Despite meticulous medico-legal investigations in SUDI cases, the COD still remains unknown in up to 80% of cases (Dempers *et al*., 2016). As such, part of the study was aimed at potentially contributing towards more SUDI cases being diagnosed as infection as opposed to a SIDS diagnosis. The serum concentrations of CRP and IL-6 were significantly elevated in the serum of SUDI cases where infection was confirmed to be the COD compared to cases that were diagnosed as SIDS. The serum levels of CRP could therefore be used as a potential indicator of prior infection in a SUDI case. This is in agreement with a study by Uhlin-Hansen in 2001 where elevated CRP levels were shown to be a good indicator of a prior inflammatory process. The suitability of CRP for PM investigations due to its stability in storage was also demonstrated in this specific study. Evidence of elevated IL-6 levels has been observed in non-SIDS cases in the literature when compared to SIDS cases (Vege *et al*., 1995). In contrast to CRP and IL-6, levels of IL-18 were significantly reduced in the serum of our SUDI cases where infection was confirmed to be the COD compared to cases with a final COD of SIDS. IL-18 is responsible for stimulating NK cells to release IFN-γ, an antiviral cytokine. Consequently, low levels of serum IL-18 render an individual more susceptible to a viral infection.

5.7 Strengths of the study

The study showed that immune biomarkers can be used to supplement the tissue histology picture when determining if infection played a role in the events leading to the death of the infant. If adopted, the use of immune biomarkers offers a more comprehensive picture of infection instead of solely depending on viral and bacterial culture results, which in most instances have a negative result.

A panel of immune biomarkers was identified that can be used as potential predictors of infection being the reason behind the death of an infant in SUDI cases.

Testing was conducted on a large number of samples collected over a full one-year period, enabling evaluation of several risk factors, including seasonal variation.

5.8 Weaknesses of the study

The lack of a control group remains the main weakness of this type of study. If a control group had been available, it would have been easier to compare the test cases with normal (control) cases and draw conclusions from the differences observed between these two groups. However, ethical restrictions prevented the study from incorporating a control group.

To the best of our knowledge, this was the first study in South Africa to investigate levels of immune biomarkers in SUDI cases. As such, there was a paucity of what constituted as normal serum levels of these biomarkers in South African infants. Studies on immune biomarkers in SUDI have been done in some developed countries and immune biomarker serum levels in these studies were used as reference points for our study. However, the inflammatory profiles of infants in those countries may not exactly be the same or truly representative of the inflammatory profile of South African infants.

5.9 Future research

As earlier indicated, there is a paucity of data on normal levels of serum cytokines in infants. This information gap needs to be addressed by conducting such a study in living, healthy infants. Once this data is available, it can be accessible to individuals conducting immunological studies involving infants.

In addition, there is a need for investigating the role of viruses in SUDI. Whether use of an assay measuring viral load in lung tissue will be possible, needs to be established. If successful, the viral load can be correlated with the severity of inflammation observed in tissue and the serum immunological profile.

CHAPTER 6: CONCLUSION

This study aimed to characterise the viruses frequently encountered in SUDI case investigations and this objective was completed as shown in Table 4.2. All but one (hMPV) of the 15 target respiratory viruses were detected in at least one of the 183 SUDI cases included in the study. However, with SVC, only a single virus (CMV) was detected and then in only 13 of the cases, further emphasising the shortcomings of SVC for viral detection in SUDI case investigations. As stated earlier, the current SUDI case investigation protocol at the Tygerberg Medico-Legal Mortuary utilises SVC for routine viral screening although results show a very low positive yield compared to multiplex PCR.

Ideally, an amendment to the institutional investigation protocol to implement the routine use of multiplex PCR in place of SVCs when investigating the COD in SUDI cases should be recommended and implemented. However, due to the higher costs of multiplex PCR, the use of a custom viral detection assay only targeting viruses that are most frequently detected in SUDI case investigations would be more suited and applicable. The use of multiplex PCR in these investigations would undoubtedly increase the chances of detecting possible viral involvement in the infant's death and more cases that would previously been assigned a COD of SIDS, could be classified as infection-related deaths. This would further ensure that infectious COD in SUDI is not underestimated and underreported. However, more research is necessary to define and validate a custom viral detection panel specifically for the South African population.

Another aim of the study was to determine the value of immune biomarkers as indicators of infection and inflammation prior to or at the time of death of an infant. From the study, a panel of three biomarkers, CRP, IL-6 and IL-18 were identified for possible use as predictors of infectious COD. These markers however need to be further investigated in a different set of SUDI cases if they are to be used routinely. More research is needed to further understand the interaction of respiratory viral infections with the immune system and how this can be related to the histological picture observed in tissue.

In conclusion, the contribution of respiratory viruses towards SUDI and the resultant immune response can no longer be underestimated and this study was able to identify several novel angles to investigate the different interactions in this multifactorial condition.

REFERENCES

Abboud G, Tahiliani V, Desai P, *et al.* Natural Killer Cells and Innate Interferon Gamma Participate in the Host Defence against Respiratory Vaccinia Virus Infection. *J Virol.* 2015;90(1):129-141

Abels S, Nadal D, Stroehle A, Bossart W. Reliable Detection of Respiratory Syncytial Virus Infection in Children for Adequate Hospital Infection Control Management. *J Clin Microbiol.* 2001;39(9):3135-3139

Adams SM, Good MW, Defranco GM. Sudden infant death syndrome. *Am Fam Physician.* 2009;79(10):870-874

Adams SM, Ward CE, Garcia KL. Sudden Infant Death Syndrome. *Am Fam Physician.* 2015;91(11):778-783

Alfelali M, Khandaker G. Infectious causes of sudden infant death syndrome. *Paediatr Respir Rev.* 2014;15(4):307-311

Allander T. Human bocavirus. *J Clin Virol.* 2008;41(1):29-33

Alvarez-Lafuente R, Aguilera B, Suarez-Mier MA, *et al*. Detection of human herpesvirus-6, Epstein-Barr virus and cytomegalovirus in formalin-fixed tissues from sudden infant death: a study with quantitative real-time PCR. *Forensic Sci Int.* 2008;178:106-111

Arend WP. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev.* 2002;13(4-5):323-340

Bajanowski T, Brinkmann B, Vennemann M. The San Diego definition of SIDS: practical application and comparison with the GeSID classification. *Int J Legal Med*. 2006;120:331-336

Bajanowski T, Rolf B, Jorch G, Brinkmann B. Detection of RNA viruses in sudden infant death (SID). *Int J Legal Med.* 2003;117:237-240

Bajanowski T, Vege A, Byard RW, *et al.* Sudden Infant Death Syndrome (SIDS) – Standardised investigation and classification: Recommendations. *Forensic Sci Int.* 2007;165(203):129-143

Beckwith JB. Discussion of terminology and definition of the sudden infant death syndrome. In: Bergman AB, Beckwith JB, Ray CG, eds. Sudden Infant Death Syndrome: Proceedings of the Second International Conference on the Causes of Sudden Death in Infants. Seattle: University of Washington Press; 1970:14-22

Beckwith JB. Defining the Sudden Infant Death Syndrome. *Arch Pediatr Adolesc Med*. 2003;157:286-290

Blackwell C, Moscovis S, Hall S, Burns C, Scott RJ. Exploring the risk factors for sudden infant deaths and their role in inflammatory responses to infection. *Front Immunol.* 2015;6(44):1-8

Blackwell CC, Gordon AE, James VS, *et al.* The role of bacterial toxins in Sudden Infant Death Syndrome (SIDS). *Int J Med Microbiol.* 2002;291(6-7):561-570

Blackwell CC, Moscovis SM, Gordon AE *et al.* Cytokine responses and sudden infant death syndrome: genetic, developmental and environmental risk factors. *J Leukoc Biol.* 2005;78(6):1242-1254

Blood-Siegfried J. The role of infection and inflammation in sudden infant death syndrome. *Immunopharmacol Immunotoxicol.* 2009;31(4):516-523

Budge PJ, Griffin MR, Edwards KM *et al.* A household-based study of acute viral respiratory illnesses in Andean children. *Pediatr Infect Dis J.* 2014;33(5):443-447

Burns JL, Emerson J, Kuypers J, *et al.* Respiratory viruses in children with cystic fibrosis: viral detection and clinical findings. *Influenza Other Respir Viruses.* 2012;6(3):218-223

Busse WW, Lemanske RF, Gern JE. The role of viral respiratory infections in asthma and asthma exacerbations. *Lancet.* 2010;376(9743):826-834

Byun SY, Kim MJ, Lee YS, Kang EJ, Jung JA. Bronchiolitis obliterans caused by CMV in a previously healthy Asian infant. *Turk J Pediatr.* 2014;56(3):316-319

Choi EH, Lee HJ, Kim SJ, *et al.* The Association of Newly Identified Respiratory Viruses with Lower Respiratory Tract Infections in Korean Children, 2000-2005. *Clin Infect Dis.* 2006;43(5):585-592

Crowe JE Jr, Williams JV. Immunology of viral respiratory tract infection in infancy. *Paediatr Respir Rev.* 2003;4(2):112-119

Dagan R, Hall CB, Powell KR, Menegus MA. Epidemiology and laboratory diagnosis of infection with viral and bacterial pathogens in infants hospitalised for suspected sepsis. *J Pediatr.* 1989;115(3):351-356

Datta S, Budhauliya R, Das B, Chatterjee S, Vanlalhmuaka, Veer V. Next-generation sequencing in clinical virology: Discovery of new viruses. *World J Virol.* 2015;4(3):265-276

Dempers JJ, Coldrey J, Burger EH, *et al*. The Institution of a Standardized Investigation Protocol for Sudden Infant Death in the Eastern Metropole, Cape Town, South Africa. *J Forensic Sci.* 2016;61(6):1508-1514

Desmons A, Terrade C, Boulagnon C, *et al*. Post-mortem diagnosis of cytomegalovirus and varicella zoster virus co-infection by combined histology and tissue molecular biology in a sudden unexplained infant death. *J Clin Virol.* 2013;58(2):486-489

Donovan C, Bourke JE, Vlahos R. Targeting the IL-33/IL-13 Axis for Respiratory Viral Infections. *Trends Pharmacol Sci.* 2016;37(4):252-261

du Toit-Prinsloo L, Dempers J, Verster J, *et al.* Toward a standardized investigation protocol in sudden unexpected deaths in infancy in South Africa: a multicentre study of medico-legal investigation procedures and outcomes. *Forensic Sci Med Pathol.* 2013;9(3):344-350

du Toit-Prinsloo L, Dempers JJ, Wadee SA, Saayman G. The medico-legal investigation of sudden, unexpected and/or unexplained infant deaths in South Africa: where are we - and where are we going? *Forensic Sci Med Pathol.* 2011;7(1):14-20

Fernandez-Rodriguez A, Ballesteros S, de Ory F, *et al*. Virological analysis in the diagnosis of sudden children death: A medico legal approach. *Forensic Sci Int*. 2006;161(1):8-14

Ferrante L, Rognum TO, Vege A, Nygard S, Opdal SH. Altered gene expression and possible immunodeficiency in cases of sudden infant death syndrome. *Pediatr Res.* 2016;80(1):77-84

Filiano JJ, Kinney HC. A perspective on neuropathologic findings in victims of the sudden infant death syndrome. *Biol Neonate.* 1994;65:194-197

Fleming PJ, Blair PS, Pease A. Sudden unexpected death in infancy: aetiology, pathophysiology, epidemiology and prevention in 2015. *Arch Dis Child.* 2015;100(10):984-988

Fleming PJ, Blair PS, Ward Platt M, Tripp J, Smith IJ, CESDI SUDI Research Group. Sudden infant death syndrome and social deprivation: assessing epidemiological factors after post-matching for deprivation. *Paediatr Perinat Epidemiol.* 2003;17(3):272-280

Forsyth KD. Immune and inflammatory responses in sudden infant death syndrome. *FEMS Immunol Med Microbiol.* 1999;25(1-2):79-83

Freymuth F, Vabret A, Cuvillon-Nimal D, *et al.* Comparison of Multiplex PCR Assays and Conventional Techniques for the Diagnostic of Respiratory Virus Infections in Children Admitted to Hospital With an Acute Respiratory Illness. *J Med Virol.* 2006;78(11):1498-1504

Fujita MQ, Zhu B-L, Ishida K, Quan L, Oritani S, Maeda H. Serum C-reactive protein levels in postmortem blood – an analysis with special reference to the cause of death and survival time. *Forensic Sci Int.* 2011;130:160-166

Ghani AS, Morrow BM, Hardie DR, Argent AC. An investigation into the prevalence and outcome of patients admitted to a pediatric intensive care unit with viral respiratory tract infections in Cape Town, South Africa. *Pediatr Crit Care Med.* 2012;13(5):e275-e281

Goenka A, Kollmann TR. Development of immunity in early life. *J Infect.* 2015;71(S1):112-120

Goldstein RD, Trachtenberg FL, Sens MA, Harty BJ, Kinney HC. Overall Postneonatal Mortality and Rates of SIDS. *Pediatrics.* 2016;137(1):1-12

Goldwater PN. SIDS pathogenesis: pathological findings indicate infection and inflammatory responses are involved. *FEMS Immunol Med Microbiol.* 2004;42(1):11-20

Guerrero-Plata A, Casola A, Garofalo RP. Human Metapneumovirus Induces a Profile of Lung Cytokines Distinct from that of Respiratory Syncytial Virus. *J Virol.* 2005;79(23):14992-14997

Gunn AJ, Gunn TR, Mitchell EA. Is changing the sleep environment enough? Current recommendations for SIDS. *Sleep Med Rev.* 2000;4(5):453-469

Guntheroth WG, Spiers PS. The Triple Risk Hypotheses in Sudden Unexpected Death Syndrome. *Pediatrics.* 2002;110(5):1-6

Harvey CE, Post JJ, Palladinetti P, *et al*. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation. *J Leukoc Biol.* 2003:74(3):360-369

Heron M. Death: Leading Causes for 2012. *Natl Vital Stat Rep.* 2015;64(10):1-93

Highet AR. An infectious aetiology of sudden infant death syndrome. *J Appl Microbiol.* 2008;105(3):625-635

Horn SD, Smout RJ. Effect of prematurity on respiratory syncytial virus hospital resource and outcomes. *J Pediatr.* 2003;143(5 Suppl):S133-141

Hunt CE, Darnall RA, McEntire BL, Hyma BA. Assigning cause for sudden unexpected infant death. *Forensic Sci Med Pathol.* 2015;11(2):283-288

Jansen RR, Wieringa J, Koekkoek SM, *et al.* Frequent Detection of Respiratory Viruses without Symptoms: Toward Defining Clinically Relevant Cutoff Values. *J Clin Microbiol.* 2011;49(7):2631-2636

Jensen LL, Rohde MC, Banner J, Byard RW. Reclassification of SIDS cases – a need for adjustment of the San Diego classification? *Int J Legal Med.* 2012;126(2):271-277

Krous HF, Beckwith JB, Byard RW, *et al*. Sudden Infant Death Syndrome and Unclassified Sudden Infant Deaths: A Definitional and Diagnostic Approach. *Pediatrics.* 2004;114(1):234-238

Krous HF. Sudden Unexpected Death in Infancy and the Dilemma of Defining the Sudden Infant Death Syndrome. *Curr Pediatr Rev.* 2010;6(1):5-12

Liu C, Xiao Y, Zhang J, *et al.* Adenovirus infection in children with acute lower respiratory tract infections in Beijing, China, 2007 to 2012. *BMC Infect Dis.* 2015;15:408. doi: 10.1186/s12879-015-1126-2.

Loebbermann J, Thornton H, Durant L, *et al.* Regulatory T cells expressing granzyme B play a critical role in controlling lung inflammation during acute viral infection. *Mucosal Immunol.* 2012;5(2):161-172

Mahony JB. Detection of respiratory viruses by molecular methods. *Clin Microbiol Rev*. 2008;21(4):716-747

Melendi GA, Laham FR, Monsalvo AC, *et al.* Cytokine Profiles in the Respiratory Tract During Primary Infection With Human Metapneumovirus, Respiratory Syncytial Virus, or Influenza Virus in Infants. *Pediatrics.* 2007;120(2):e410-415

Mentel R, Ilgert U, Wegner U, Zimmerman K, Bruns R, Gurtler L. Molecular and clinical characteristics of respiratory syncytial virus infections in hospitalized children. *Med Microbiol Immunol.* 2005;194(1-2):67-71

Mistchenko AS, Diez RA, Mariani AL, *et al.* Cytokines in adenoviral disease in children: association of interleukin-6, interleukin-8, and tumor necrosis factor alpha levels with clinical outcome. *J Pediatr.* 1994;124(5 Pt 1):714-720

Mitchell EA, Krous HF. Sudden unexpected death in infancy: a historical perspective. *J Paediatr Child Health.* 2015;51(1):108-112

Morris JA. The common bacterial toxins hypothesis of sudden infant death syndrome. *FEMS Immunol Med Microbiol.* 1999;25(1-2):11-17

Moscovis SM, Hall ST, Burns CJ, Scott RJ, Blackwell CC. The male excess in sudden infant deaths. *Innate Immun.* 2014;20(1):24-29

Newton AH, Cardani A, Braciale TJ. The host immune response in respiratory virus infection: balancing virus clearance and immunopathology. *Semin Immunopathol.* 2016;38(4):471-482

Noveroske DB, Warren JL, Pitzer VE, Weinberger DM. Local variations in the timing of RSV epidemics. *BMC Infect Dis.* 2016;16(1):674-680

Opal SM, DePalo VA. Anti-Inflammatory Cytokines. *Chest.* 2000;117(4):1162-1172

Opdal SH, Rognum TO. The sudden infant death syndrome gene: does it exist? *Pediatrics.* 2004;114(4):506-512

Piper SC, Ferguson J, Kay L, *et al.* The role of interleukin-1 and interleukin 18 in pro-inflammatory and anti-viral responses to rhinovirus in primary bronchial epithelial cells. *PLOS ONE.* 2013;8(5):1-11

Pryce JW, Bamber AR, Ashworth MT, Klein NJ, Sebire NJ. Immunohistochemical expression of inflammatory markers in sudden infant death; ancillary tests for identification of infection. *J Clin Pathol.* 2014;67(12):1044-1051

Rambaud C, Guibert M, Briand E, Grangeot-Keros L, Coulomb-L'Hermine A, Dehan M. Microbiology in sudden infant death syndrome (SIDS) and other childhood deaths. *FEMS Immunol Med Microbiol.* 1999;25(1-2):59-66

Raza MW, Blackwell CC. Sudden infant death syndrome, virus infections and cytokines. *FEMS Immunol Med Microbiol.* 1999;25(1-2):85-96

Rognum TO, Saugstad OD. Biochemical and immunological studies in SIDS victims. Clues to understanding the death mechanism. *Acta Paediatr.* 1993;389(suppl):82-85

Samuels M. Viruses and sudden infant death. *Pediatr Resp Rev.* 2003;4:178-183

Smuts HE, Workman LJ, Zar HJ. Human rhinovirus infection in young African children with acute wheezing. *BMC Infect Dis.* 2011;11:65:1-8

Sumino KC, Walter MJ, Mikols CL, *et al.* Detection of respiratory viruses and the associated chemokine responses in serious acute respiratory illness. *Thorax.* 2010;65(7):639-644

Sung RY, Hui SH, Wong CK, Lam CW, Yin J. A comparison of cytokine responses in respiratory syncytial virus and influenza A infections in infants. *Eur J Pediatr.* 2001;160(2):117-122

Toro K, Voros K, Meszner Z, Varadi-T A, Toth A, Kovacs K. Evidence for Infection and Inflammation in Infant Deaths in a Country with Historically Low Incidences of Sudden Infant Death Syndrome. *Front Immunol.* 2015;6(389):1-6

Uhlin-Hansen L. C-reactive protein (CRP), a comparison of pre- and PM blood levels. *Forensic Sci Int.* 2001;124(1):32-35

UN Millenium Development Goals Report, 2014. Available from: http://www.un.org/millenniumgoals/2014%20MDG%20report/MDG%202014%20English%20web.pdf

van de Pol AC, van Loon AM, Wolfs TF, *et al.* Increased Detection of Respiratory Syncytial Virus, Influenza Viruses, Parainfluenza Viruses and Adenoviruses with Real-Time PCR in Samples from Patients with Respiratory Symptoms. *J Clin Microbiol.* 2007;45(7):2260-2262

van den Broek M, Bachmann MF, Kohler G, *et al.* IL-4 and IL-10 Antagonize IL-12-mediated Protection Against Acute Vaccinia Virus Infection with a Limited Role of IFN-γ and Nitric Oxide Synthetase 2. *J Immunol.* 2000;164(1):371-378

van Elden LJR, van Kraaij MGJ, Nijhuis M, *et al.* Polymerase chain Reaction Is More Sensitive than Viral Culture and Antigen Testing for the Detection of Respiratory Viruses in Adults with Hematological Cancer and Pneumonia. *Clin Infect Dis.* 2002;34(2):177-183

Vege A, Rognum TO, Scott H, Aasen AO, Saugstad OD. SIDS cases have increased levels of interleukin-6 in cerebrospinal fluid. *Acta Paediatr.* 1995;84(2):193-196

Vege A, Rognum TO. Inflammatory responses in sudden infant death syndrome – past and present views. *FEMS Immunol Med Microbiol.* 1999;25(1-2):67-78

Vennemann M, Bajanowski T, Butterfaß-Bahloul T, *et al.* Do risk factors differ between explained sudden unexpected death in infancy and sudden infant death syndrome? *Arch Dis Child.* 2007;92:133-136

Vennemann MM, Loddenkotter B, Fracasso T, *et al.* Cytokines and sudden infant death. *Int J Legal Med.* 2012;126(2):279-284

Vennemann MM. Bajanowski T, Brinkmann B, *et al.* Sleep environment risk factors for sudden infant death syndrome: the German Sudden Infant Death Syndrome Study. *Pediatrics.* 2009;123(4):1162-1170

Weber MA, Hartley JC, Ashworth MT, Malone M, Sebire NJ. Virological investigations in sudden unexpected death in infancy. *Forensic Sci Med Pathol.* 2010;6(4):261-267

Weber MA, Klein NJ, Hartley JC, Lock PE, Malone M, Sebire NJ. Infection and sudden unexpected death in infancy: a systematic retrospective case review. *Lancet.* 2008;371(9627):1848-1853

Weber MA, Sebire NJ. Postmortem investigation of sudden unexpected death in infancy: current issues and autopsy protocol. *Diagn Pathol.* 2009:15(11):510-523

Welliver RC. Review of epidemiology and clinical risk factors for severe respiratory syncytial virus (RSV) infection. *J Pediatr.* 2003;143(S5):S112-117

Williams Al, Uren EC, Bretherton L. Respiratory viruses and sudden infant death. *Br Med J.* 1984;288(6429):1491-1493

Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis.* 2002;2(1):25-32

ADDENDA

A1 Form FSP006(b) - Questionnaire

DEPARTMENT of HEALTH
Provincial Government of the Western Cape

FPS006(b)

FORENSIC PATHOLOGY SERVICE

SUDI (Complete If A Baby Should Suddenly And Unexpectedly Die)

FPS laboratory_____ WC _____

Name of baby _____

Part 1: Scene Questionnaire and Observations

Date: Time: Name of Forensic officer:

Section A. **Who gives the history/ information in this case e.g. mother/father/granny/grandpa/other relative(give details)**	
Name:	Relationship:
Address:	Contact telephone number:
ID Number:	
Infants full name:	
Home Address:	
Age of Baby	Date of birth:
Race:	Sex:

Section B **Person(s) at/called to the scene and relationship**		
Name/relationship	Date	Time
Name/relationship	Date	Time
Name/relationship	Date	Time
Police response/name	Date	Time
Paramedic response/name	Date	Time
When was the death certified/by whom	Date	Time
If the baby was taken to hospital		
Name of hospital		

WC_____

Date of arrival: Time of arrival:		
Name of doctor seen / declared death:		
Comment: Get copies of doctors notes		
Was resuscitation done on the baby by the paramedic or the doctors at the hospital?		
Section C **Household environment:**		
Place where baby lives: house shack other –		
Number of bedrooms Is the room in which the baby is found well ventilated?		
Odour(s) present in the room the baby slept in?	Yes	No
Peeling paint in the room the baby slept in?	Yes	No
Fungal growth (mould) in the room the baby slept in?	Yes	No
Did people smoke cigarettes in the room the baby slept?	Yes	No
Are there pets in the house? If yes – type and number:	Yes	No
Did caregiver use alcohol or drugs on the night baby died?	Yes	No
Was there a heater or open fire or galley blik or other heating device in room where baby slept?	Yes	No
In what position was the baby found lying? Has the baby been moved? Were there any covers/ clothing etc over the baby's head?		
Was the baby squashed/wedged between anything (object)?	Yes	No
Was there overlaying (someone lay on top of the baby)?	Yes	No
Comments from forensic officer who attended the scene:		

WC_____

Part 2: Facility Questionnaire

Date: ***Time:*** ***Name of Forensic officer:***

Section D
Circumstances of death / details about events before death

1. When was the baby last seen alive				Date	Time
2. Who last saw the baby alive					
3. When was the baby found dead				Date	Time
4. Who found the baby dead at the scene					
5. Was the baby ill?				Yes	No
a) If yes – What was wrong and for how long?					
b) Was the baby taken to the doctor or pharmacy or clinic or traditional healer for the illness? When (date and time)?				Yes	No
c) If not, why not:					
d) Was the baby admitted to a hospital or clinic for the illness: When (date and time)?				Yes	No
e) If not, do you know why not?					
f) What medication was given (names please					
6. Where was the baby found dead	Bed	Couch	Cot	Floor	Other
Other:					
7. Did the baby sustain any injuries – eg by falling or being hit: If yes:				Yes	No
a) When did it happen?					
b) How did it happen?					
c) Where did it happen?					
d) What did the caretaker do about it?					

3

WC_____

8. a) On what was the baby placed to sleep	Bed with a pillow	Bed without a pillow	Couch with a pillow	Couch without pillow	Cot with pillow
	Cot without pillow	Floor with pillow	Floor without pillow	Other	
b) If placed on a bed/cot, what was the mattress type			Foam rubber	Inner spring	Other
c) Was the mattress covered with a blanket or sheet				Yes	No
d) What position was the baby placed when put to sleep?		Back	Stomach	Side	Other
Other -					
e) what was used to cover the baby: List items					
e) What position was the baby found dead?		Back	Stomach	Side	Other
Other –					
f) Has the baby been moved?				Yes	No
g) Face position when the baby was found dead			To the left	To the right	Face down
			Face up	Unknown	
h) Face and or chest squashed / wedged between any object(s) when the baby was found dead?			Yes	No	Unknown
If yes – details please –					
i) Was the nose and mouth of the baby covered by anything – eg blankets or anything else			Yes	No	Unknown
j) Were there other items in contact with the baby – eg pillow			Yes	No	Unknown
k) Did the baby use a Dummy (pacifier)?				Yes	No
l) Did the baby sleep in the same bed as the mother?				Yes	No
m) Did the baby sleep in her arms?				Yes	No
n) Did the baby sleep on her chest?				Yes	No
o) Did the baby sleep with the mother on a couch?				Yes	No
p) How many other people slept on the same bed as the baby at the time the baby died?					
q) Was anyone found on top of the baby while in the bed (Overlaying)?				Yes	No
r) Was the window where the baby slept on the day /night the baby died				Open	Closed
s) Did the mother or anyone in the house smoke while the baby slept on the night/day of death?					
t) When was the baby last fed?				Date	Time

4

WC_____

u) Did the mother/caregiver use alcohol before going to bed with the baby on the night/day the baby was found dead? If yes, how much?			Yes	No
v) Did the mother/caregiver use drugs before going to bed with the baby on the night/day the baby was found dead? If yes, what drugs?			Yes	No
w) Did the mother/caregiver give the baby medication on the night/day of death? If yes, name of medication:			Yes	No
Section E **About the baby**				
1. Where was the baby born?	Hospital	Clinic	Home	Other
Name of hospital/clinic/other				
2. How was the baby born?			Normal vaginal delivery	Caesarian section
3. How much did the baby weigh at birth?				
4. Was the baby		Premature	Full term	Post dates (Overdue)
5. If the baby was premature, how premature was it?				
6. Did the baby receive Kangaroo care (KMC)			Yes	No
7. Did the mother carry the baby on her back?			Yes	No
8. Was the baby		Breast fed	Bottle/formula fed	Both breast and bottle fed
If formula, name of the milk –				
9. Was boiling water used to make the bottle?			Yes	No
10. What other food was use to feed the baby?				
11. Does the mother have the clinic card?			Yes	No
If yes – keep the card for the pathologist. If no – ask the mother to bring it to the facility				
12. Was the baby sick before it died?			Yes	No
If yes	<24h	>24h	> 2 weeks	Never
a) Did the baby have a cold/ runny nose?				
b) was the baby coughing?				
c) did the baby have diarrhea (runny tummy)?				

5

WC_____

d) Was the baby unusually restless / irritable?				
e) Was the baby crying more than usual?				
f) Was there a difference /change in the appetite / feeding?				
g) Was the baby vomiting?				
h) Any fits / seizures?				
i) did the baby have a fever / showed increased sweating?				
j) Was the baby listless? (floppy)				
k) did the baby turn blue?				
13. Was the now deceased baby taken to	Hospital	clinic	doctor	Pharmacy
	Traditional healer	Other		
14. Did the baby come in contact with someone who is sick in the past two weeks?			Yes	No
If yes – who?				
15. Did the baby ever suddenly stopped breathing?		Yes	No	Unknown
16. When was the baby's last vaccination?				
18. Is the baby known to be allergic to anything?		Yes	No	Unknown
If yes, what?				
19. Did the family visit another country prior to the death of the baby?			Yes	No
If yes, give details				
20. Was the baby admitted to hospital in the past week before the death?			Yes	No
a) If yes, for how long and where:				
b) Why?				
c) Discharge date?				
d) Condition of baby after discharge:				
e) Medication after discharge from the hospital (names please)				
21. Was the baby taken to a traditional healer?			Yes	No
a)If yes, date when the baby was taken to the healer:				

WC_____

b) What was given?		
c) Ask for the medication to be given to the pathologist.		
d) Condition of the baby after going to the healer?		
21. What did the baby wear when it died? (list clothing)		
Section F **About the mother**		
1. Is the mother	Married	Single
2. Is the mother employed?	Yes	No
3. Age of the mother?		
4. What standard of schooling did she achieve?		
5. Was she on contraception before she fell pregnant?	Yes	No
6. Did she take iron and vitamin tablets during her pregnancy?	Yes	No
7. Did she receive antenatal care?	Yes	No
8. Did the mother have diabetes in pregnancy?	Yes	No
9. Did the mother have high blood pressure in pregnancy?	Yes	No
10. Did the mother gain weight adequately in pregnancy?	Yes	No
11. Was she diagnosed with any illness during the pregnancy eg. HIV?	Yes	No
12. Was the mother on any medication during the pregnancy?	Yes	No
If yes, what medication:		
13. Were there any difficulties during the delivery?	Yes	No
If yes, what?		
14. Were there any problems with the baby after the delivery?	Yes	No
If yes, what?		

WC_____

15. Was any specific instruction given about specific health care for the baby?			Yes	No
If yes, what?				
16. Was she depressed after the pregnancy?			Yes	No
17. Did she get any treatment?			Yes	No
18. How many babyren does she have?				
19. How old are they?				
20. Are they healthy?			Yes	No
21. Do any of the babyren have learning disability?			Yes	No
22. Do the living babyren have the same father as the deceased baby?			Yes	No
23. Does she look after the baby?			Yes	No
24. If not, who looks after the baby?				
25. Why is the mother unable to look after the baby?				
26. Did the mother smoke during the pregnancy?			Yes	No
If yes, how many per day?				
27. Did the mother drink during the pregnancy?			Yes	No
a) What did she drink?	Beer	Wine	Spirits	Other
b) how much did she drink?	Every day		Now and again	Weekends
1 glass	Every day		Now and again	Weekends
> 1 glass	Every day		Now and again	Weekends
A bottle of alcohol	Every day		Now and again	Weekends
> 1 bottle	Every day		Now and again	Weekends
28. Does she use drugs?			Yes	No
a) If yes, what drugs does she use?	Tik	Cocaine / Heroin	Mandrax	Other
b) How often does she use drugs?	Every day		Now and again	Weekends
29. Does the mother smoke after the pregnancy?			Yes	No
30. Does the mother know that smoking harms the unborn baby?			Yes	No
31. Does the husband/partner drinks?			Yes	No

8

WC_____

32. Does the mother drink after the pregnancy?	Yes	No
33. Do the parents of the mother drink?	Yes	No
34. Does the mother know that alcohol harms the unborn baby?	Yes	No
35. Did the mother have a previous baby that died suddenly?	Yes	No
a) If yes, how many died?		
b) At what age?		
c) Was a PM done?	Yes	No
If yes, where was it done?		
36. Did the mother have a previous stillbirth?	Yes	No

Section G
Household environment

1. Place where the baby lives	House		Shack	Other
2. Number of bedrooms?				
3. Is the room in which the baby was found well ventilated?			Yes	No
4. Odour(s) present in the room the baby slept in?			Yes	No
5. Peeling paint in the room the baby slept in?			Yes	No
6. Fungal growth (mould) in the room the baby slept in?			Yes	No
7. Are there pets in the house?			Yes	No
If yes, type and number:				
8. Was the following in the room where the baby slept to heat the room?	Electric heater	"Galley"	Fire	Other
Describe other –				
9. Number of adults in the dwelling?				
10. Number of babyren in the dwelling?				
11. Total number of people in the dwelling?				
12. Estimated monthly income?				
13. Number of smokers in the dwelling?				
14. Are there mentally retarded/ challenged people in the dwelling?			Yes	No

WC_____

COMMENTS TO PATHOLOGIST FROM THE FORENSIC OFFICER WHO ATTENDED THE SCENE AND INTERVIEWED DURING ID PROCESS:

ITEMS RETAINED AT THE SCENE OR FROM THE MOTHER DURING INTERVIEW

Date:

Signature / Thumbprint of deponent

I certify that the above statement was taken down by myself and that the deponent has acknowledged that he / she knows and understands the contents hereof.

Date _____ Time: _____

Place: _____

_____

Department of Health
Forensic Pathology Laboratory

10

A2 Raw data for statistical analysis

Don Matshazi SUDI Data 24 Nov 17.xlsx - Cleaned Up Data

Case_Nr	Gender	Age_Wks	Race	birthweight_gram	PMI_Days	season	Symptoms	bed_sharing	Housing	Smoking	Alcohol_Usage	Sleeping_Position	Position_Found	TNF-alpha	IL-18	IP-10	IL-1beta	IL-1RA	IL-4	IL-5	IL-13	IL-6	IL-8	IL-10	IFN-gamma	IL-1alpha	IL-2	IL-12p70	CRP	HIV	Tr_HADV	Tr_HBoV	Tr_HCoV_OC43	Tr_HCoV_229E_NL63	Tr_HEV	Tr_FluA	Tr_FluB	HMPV
1	1	50.4	3	2900	2	1	1		2	2	2	1	1	51.18	620.32	563.7	103.13	5182.1	286.03	0	614.98	55.59	172.91	0	0	21.41	1129.9	356.39	18.65	0	0	0	0	0	0	0	0	0
2	1	5.7	3	3060	2	1	0	1	1	2	2	1	1	0	183.84	93.03	65.95	9090	32.04	0	56.25	7.19	38.94	2.93	0	0	76.86	12.6	0.4	0	0	0	0	0	0	0	0	0	0
3	1	10.7	2	2780	5	1	0	1	1	1	2	1	1	0.91	436.53	141.64	125.63	8863.4	0	0	122.72	9.04	91.18	2.72	0.91	3.77	123.01	16.09	2.5	0	0	0	0	0	0	0	1	0	0
4	1	3.1	3	2260	5	1	0	1	2	2	2	2	2	0	52	55.05	68.37	17681	6.81	0	42.96	1.11	61.15	3.93	1.82	5.23	0	10.86	1.5	0	0	0	0	0	0	0	0	0	0
5	2	0.9	2	2900	6	1	1	1	1	1	1	2	2	7.69	536.84	114.64	151.39	23912	10.76	0	102.77	13.31	221.51	3.36	1.97	7.12	103.39	10	6.35	0	0	0	0	0	0	0	0	0	0
6	2	50.9	3	3310	6	1	1	1	1	1	1	2	1	4.09	26.26	60.39	85.5	5337.3	97.62	0	249.06	491.78	255.08	4.93	1.67	5.89	990.68	105.55	1.35	0	0	0	0	0	0	0	0	0	0
7	1	10	2	3210	6	1	1	1	1	1	2	1	1																	0	0	0	0	0	0	0	0	0	0
8	2	6.4	2	2400	7	1	0	1	2	2	2	2	2																	0	0	0	0	0	0	0	0	0	0
9	2	4	3	1590	5	1	0		2	2	2		1																	1	0	0	0	0	0	0	0	0	0
10	2	6.6	3	1610	6	1	1	1	1	2	2	2																		1	0	0	0	0	0	0	0	0	0
11	1	14	3	3800	5	1	0	1	2	2	2	2	2	0	0	17.32	146.75	10162	1.88	0	162.61	51.91	280.66	2.51	1.6	6.52	59.97	10.86	2.35		0	0	0	0	0	0	0	0	0
12	1	7.1	3	3000	7	1	0						2	16.97	657.3	58.87	90.56	3833.6	66.23	3.56	721.93	887.23	343.73	14.97	761.83	13.87	400.69	121.85	1.4	1	0	0	0	0	0	0	0	0	0
13	2	6	3	1400	5	1	0	1	2	2	2	1	1																	0	0	0	0	0	0	0	0	0	0
14	1	33.9	3	2950	6	1	0		2	2	2	2		67.57	114.53	55.83	138.28	4088.3	361.44	3.31	1167.4	28.78	227.96	3.07	4.24	19.38	1335.5	296.45	3.95	0	0	0	0	0	0	0	0	0	0
15	2	4.9	2		17	1	0																							0	0	0	0	0	0	0	0	0	0
16	2	12.3	3	1000	3	1	0	1	1	2	2	1		2.95	514.64	238.06	82.02	9521	14.3	0	109.42	32.08	37.87	3.36	2.94	6.52	82.58	17.84	0.85	0	0	0	0	0	0	0	0	0	0
17	2	6.3	2		4	1	1	1	1	2	2	3																		0	0	0	0	0	0	0	0	0	0
18	1	31.9	3	3700	4	1	0	1	1	2	2			41.35	201.96	54.28	42.77	7623.9	161.42	25.29	555.09	18.02	84.24	9.21	1.82	41.86	1490	488.81	0.2	0	0	0	0	0	0	0	0	0	0
19	2	8	2	3100	2	1	0						3	1.77	32.83	5.83	175.32	9090	17.1	1.28	62.81	9.11	99	0	10.78	1.3	229.02	0	0.35	0	0	0	0	0	0	0	0	0	0
20	1	2.7	3	1720	8	1	0						1	3.05	13.62	12.19	144.98	9090	21.77	0.41	51.12	4.37	24.18	17.11	18.47	29.98	209.44	0	1	0	0	0	0	0	0	0	0	0	0
21	1	1.6	3	3083	6	1	0							3.05	186.25	15.31	53.55	9090	0.24	2.53	32.74	15.78	212.41	9.65	15.87	2.33	99.18	0	2.1	1	0	0	0	0	0	0	0	0	0
22	2	6.4	3	3000	5	1	0	1	2	2	2	1	1	0.91	242.83	8.13	387.07	10310	22.21	0	96.13	669.33	925.54	2.79	1.67	12.83	115.77	12.6	0	1	0	0	0	0	0	0	0	0	0
23	2	11.1	2	3070	4	1	0	1	2	2	2	2	3	3.71	441.3	101.71	53.57	19080	17.59	0	122.72	6.53	12.93	2.93	1.52	1.9	98.32	30.26	0.1	1	0	0	0	0	0	0	0	0	0
24	1	4	3	3220	6	1	0	1	1	1	2	2	2	0	13.45	14.09	217.03	20659	37.26	0	76.18	2.08	40.01	3.78	2.67	1.9	82.58	10.86	0	0	0	0	0	0	0	0	0	0	0
25	1	28.4	3	2635	4	1	0	1	2	1	2	2	3	0	0	12.05	168.41	13149	6.81	0	23.03	62.55	77.36	2.23	1.37	4.53	41.07	15.21	17.35	0	0	0	0	0	0	0	0	0	0
26	1	18	3	2300	3	1	0	1	2	1	1	3	2	9.79	345.76	14.01	31.36	7018	60.82	0	209.16	2.9	42.16	3.5	1.67	3.77	357.94	72.11	0	0	0	0	0	0	0	0	0	0	0
27	1	6.9	3	3220	3	1	0	1	2	2	2	2	2	7.34	235.93	23	44.84	5947.8	73.67	0	209.16	73.8	329.7	2.93	2.25	10.89	546.44	83.2	0	0	0	0	0	0	0	0	0	0	0
28	2	8.9	2	2090	3	1	1						3	0	264.88	207.63	139.66	21998	8.85	0	49.6	888.68	215.82	2.51	1.75	9.08	16.29	10	2.85	0	0	0	0	0	0	1	0	0	0
29	2	9.4	3	3375	4	1	0	1	1	2	2	2	2																	0	0	0	0	0	0	0	0	0	0
30	1	8.9	3	1150	6	1	0	1	2	2	2	2	3	0	0	21.3	145.05	15951	6.81	0	9.75	8.37	61.29	2.79	1.82	0	50.8	9.14	0	0	0	0	0	0	0	0	0	0
31	2	10.4	3	2600	5	1	0						2	3.34	150.83	24.57	61.14	5825.7	79.85	0	96.13	13.83	33.6	3.07	1.44	0	490.13	39.24	12.7	0	0	0	0	0	0	0	0	0	0
32	2	11.1	2	1360	4	1	1	1	1	2	2	1	1																0.05	1	0	0	0	0	0	0	0	0	0
33	2	8	3	1400	6	1	0	1	1	2	2	2	2	1.99	129	18.25	277.12	9090	25.86	1.28	504.67	3370	1208.6	15.61	25.09	22.23	159.26	0	2.9	0	0	0	0	0	0	0	0	0	0
34	1	2.6	3	4900	4	1	0	1	2	2	2	2	2	0	70.41	7.68	90.17	16706	1.88	0	0	0.7	109.94	2.65	1.52	0	13.94	7.43	0	0	0	0	0	0	0	0	0	0	0
35	2	8.1	3	2550	3	1	0	1	1	2	2	1	1	0.91	224.86	82.66	51.9	16273	12.57	0	42.96	0.94	41.01	2.51	1.52	0	8.75	7.43	0.15	0	0	0	0	0	0	0	0	0	0
36	2	4.1	3	3020	4	1	0	1	2			2	2	2.17	95.75	14.88	136.9	10141	20.71	0	76.18	15.04	23.2	2.51	1.97	0	240.8	66.58	0	0	0	0	0	0	0	0	0	0	0
37	2	15.7	3	4900	7	1	0	1	2	2	2	1	1																	0	0	0	0	0	0	0	0	0	0
38	2	4.6	3	3305	6	1	0	2	1	2	2	2	3	0	660.74	35.47	32.41	14738	0	0	0	46.1	67.51	0	0	0	15.2	0		0	0	0	0	0	0	0	0	0	0
39	1	8.4	3	4670	5	1	0	1	2	2	2	1	1																	0	0	0	0	0	1	0	0	0	0
40	2	25.7	2	2900	3	1	0	1	2	1	2	2	2	0	261.35	17.02	85.08	4024.3	44.71	0	0	1240.6	856.62	0	0	0	116.06	0	0	0	0	0	0	0	0	0	0	0	0
41	2	11.6	2	3500	4	1	0	1	1	2	2	1	1																	0	0	0	0	0	0	0	0	0	0
42	1	9.9	2	1088	4	1	0						2																	0	1	0	0	0	0	0	0	0	0
43	1	17.3	3	3020	3	1	0	1	2	2	2	3	1																	0	0	0	0	0	1	0	0	0	0
44	2	3.1	2	1760	4	1	0	1	1	2	2	2																		0	0	0	0	0	0	0	0	0	0
45	2	37.7	0	1900	9	1	1	1	2	2	2	2																		0	0	0	0	0	1	0	0	0	0
46	2	14.1	2	1090	5	1	1	1	1	2	2	1																	9.9	1	0	0	0	0	0	0	0	0	0
47	1	13.6	3	2470	3	1	1		2	1	1		2																	1	0	0	0	0	0	0	0	0	0
48	2	11.3	3	3485	3	1	0	1		2	2	2	2	0	384.65	34.6	14.95	6220.6	0	0	0	0	64.51	0	0	0	315.04	0	0.065	0	0	0	0	0	0	0	0	0	0
49	1	15.4	3	3625	4	1	0	1	1	2	2	2	2																	0	0	0	0	0	0	0	0	0	0
50	2	37.3	0		5	1	0							14.23	0	14.13	283.26	7063.5	151.37	0	388.75	184.24	1475.6	2.93	4.24	25.71	1129.2	208.14	5.5	0	0	0	0	0	0	0	0	0	0
51	2	2.3	2	2375	4	1	1						3	3.71	297.17	9.01	75.09	17261	23.69	0	202.51	5.53	48.67	2.1	1.37	3.77	141.72	33.84	0	0	0	0	0	0	0	0	0	0	0
52	2	9	2	1710	2	1	0						1	9.44	410.61	275.21	114.47	8546.5	51.79	0	342.19	6.36	69.58	2.93	1.52	11.39	689.73	59.24	0	0	0	0	0	0	0	0	0	0	0

1 of 8

Don Matshazi SUDI Data 24 Nov 17.xlsx - Cleaned Up Data

Case_Nr	Gender	Age_Wks	Race	Birthweight_Gram	PMI_Days	Season	Symptoms	Bed_sharing	Housing	Smoking	Alcohol_Usage	Sleeping_Position	Position_Found	TNF-alpha	IL-18	IP-10	IL-1beta	IL-1RA	IL-4	IL-5	IL-23	IL-6	IL-8	IL-10	IFN-gamma	IL-1alpha	IL-2	IL-12p70	CRP	HIV	Tr_HADV	Tr_HBoV	Tr_HCoV_OC43	Tr_HCoV_229E_NL63	Tr_HEV	Tr_Flu A	Tr_Flu B	HMPV
53	2	27.3	3	1700	4	1	0	1	1	2	2	1	3	0	0	2.92	38.4	11744	17.59	0	29.67	5.53	84.55	2.37	2.53	0	86.59	16.09	3.6	0	0	0	0	0	0	0	0	0	0
54	1	8.9	2		8	1	0	1	1	2	2	1	1	0	0	2.92	38.4	11744	17.59	0	29.67	5.53	84.55	2.37	2.53	0	86.59	16.09	3.6	0	0	0	0	0	0	0	0	0	0
55	1	6.3	3	3680	4	2	0	1	1	2	2		2																									
56	1	11.4	3	2980	3	2	0	1	2	2	2	2	2																									
57	1	12.9	2	860	5	2	0						3																									
58	2	10.6	2	1235	6	2	1	1	2	2	2	2	3																									
59	2	3.3	3	3130	4	2	1						2	0	0	173.19	100.17	14337	0	0	0	386.45	212.01	2.1	1.37	13.06	13.94	14.34		1	0	0	0	0	0	0	0	0	0
60	2	24	3	3070	3	2	0						2	31.04	320.77	16.67	45.33	5615.4	162.14	0	824.76	0	72.56	0	0	0	1497.4	0	2.65	1	0	0	0	0	0	0	0	0	0
61	1	6.6	3	2970	5	2	0						2																									
62	2	3.4	2	2460	5	2	0		1	2	2		2	0	0	3.97	104.02	24138	23.69	0	56.25	16.26	263.09	2.65	2.46	1.9	148.55	24.91	0	0	0	0	0	0	0	0	0	0	0
63	2	39	3	1800	4	2	0						2	13.55	103.73	22.43	86.38	5478.4	73.67	0	335.53	191.55	149.9	4.07	2.94	7.12	787.13	99.94	0.1	0	0	0	0	0	0	0	0	0	0
64	1	10.4	3	2380	6	2	0						3	0	0	27.78	170.92	10066	143	0	126.04	7.03	85.47	3.93	2.18	13.76	137.12	17.84	2.2	0	0	0	0	0	0	0	0	0	0
65	1	6	3		13	2	0							0	0	43.19	141.51	11875	6.81	0	86.15	2.74	63.52	2.51	1.67	9.08	83.92	7.43	1.3	0	0	0	0	0	0	0	0	0	0
66	2	38.9	2	2840	5	2	1	1	1	1	2	2	3	10.32	328.55	51.74	39.28	3419.5	172.16	41.8	909.6	17.71	46.29	0	0	0	1115.3	0	11.4	0	0	0	0	0	0	0	0	0	0
67	2	15.9	2	3170	4	2	0	1	1	1	2	2	2	23.8	996.27	55.06	197.45	3888.3	83.11	0	686.69	1761.7	1849.1	0	0	10.68	372.71	0	3.85	0	1	0	0	0	0	0	0	0	0
68	1	17.7	2	2800	3	2	0						2	0	126.5	86.64	89.1	4092.3	0	0	0	0	33.58	0	0	0	139.77	0	10.8	0	0	0	0	0	0	1	0	0	0
69	1	2.9	3	3600	3	2	0	1	1	2	1	2	2	0	445.44	21.12	42.07	26822	0	0	0	0	25.99	0	0	0	0	0	3.55	1	0	0	0	0	0	0	0	0	0
70	1	6.6	3	2080	2	2	0						2	0	180.68	120.32	68.88	43323	0	0	0	0	68.51	30.8	0	0	0	99.62	0	3.15	0	0	0	0	0	0	0	0	0	0
71	2	12.9	3	2720	5	2	1						2																									
72	1	5.6	2	2630	5	2	1						3	0	256.36	16.24	90.43	24954	0	0	0	0	29.79	0	0	0	36.18	0	0.5	0	0	0	0	0	0	0	0	0	0
73	2	6.7	2	2560	6	2	0						3																4.4	0	0	0	0	0	0	0	0	0	0
74	1	1.4	2	1970	8	2	0	1	1	1	1	1	1	0	0	203.5	164.48	16831	143	0	119.39	1.92	32.18	3.43	1.67	5.89	50.8	15.21	0.2	0	0	0	0	0	1	0	0	0	0
75	1	14.7	3	1410	3	2	1		1	2	2	1																	3.75	0	0	0	0	0	0	0	0	0	0
76	2	50.1	3	3355	4	2	0		2	2	2			61.58	244.66	41.81	75.09	3546.6	234.89	15.01	1470.9	9	103.85	0	0	15.43	1679.6	1397.6	0	0	1	0	0	0	0	1	0	0	0
77	1	18	3	730	3	2	1		1	2	2			0	0	100.09	355.04	46649	0	0	0	90.91	730.16	0	0	4.85	31.58	0	2.15	0	0	0	0	0	0	1	0	0	0
78	2	1.1	3	2430	3	2	0	1	2	2	2	2	2	11.85	257.66	21.38	63.12	11575	92.78	0.83	66.22	6.86	48.59	3.22	1.21	17.29	0	121.5	2.55	1	0	0	0	0	0	0	0	0	0
79	1		0	1530	2	2	1	1	1	1	2	1	1	0	358.7	193.69	50.24	26625	0	0	0	28.39	74.7	0	0	0	36.18	0	4.45	0	0	0	0	0	0	0	0	0	0
80	1	26	3	2650	2	2	1	1	1	2	2	2		38.16	144.19	243.09	117.75	3633.1	205.33	0	4199.4	153.78	169.68	0	0	3.94	1055.8	82.32	10.9	0	1	0	0	0	0	0	0	0	0
81	2	19.7	2		6	2	1	1	1	1	2	2	2																	0	1	0	0	0	0	0	0	0	0
82	1	8.9	2	2600	3	2	1	1	1	1	2	1	2	0	305.59	36.34	0	3785.9	60.62	0	136.76	0	33.93	0	0	0	273.65	0	1.35	0	0	0	0	0	0	0	0	0	0
83	1	46.7	3	2800	4	2	1	1	1	2	2	1		35.1	1957.3	2074.9	354.45	5603.4	125.25	0	1082.4	2823.4	1735.5	0	0	28.06	434.08	0	6	0	0	0	0	0	0	0	0	0	0
84	2	19.6	2	1080	12	2	0	1	1	1	2	1	1	0	0	0	122.42	11127	0	0	0	41.75	56.47	0	0	0	78.74	0	14.5	0	0	0	0	0	0	0	0	0	0
85	2	39.9	2	2640	5	2	0	1	1	1	2		2	273.32	315.04	238.9	9750.9	10970	287.67	19.82	2865.9	19983	4156.8	15.99	0	266.52	2335.1	834.06	15.85	0	0	0	0	0	0	0	0	0	0
86	2	10.7	2	2580	3	2	1		1	2	2		1	0	230.82	46.89	17.35	6618.1	0	0	0	0	45.33	0	0	0	39.11	0	1.75	0	0	0	0	0	0	0	0	0	0
87	1	34.9	3	2900	3	2	1	1	1	2	2	2	2																	0	0	0	0	0	0	0	0	0	0
88	2	6	3	1530	2	2	0	1	1	2	2	1	1	0	285.81	19.22	44.97	27327	15.1	0	0	0	79.49	0	0	0	103.37	0	0	1	0	0	0	0	0	0	0	0	0
89	2	3.4	2	1510	2	2	0		2	1	2	2	1																	0	0	0	0	0	0	0	0	0	0
90	1	4.7	2	3200	2	2	1	1	1	2	2	2	2																	0	0	0	0	0	0	0	0	0	0
91	2	6.7	2	2780	4	2	0	1	1	1	2	2	2	0	141.55	18.27	1.93	6670.7	41.13	0	0	0	27.63	0	0	0	256.35	0	8.35	1	0	0	0	0	0	0	0	0	0
92	2	1.4	2		3	2	1	1	1	1	1	1	2	0	20.12	3.36	53.31	27342	58.06	0	0	0	79.49	0	0	0	59.83	0	0	0	0	0	0	0	0	0	0	0	0
93	2	5.4	3	1290	3	2	0	1	2	2	2	1	1	0	369.26	24.62	30.75	32388	0	0	0	0	27.15	0	0	0	41.93	0	0	1	0	0	0	0	0	0	0	0	0
94	1	5.9	2	3495	2	2	1	1	1	1	2	2	3	0	232.5	20.8	13.64	18275	55.38	0	115.08	61.14	256.73	0	0	0	217.47	0	0.1	0	0	0	0	0	0	0	0	0	0
95	2	2.3	2	3100	4	2	1	1	1	1	2	2	2	0	0	24.26	86.57	24831	15.1	0	0	62.71	118.42	0	0	0	0	0	2.5	0	0	0	0	0	0	0	0	0	0
96	2	17.7	3	720	3	2	0	1	1	2	2	3	3	0	0	96.99	115.61	9377.6	0	0	0	23.58	105.59	0	0	11.57	29.98	0	3.5	0	0	0	0	0	0	0	0	0	0
97	2	9.4	2	2430	6	2	1						2																	0	0	0	0	0	0	0	0	0	0
98	2	52	3	2930	4	2	1	1	1	2	2	2	3	37.73	1239.2	176.21	12892	28407	59.35	0	0	22733	5411.6	0	0	136.32	482.55	0	10.3	0	1	0	0	0	0	0	0	0	0
99	2	4.7	3	1640	2	2	0						1																	1	0	0	0	0	0	0	0	0	0
100	1	6.6	3	1380	2	2	0	1	2	2	2	1	1																	0	0	0	0	0	0	1	0	0	0
101	1	11.1	3	3420	1	2	1	1	1	2	2	1		23.3	57.27	53	40.96	3938.5	185	0	2380.3	0	70.56	0	0	0	927.73	0	0	0	0	0	0	0	0	0	0	0
102	1	7	3		4	2	0							7.99	27.68	49.67	2346.9	9090	19.63	4.75	131.07	3370	1880	2.17	3.28	13.42	94.18	31.43	10.1		0	0	0	0	0	0	0	0	0
103	1	1.6	3	2620	3	2	0	1	2	2	2	2	2	3.01	42.84	5.44	70.57	9090	11.63	5.46	101.95	21.45	291.75	0.48	2.03	5.66	0	28.43	0.2		0	0	0	0	0	0	0	0
104	2	5.6	2	1480	2	2	1	1	1	1	2	2		2.62	141.05	21.58	26.1	9090	136.75	5.11	67.95	6.49	58.29	0	7.77	4.48	0	29.93	0	1	0	0	0	0	0	0	0	0

Don Matshazi SUDI Data 24 Nov 17.xlsx - Cleaned Up Data

Case_Nr	Gender	Age_Wks	Race	Birthweight_Gram	PMI_Days	Season	Symptoms	Bed_sharing	Housing	Smoking	Alcohol_Usage	Sleeping_Position	Position_Found	TNF-alpha	IL-18	IP-10	IL-1beta	IL-1RA	IL-4	IL-5	IL-23	IL-6	IL-8	IL-10	IFN-gamma	IL-1alpha	IL-2	IL-12p70	CRP	HIV	Tr_HADV	Tr_HBoV	Tr_HCoV_OC43	Tr_HCoV_229E_NL63	Tr_HEV	Tr_Flu A	Tr_Flu B	HMPV
105	2		3	2040	4	2	0	1	2	2	2	2	2	0	10.52	116.66	50.24	11265	0	0	453.51	0	9.52	0	0	6.34	10.45	0	0		0	0	0	0	0	0	0	0	0
106	1	2.7	2	2510	3	2	0	1	1	1	2	1	1	0	414.51	27.49	0	24690	0	0	0	0	34.11	0	0	0	0	0.1		0	0	0	0	0	0	0	0	0
107	1	6	3	1370	3	2	0	1	1	2	2	3	3	0	46.98	49.18	78.45	28700	0	0	0	0	26.19	0	0	0	33.15	0	2.5		0	0	0	0	0	0	0	0	0
108	1	8	3	2820	5	2	0	1	2	1	2	2	3	0	0	8.42	72.93	4763	139.55	0	809	0	63.16	0	0	0	560.73	0	0	0	0	0	0	0	0	0	0	0	0
109	1	8.1	3	2050	5	2	0	1	2	2	2	2	2	0	197.81	66.28	96.09	22234	0	0	0	0	11.52	0	0	44.75	23.14	0	0.3	1	0	0	0	0	0	0	0	0	0
110	2	6	3		7	2	0	1	1	2	1	2	1	0	0	22.65	128.35	10377	51.09	0	258	0	46.45	0	0	2.81	39.11	0	0	1	0	0	0	0	0	0	0	0	0
111	1	6	3	2125	7	2	0	1	2	1	1	2	2	0	0	39.63	112.82	17831	14.3	0	29.67	2.41	39.65	28.35	0	13.31	4.16	0	0		0	0	0	0	0	0	0	0	0
112	1	2	3		5	2	0	1	2	2	1	1	1	2.63	170.8	7.28	60.52	9090	11.32	1.28	85.28	9.29	28.35	0	413.13	1.3	71.47	0	0		0	0	0	0	0	0	0	0	0
113	1	14.1	2	2460	3	1	1						1	5.7	578.38	36.4	67.46	4534	42.1	0.41	188.2	5.02	42.89	0	413.13	1.3	71.47	0	0		0	0	0	0	0	0	0	0	0
114	1	18.7	3	2805	3	1	0						2	22.83	247.9	7.41	66.47	4990.5	124.57	10.4	762.71	11.57	64.11	0	2631.6	15.88	797	170.68	0.3		0	0	0	0	0	0	0	0	0
115	1	10.9	3	3050	2	1	0	1	2	2	2	3	3																		0	0	0	0	0	0	0	0	0
116	2	19.7	3	3000	3	1	1	1	2	2	2	1	2																		0	0	0	0	0	0	0	0	0
117	1	12.6	2	1750	7	1	0	1	1	1	2	1	1	3.88	211.98	88.52	54.55	9090	46.21	1.28	391.6	143.62	419.26	0	105.46	6.63	130.84	0	0.4		0	0	0	0	0	0	0	0	0
118	1	4.9	2	2010	6	1	0						1	5.5	150.79	25.19	111.48	9090	36.19	1.28	283.17	10.15	87.64	0	22.42	4.16	152.62	0	0		0	0	0	0	0	0	0	0	0
119	1	0.7	3	2480	5	1	0	1				2	2	176.74	1095.8	69.28	4080	9090	64.09	0.41	1057.4	3370	1880	39.06	27.77	250.4	183.78	0	17.1		0	0	0	0	0	0	0	0	0
120	1	6.9	3	2240	4	1	0	1	2	2	2	3	3	7.87	1121	49.89	44.03	9090	21.77	1.94	207.65	27.23	23.08	0	26.43	6.63	0	0	9.2		0	0	0	0	0	0	0	0	0
121	2	4.7	2	2155	4	1	1						2																		0	0	0	0	0	0	0	0	0
122	1	16.4	3		16	1	0							13.99	1054.7	112.99	342.72	6712	68.34	1.94	452.95	715.08	1880	0	205.89	42.25	236.18	54.19	15.5		0	0	0	0	0	0	0	0	0
123	2	57.3	3		3	1	0	1	2	2	2	2		98.2	217.43	177.58	198.58	9090	258.32	5.35	1049.6	117.22	145.15	0	997.99	59.9	652.91	374.87	13.9		1	0	0	0	0	0	0	0	0
124	2	15.7	3	3290	2	1	0	2	1	2	2	2	1	3.46	1000.6	128.91	13.27	9090	64.09	1.28	301.59	42.46	24.83	0	45.69	3.27	0	0	11.5		0	0	0	0	0	0	0	0	0
125	1	3.9	3	2780	4	1	0	1	2	1	1	3	3	4.29	490.18	130.43	30.1	9090	25.86	0.41	301.59	374.83	99.55	0	30.48	3.27	0	0	11.5		0	0	0	0	0	0	0	0	0
126	1	22.9	2		9	1	1	1	1	1	1	3	3	8.85	11.38	49.63	101.28	9090	39.22	1.28	500.39	11.95	49.72	0	17.17	5.01	343.94	16.8	3.8		0	0	0	0	0	0	0	0	0
127	1		0			1	0							3.46	252.37	31.21	48.05	9090	21.77	1.28	148.37	2.48	21.63	0	14.59	12.5	0	0	0.2		0	0	0	0	0	0	0	0	0
128	2	0	3	3410	3	1	0		2	2	2	1		36.02	889.58	192.75	61.51	3537.3	170.04	3.06	899.51	8.37	78.71	0	775.89	6.63	563.48	199.92	6.1		0	0	0	0	0	1	0	0	0
129	2	3.9	3	2420	5	1	1							2.63	19.89	16.33	393.32	9090	29.56	0	236.36	3370	362.41	0	13.31	17.19	109.46	0	13.3		0	0	0	0	0	0	0	0	0
130	1	4.6	2	2410	2	1	0	1	1	2	2	1	1	3.05	275.18	26.72	26.52	9090	21.77	1.94	0	4.55	6.27	0	14.59	2.33	0	0	1		0	0	0	0	0	0	0	0	0
131	2	23.7	2	3660	4	1	1	1	1	1	2	1		18.08	2255.5	58.58	45.54	9090	29.56	1.28	297	489.2	290.43	0	108.46	5.01	12.93	0	16.1		0	0	0	0	0	0	0	0	0
132	2	22	3	2770	5	1	1							197.23	347.2	72.87	502.22	9090	456.1	40.91	7293.3	663.07	834.79	31.22	1750	73.17	6830.6	1640.2	2.7		0	0	0	0	0	0	0	0	0
133	1	49.9	3	3710	5	1	0	1	2	2	2	2		124	224.93	111.18	727.26	9090	386.53	17.4	3864.8	483.22	525.42	0	1750	64.38	3742.8	1119	8.2		1	0	0	0	0	0	0	0	0
134	2		0			1	0							4.29	260.65	39.78	204.26	9090	515.31	1.94	245.82	10.33	77.02	0	15.87	19.74	135.93	0	0		0	0	0	0	0	0	0	0	0
135	1	12.6	3	2800	5	1	0	1	1	2	1	1	1	2.63	102.93	75.49	61.02	9090	19.53	1.28	273.9	3.16	19.02	0	26.43	13.19	62.99	0	0		0	0	0	0	0	0	0	0	0
136	1	1.6	2	2995	4	1	0	1	1	1	2			12.86	196.38	13.6	303.71	9090	25.86	1.94	173.42	43.82	41.85	0	19.78	7.41	0	6		0	0	0	0	0	0	0	0	0
137	2		0			1	0						2	27.51	1454.9	118.85	548	9090	90.26	1.94	444.26	403.53	1880	0	45.69	28.23	201.89	0	14.6		0	0	0	0	0	0	0	0	0
138	1	4.7	2	2060	5	1	1						2	9.04	728.05	26.78	92.51	9090	43.5	1.28	337.98	22.01	35	0	10.78	5.83	60.03	0	0.3		0	0	0	0	0	0	0	0	0
139	1	9.3	2	2500	4	1	1						2	7.48	155.44	26.39	147.62	4621.4	32.98	2.53	431.17	466.35	448.21	0	139.61	8.92	206.94	0	0.6		0	0	0	0	0	0	0	0	0
140	2	38.1	3	2155	4	1	0	1	2	1	1	2	2	72.91	86.22	80.26	2535.6	9090	204.2	13.18	1771.2	3370	1880	0	2062.4	187.65	1085.1	706.4	16.5		0	0	0	0	0	0	0	0	0
141	1	10.1	3	2400	3	1	0	1	1	1	2	1		3.46	764.77	89.73	32.9	9090	168.8	0.41	188.2	2.6	45.74	0	5.89	1.3	96.82	0	0		0	0	0	0	0	0	0	0	0
142	1	25	3	1180	3	1	1		1	1	1			7.48	850.82	311.86	216.31	9090	31.3	0.41	264.59	6.32	15.28	8.68	112.22	3.27	80.78	0	1.6		1	1	0	0	0	0	0	0	0
143	1		0			1	0							10.2	715.34	82.8	16.16	9090	19.53	1.28	255.23	43.14	47.69	0	12.04	0	43.95	0	0		0	0	0	0	0	0	0	0	0
144	1	3.1	3	2165	5	1	0		2	2	2																				0	0	0	0	0	0	0	0	0
145	2	4	2	2500	3	1	0						2	21.38	218.79	6.49	33.67	9090	96.32	4.49	783	1.27	48.14	0	1220.5	8.92	494.59	281.22	0		0	0	0	0	0	0	0	0	0
146	1	0.7	2	2540	2	1	0						2	9.04	325.4	61.1	81.74	9090	43.5	1.28	0	8.62	47.69	0	14.59	3.27	159.26	0	0.5		0	0	0	0	0	0	0	0	0
147	2	12.3	3	900	2	1	0	1	1	2	2	3	3	5.5	995.23	346.58	7.94	1328.6	29.56	1.94	513.22	3.28	29.53	0	123.58	1.3	18.86	0	0		0	0	0	0	0	0	0	0	0
148	2		2		19	1	0						3	2.63	10.83	38.02	336.23	9090	25.86	1.94	127.88	2.37	79.86	0	14.59	19.11	315.61	0	1.3		0	0	0	0	0	0	0	0	0
149	1	7.1	3		3	1	0	1	1	1	2	1	1	3.88	163.78	23.5	36.46	3582.7	25.86	1.28	197.96	3.56	11.72	0	361.55	0	259.43	0	7.7		0	0	0	0	0	0	0	0	0
150	1	5.7	2	2190	3	1	1							6.3	415.36	64.12	18.51	9090	25.86	3.06	337.98	21.62	29.68	0	10.78	1.3	23.92	0	1		0	0	0	0	0	0	0	0	0
151	1	6.1	3	1280	2	1	0						1	3.46	493.08	30.87	40	9090	29.56	1.28	217.28	7.52	60.99	0	27.77	8.18	0	0	3.9		0	0	0	0	0	0	0	0	0
152	2	16.9	3	1295	4	1	1	1	2	2	2	2	2	13.99	301.95	17.33	26.77	2779.6	69.37	1.94	324.4	1.49	27.18	0	546.8	5.83	267.74	59.73	0.2		0	0	0	1	0	0	0	0	0
153	2		0			1	0							10.2	584.98	308.61	779.34	9090	101.33	1.94	337.98	3.74	49.5	0	21.1	18.47	92.03	0	0.8		0	0	0	0	0	0	0	0	0
154	1	4.3	0	3300	5	1	1	1	2	2	2	1	3																		0	0	0	0	0	0	0	0	0
155	1	9	3	985	3	1	1						2	3.46	900.24	41.11	188.62	9090	34.61	0.41	168.45	106.54	176.73	0	14.59	10.39	0	7.1		0	0	0	0	0	0	0	0	0
156	1	5	3	2330	3	1	0	1	2	2	1	2	2																		0	0	0	0	0	0	0	0	0

Case_Nr	Tr_HPIV1	Tr_HPIV2	Tr_HPIV3	Tr_HPIV4	Tr_HRV_ABC	Tr_HRSV_A	Tr_HRSV_B	Lung_HAdV	Lung_HBoV	Lung_HCoV_OC43	Lung_HCoV_229E_NL63	Lung_HEV	Lung_Flu_A	Lung_Flu_B	Lung_HMPV	Lung_HPIV1	Lung_HPIV2	Lung_HPIV3	Lung_HPIV4	Lung_HRV_ABC	Lung_HRSV_A	Lung_HRSV_B	Nr_PCR_Viruses	Liver_SVC_HAdV	Liver_SVC_CMV	Lung_SVC_HAdV	Lung_SVC_CMV	Micro_Left_Lung	Micro_Right_Lung	Micro_Heart	Interstitial_Pneumonitis	Edema	Congestion	Broncho-pneumonia	Focal_Collapse	Pigment	Alveolar_Debris	Cause_Of_Death
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	2
3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1	0	0	1	1	0	0	0	1	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	3	1	2	0	2	2	2	2
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	2
6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0								2
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	0	2
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	1	3	1	0	1
9	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	1	3	0	1	0	0	2
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	0	1	0	0	0	1
11	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	3
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2
14	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	1	1	0								2
15	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	1	1	1	0	1	1	0	2	2	0	1
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	2	0	1	0	1	0	0	1
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	1	0	0	2
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1								2
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	2
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1	0	1
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								3
22	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2	0	1	0	0	1
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	2	0	0	2
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	3	0	0	0	0	3	0	1
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	2	0	0	1
26	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	2	2	0	0	2	0	2
27	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	3	0	3	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	1	0	2	0	0	0	0	1
29	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	0	0	0	0	2
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1	2	0	0	0	2	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0		0	0	0	0	0	0	1
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	0	0	0	0	0	0	2
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	3	0	0	0	0	1
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	2	0	0	1
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								2
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	3	2
38	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	2
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	1	2	0	1	0	0	2
40	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	2	3	0	1
41	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1								0
43	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	2	3	0	0	3	0	1
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	3	2	3	0	0	1	3	1
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
46	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0								1
47	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	0	2
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	2	0	0	3
50	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	3	0	0	3
51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	1	0	1	0	0	2
52	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	1	0	2	0	2	0	0	2

Don Matshazi SUDI Data 24 Nov 17.xlsx - Cleaned Up Data

Case_Nr	Tr_HPIV1	Tr_HPIV2	Tr_HPIV3	Tr_HPIV4	Tr_HRV_ABC	Tr_HRSV_A	Tr_HRSV_B	lung_HAdV	lung_HBoV	lung_HCoV_OC43	lung_HCoV_229E_NL63	lung_HEV	lung_Flu_A	lung_Flu_B	lung_HMPV	lung_HPIV1	lung_HPIV2	lung_HPIV3	lung_HPIV4	lung_HRV_ABC	lung_HRSV_A	lung_HRSV_B	Nr_PCR_Viruses	Liver_SVC_HAdV	Liver_SVC_CMV	Lung_SVC_HAdV	Lung_SVC_CMV	Micro_Left_Lung	Micro_Right_Lung	Micro_Heart	Interstitial_Pneumonitis	Edema	Congestion	Broncho-pneumonia	Focal_Collapse	Pigment	Alveolar_Debris	Cause_Of_Death
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	2
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	2	2
55	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	1	1	0	1	2	1
56	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	1	2	3	2
57	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	2	0	3	0	0	0	3	2
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	1
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	0	0	0	0	1
63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	1	0	0	0	2	2
66	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	1	2	0	2
68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	2	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	1
71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
73	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	2	2	0	0	0	2	2
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	1	0	0	0	2
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2
77	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1
78	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	2
79	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	3	0	1	0	0	0	0	2
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	0	1	1	0	0	1	1	0	0	0	0	1
81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	1	0	0	0	0	1
83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	2
84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	0	0	2
87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	3	2	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	2
91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	3	0	0	2
95	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	1	1	0	2	1	3	2
96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	2	2	1	0	2	0	0	1
97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	4	0	0	0	1	0	0	0	1	0	0	0	1	0	0	2
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
101	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2
102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2
103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	3
104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	1	1	0	0	0	0	2

Case_Nr	TT_HPIV1	TT_HPIV2	TT_HPIV3	TT_HPIV4	TT_HRV_ABC	TT_HRSV_A	TT_HRSV_B	Lung_HAdV	Lung_HBoV	Lung_HCoV_OC43	Lung_HCoV_229E_NL63	Lung_HEV	Lung_Flu_A	Lung_Flu_B	Lung_HMPV	Lung_HPIV1	Lung_HPIV2	Lung_HPIV3	Lung_HPIV4	Lung_HRV_ABC	Lung_HRSV_A	Lung_HRSV_B	Nr_PCR_Viruses	Liver_SVC_HADV	Liver_SVC_CMV	Lung_SVC_HADV	Lung_SVC_CMV	Micro_Left_Lung	Micro_Right_Lung	Micro_Heart	Interstitial_Pneumonitis	Edema	Congestion	Bronchio-pneumonia	Focal_Collapse	PEgment	AlVeolar_Debris	Cause_Of_Death
105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2
106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	2
107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	2
108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	1	0	0	1	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
110	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
111	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2
113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	2	1	0	0	2
114	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
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116	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0	2
117	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	4	0	0	0	2	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
119	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	3	0	0	0	0
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121	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
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124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1
126	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2
127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
128	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
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131	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2
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138	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
139	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1
140	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1
141	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	2
142	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	1	0	0	0	1	1	0	0	0	3
143	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	1	0	1
144	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	2
145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2
146	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
147	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	3	0	0	0	1	0	2
148	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	2
149	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0
150	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
151	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
152	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	2	0	2	0	2
153	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	2	0	1	0	2
154	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	2	0	0	1	0	0	2
156	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2

Case_Nr	Tr_HPIV1	Tr_HPIV2	Tr_HPIV3	Tr_HPIV4	Tr_HRV_ABC	Tr_HRSV_A	Tr_HRSV_B	Lung_HAdV	Lung_HBoV	Lung_HCoV_OC43	Lung_HCoV_229E_NL63	Lung_HEV	Lung_Flu_A	Lung_Flu_B	Lung_HMPV	Lung_HPIV1	Lung_HPIV2	Lung_HPIV3	Lung_HPIV4	Lung_HRV_ABC	Lung_HRSV_A	Lung_HRSV_B	Nr_PCR_Viruses	Liver_SVC_HAdV	Liver_SVC_CMV	Lung_SVC_HAdV	Lung_SVC_CMV	Micro_Left_Lung	Micro_Right_Lung	Micro_Heart	Interstitial_Pneumonitis	Edema	Congestion	Broncho-pneumonia	Focal_Collapse	Pigment	Alveolar_Debris	Cause_Of_Death
157	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	2
158	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	0	1	0	1	0	0	1
159	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								2
161	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	3	0	0	0	1	0	2	2
162	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	2
163	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	1	1	0	0	1	1	0	1	0	0	1
164	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2
165	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	2
166	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2
167	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	3	2	0	0	0	3	1
168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2
169	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	1	0	0	2
170	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	0	0	0	1	1	0	0	0	0	0	0	0	0	3
171	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	3	0	3	0	0	0	1	1
172	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	3	1	0	1	0	0	2
173	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	1	0	1	0	0	2
174	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
175	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	2	1	0	1	0	0	2
176	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	3
177	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	2	1	0	1	0	2	1
178	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1
179	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1
180	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	1	0	1	1	0	1	0	1	0	0	1
181	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	1	1	1	1	0	1	0	1	0	1	2
182	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
183	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	1	0	1	1	1	1
n	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	183	140	144	144	144	144	144	144	183
Avg																							1																
SD																							0.99																
Me																							1																
Min																							0																
Max																							4																
0	182	182	174	182	126	182	176	178	183	182	183	182	179	183	183	183	183	176	183	160	181	180	81	183	179	183	170	123	122	152	83	84	47	138	69	127	114	23
1	1	1	9	1	57	1	7	5	0	1	0	1	4	0	0	0	0	7	0	23	2	3	60	0	4	0	13	60	61	31	19	35	66	6	58	8	11	50
2																							29								22	20	22	0	13	5	12	98
3																							9								16	5	9	0	4	4	7	12
4																							4															
High																																						