# Measuring Epidemiology and Sero-Correlates of Protection Against Severe Respiratory Syncytial Virus (RSV) Associated Hospitalisation in HIV Exposed and Unexposed South African Children

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, South Africa, 2020

**Declaration**

I, Yasmeen M. Agosti, declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature:

Date: 9 Dec 2020

Place: Johannesburg, South Africa

**Dedication page:**

I dedicate this thesis to:

My husband and children who have so patiently allowed me to pursue this degree and

My parents who fostered a love for lifelong learning

**Publications arising from this thesis:**

Jallow S, **Agosti Y**, Kgagudi P, Vandecar M, Cutland CL, Simões EAF, et al. Impaired Transplacental Transfer of Respiratory Syncytial Virus-neutralizing Antibodies in Human Immunodeficiency Virus-infected Versus Uninfected Pregnant Women. Clin Infect Dis. 2019;69(1):151-4.

**Abstract:**

**Introduction:** RSV is a leading cause of respiratory related hospitalisations in children less than five years globally. The majority of the morbidity and mortality of RSV disease occurs in lower and middle income countries. Decades of epidemiological work have demonstrated that the risk for RSV hospitalisation is highly concentrated in the first six months of life, when infants' immune systems are immature and the protective effects of maternally derived antibody are waning. The South African paediatric population is comprised of a significant percentage of HIV exposed uninfected (HEU) infants who reportedly have higher risk for infectious morbidity and mortality.

**Methods:** This thesis utilized a prospective, inpatient paediatric surveillance program; and a prospective birth cohort to describe RSV hospitalisations, maternally derived immunity and explore for a sero-correlate of protection against RSV LRTI hospitalisation among HIV unexposed and HEU infants.

**Results:** RSV hospitalisation incidence was 21.4 per 1000 live births among Sowetan infants. A peak of disease occurred in the first month of life and constituted a large percentage (53%) of all-cause LRTI hospitalisation during the RSV epidemic period. The majority of hospitalisations (69%) occurred among infants in their first six months of life. RSV hospitalisation incidence of HIV unexposed and exposed infants did not differ significantly (21 vs 20 per 1000 live births, respectively). Most RSV hospitalisation cases were discharged home, with a low cases fatality risk (0.002). The majority of RSV-associated hospitalisations received antibiotics (69%). Infants without and with HIV exposure acquired maternally derived neutralizing RSV antibody via transplacental transfer (HUU 0.82 vs HEU 0.67, $p_{adj}$ 0.1222). The cord to maternal blood ratio (CMR) of RSV neutralizing antibody was 0.74. The CMR was significantly associated with maternal hypergammaglobulinemia. Cord blood titres demonstrated an inverse relationship between maternally derived neutralising RSV antibody and risk of RSV hospitalisation in infants up to six months of age. While a definitive threshold of protection was not identified, it was observed that for every unit rise in $\log_2$ titre, there was a 43% reduction in odds for hospitalisation

**Conclusion:** RSV hospitalisation among Sowetan infants represents a significant burden of disease that is highly concentrated within the first six months of life. Maternally derived neutralising RSV antibody is present in infants at the time of birth, albeit at levels lower than

what has been described in other parts of the world. Maternally derived neutralising RSV antibody is associated with protection against disease but a definitive correlate of protection has not yet been identified.

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Doctoral work is not a solo act. There a many individuals who played an important role in this journey:

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And last by certainly not least – the mothers and children who participated in this study. My hope is that these data will contribute towards a larger effort to find a solution to serious respiratory infection.

**Table of Contents**

**List of abbreviations:**

| | |
|---|---|
| ALRI | acute lower respiratory tract infection |
| ARI | acute respiratory infection |
| ART | anti-retroviral therapy |
| BMDH | Bheki Mlangeni District Hospital |
| CFR | case fatality risk |
| CHBAH | Chris Hani Baragwanath Academic Hospital |
| CI | confidence interval |
| CLWH | children living with HIV |
| CMR | cord maternal ratio |
| CRF | case report form |
| Ct | cycle threshold |
| ED 50 / ED 80 | end dose 50 / end dose 80 |
| EIA | enzyme immunoassay |
| ELISA | enzyme-linked immunosorbent assay |
| FcRN | neonatal Fc receptor |
| GBS | Group B Streptococcal |
| GMT | geometric mean titre |
| HAART | highly active anti-retroviral therapy |
| hCFR | In-hospital case fatality ratios |
| HEU | HIV exposed uninfected |
| HIV | human immunodeficiency virus |
| HUU | HIV unexposed uninfected |
| ICD | international classification of diseases |
| ICU | intensive care unit |
| IFAT | immunofluorescence antibody test |
| IgG | immunoglobulin G |
| ILI | influenza like illness |
| IMCI | integrated management of childhood illness |
| IQR | interquartile range |
| LMIC | lower and middle income countries |
| LRTI | lower respiratory tract infection |
| MEM | moving epidemic method |
| MOU | midwife operated units |
| MUAC | mid upper arm circumference |
| NHLS | National Health Laboratory System |
| NICD | National Institute of Communicable Diseases |
| NPS | nasopharyngeal swab |
| NPV | negative predictive value |
| OR | odds ratio |
| PCV13 | pneumococcal conjugate vaccine 13 |
| PCV7 | pneumococcal conjugate vaccine 7 |
| PMTCT | prevention of mother to child transmission |
| PPV | positive predictive value |
| RMPRU | Respiratory and Meningeal Pathogens Research Unit |
| RR | risk ratio |
| RSV | respiratory syncytial virus |
| RT-PCR | reverse transcription polymerase chain reaction |

| | |
|---|---|
| SARI | severe acute respiratory illness |
| SRI | serious respiratory infection |
| U.K. | United Kingdom |
| UR | uncertainty range |
| U.S.A. / U.S. | United States of America / United States |
| UTM | universal transport medium |
| WHO | World Health Organization |

## Preface

I came to South Africa shortly after completing my paediatric residency in Philadelphia (U.S.A.). My original plan was to do a fellowship in paediatric infectious disease, and I had secured a position at the hospital where I had trained. Shortly thereafter, my husband was offered a unique opportunity to work with the Red Cross based out of Johannesburg. As a family of two adults and two young children, we had just endured a gruelling set of years while I worked my way through medical school and residency. After careful consideration, placing fellowship on hold in order to come to South Africa seemed a kinder option. I naively assumed that I could find work as a general paediatrician at one of the city hospitals, but it quickly became apparent that this would not be feasible during my several year stay in South Africa. So I began to explore opportunities in research thinking that it would be nice to gain experience in a particular infectious disease before ultimately returning the States to do fellowship. I met Professor Madhi and the Research and Meningeal Pathogens Research Unit (RMPRU) shortly thereafter. I left the RMPRU intrigued by the possibility of approaching my interest in infectious diseases from a completely different angle – through doctoral research.

I began the PhD program in Clinical Microbiology and Infectious Diseases five months after arriving in South Africa. Professor Madhi had asked me to select a pathogen of interest, as long as it was already incorporated into one the RMPRU's existing surveillance programs. As one could easily surmise by the title of this thesis, I selected Respiratory Syncytial Virus (RSV). I did so because RSV was an infection that defined my training as a general paediatrician who came of age after the introduction of *Haemophilus influenzae* type b and pneumococcal vaccination. My fall and winter seasons were almost entirely dedicated to trying to help infants (and their parents) manage RSV bronchiolitis with few options at hand. Supportive care measures seemed frustratingly meagre while standing by the bedside of young patients struggling to breath. When I started to prepare for the PhD proposal by diving into the epidemiological literature, I was stunned by the size of the burden estimated on the global scale. I was further taken aback by its estimated mortality which largely occurs in lower- and middle-income countries. How could we not yet have found a solution to such an important and large unmet medical need? This realization imbued my thesis work with a deep sense of purpose. My underlying goal through this thesis work would be to contribute to the

knowledge base in hopes that someday these data could help to inform a vaccine or anti-viral medication in development.

I returned to the U.S. after several years to resume clinical work (all that RSV to take care of!) and was unexpectedly offered the opportunity to work on a maternal RSV vaccine program in industry. I made the difficult decision to leave my clinical career believing that perhaps I could contribute my experiences towards a concrete solution. For the past three years, I have been the medical director of the RSV program at Pfizer. I have worked on this thesis in parallel to my job and believe that my academic and professional endeavours have come together in a very meaningful way. My contribution to a developing RSV vaccine program has been deeply informed by both my experiences as a clinician and as a doctoral student. The rationale of a vaccine program relies intensely upon a solid understanding of the disease and its epidemiological/sero-epidemiological backdrop. At the same time, this thesis has also been deeply informed by my experiences working on an RSV vaccine program. I am very fortunate to work alongside a multi-disciplinary team with basic science and epidemiological expertise. I never had trouble finding an interested ear to discuss my latest thoughts and questions as I worked through each successive thesis objective. Many of the papers cited in this thesis are papers that I have also used in my job to help inform clinical trial case definitions, the design of surveillance programs, and policy considerations.

Working a full time and raising a family have extended the time it took to reach the end of this thesis – six years in total. However, despite the snail's pace, I am truly grateful that I was able to do it in such a meaningful way and with the patient and valuable guidance of my thesis advisors.

# Chapter 1 Introduction

## 1.1 The global burden of respiratory syncytial virus infections in children under five

Respiratory syncytial virus (RSV) is a leading cause of respiratory related associated morbidity in children under the age of five years around the world and yet there is no effective treatment or vaccine. The only form of prevention, a passively administered monoclonal antibody, is generally unaffordable and reserved for high risk infants. These three facts depict an important unmet medical need and backdrop for understanding how local data can help to inform future decisions once interventions do become available.

The most recent systematic review of the global RSV burden in children published by Shi et al. in 2017 highlights the key measures, uncertainties and gaps in our understanding (1). The review provides global, regional and age stratified estimates with uncertainty ranges* (UR) for illness episodes, hospitalisations, in-hospital mortality and total mortality based upon 250 publications and 76 unpublished datasets from high, middle and low income settings (LMIC). The annual incidence of under-five RSV associated acute lower respiratory tract infections (ALRI) is estimated to be 33.1 million episodes (UR 21.6–50.3), which amounts to approximately 28% of all ALRI episodes. Furthermore, there are approximately 3.2 million hospitalisations (UR 2.7–3.8) and 59,600 in-hospital deaths attributed to RSV, which are thought to be conservative estimates. When community deaths are taken into consideration, RSV mortality is estimated to be 118,200 (UR 94,000 and 149,000) deaths per year (1). The analysis further highlights that the burden is concentrated among the youngest infants with approximately 1.4 million or 45% of the total hospitalisations and 27,300 RSV associated deaths occurring in those less than six months of age. Of note, the vast majority of these hospitalisations and deaths are believed to occur in developing countries, although robust sources of data are lacking. The size, volume and heterogeneity of the data utilized by Shi et al. has helped to depict the burden in children in broad brushstrokes. But more granular data organized by chronological age, risk groups and from LMIC settings are needed.

* Uncertainty ranges are used for Bayesian analysis where probabilities are assigned to represent an uncertainty about the data. This statistical approach stands in contrast to the frequentist framework where confidence intervals and p-values are used to represent uncertainty about the data.

## 1.2 Viewing RSV burden by chronological age

The first of only two studies to provide RSV associated hospitalisation incidence by month of age was conducted by Hall et al. in the United States (2). This prospective, population-based surveillance of lab confirmed RSV hospitalisations was conducted over five years at three sites in different states. The data demonstrated a distribution of disease over the course of the first 24 months of life, within which the highest incidence of disease occurred among one-month old infants at a rate of 25.9 per 1000 children; **Figure 1.0** illustrates Hall incidence rates by month of age. The next highest incidence of approximately 14 per 1000 children occurred just before and after the one-month old age group, thereby concentrating the largest proportion of disease to the first three months of life. Thereafter, the disease rates gradually reduced with each successive month of life from 8.9 per 1000 at four months of age to 2.9 per 1000 children at 11 months of age. Although there was evidence of year to year variation in the incidence by as much as four-fold, the distribution of disease remained unchanged. Notably, the study by Hall et all included preterm infants who have a higher risk of RSV hospitalisation, which could have impacted this distribution.

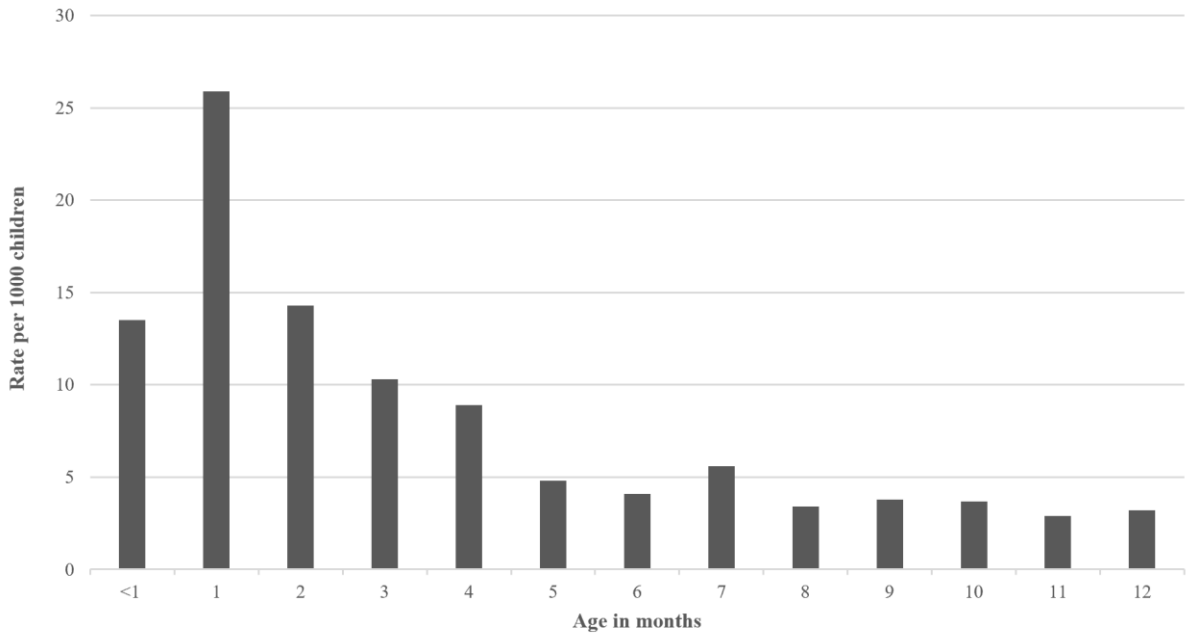


**Figure 1.0** Incidence rates of RSV hospitalisation in U.S. infants by month of age [2]

A 2017 meta-analysis by Parikh et al. resolves this issue (3). Disease distribution across the first year of life was identical for both term and preterm infants with the peak incidence of disease occurring between 1 and <2 months of age; **Figure 1.1**. Note the proportions sum to

100%. The Parikh data demonstrated that approximately 50% of RSV hospitalisations occurred in the first three months of life and between 75-80% in the first six months (3).

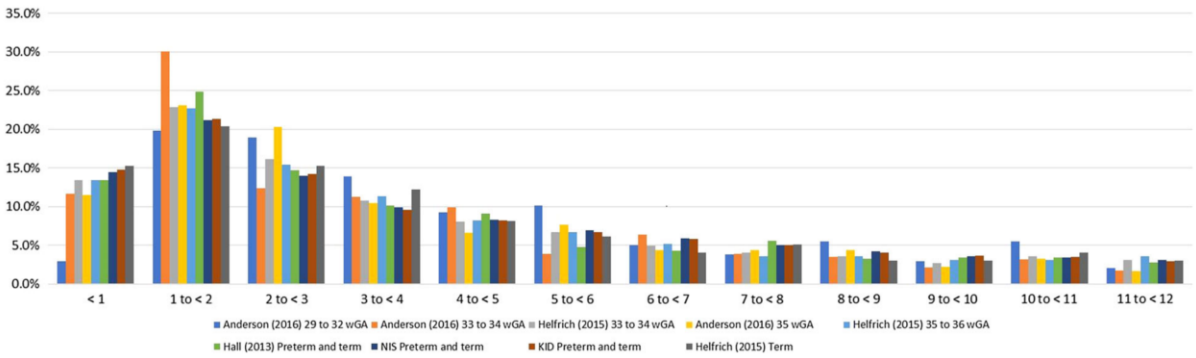


**Figure 1.1** Proportion of U.S. infant RSV hospitalisations by month of age, across various data sources [3]

### 1.3 Age related RSV burden and seasonality

The distribution of age related RSV disease must also be interpreted in the context of seasonality. In the study by Hall et al., 73% of hospitalisations occurred during the peak winter months of the northern hemisphere including December, January and February (2); however, the distribution of age related hospitalisation incidence in relation to RSV seasonality was not analysed. Such an analysis was undertaken in a Danish study, in which lab confirmed RSV hospitalisations in children less than five years were identified through a national patient registry over the course of five seasons (4). The majority of hospitalisations (60%) occurred in children under six months of age, with the highest annual incidence at one month of age; **Figure 1.2**. The age specific hospitalisation incidence rates changed by month of the RSV season in Denmark. Peak seasonal incidence occurred in tandem across all age strata. The highest rates of hospitalized disease occurred in one-month old infants. This visualization of the data reaffirms that severe RSV illness is concentrated in the youngest infants and maintains a seasonal pattern and peak across all age groups.

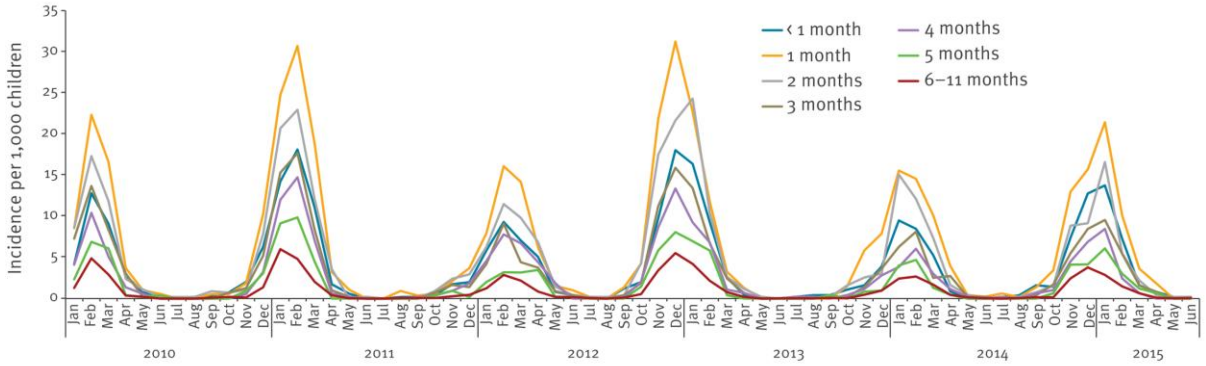


**Figure 1.2** Monthly RSV hospitalisation incidence in Denmark by month of age and month of season [4]

### 1.4 RSV associated mortality

Ninety-nine percent of RSV associated mortality is estimated to occur in developing countries, although there is limited data which informs these estimates (5). Shi et al. estimates for paediatric mortality were: 59,600 in-hospital deaths for all children less than five years, 27,300 in-hospital deaths in infants less than six months and 118,200 total RSV deaths for children less than five years. The uncertainty ranges provided alongside these point estimates are wide and reflect the variability and incomplete nature of the data. These modelled death counts can be further contextualized by looking at case fatality rates.

A systematic literature review and modelling study conducted by Shi et al. in 2017 provides the most current assessment of global case fatality rates (1). This analysis provided CFR and number of in-hospital deaths in children with RSV-ALRI reported by World Bank regions and different age strata. For infants 0–5 months of age from upper middle income countries the CFR was 1.8% (1.2–2.6) with an estimated 7,200 (4,200–12,300) in-hospital deaths. For infants 6–11 months of age from upper middle income countries the CFR was 2.4% (1.1–5.4) with an estimated 8,000 (2,800–22,100). These are likely to be under-estimates due to lack of virologic testing.

A retrospective, descriptive case-series analysis conducted by Scheltema et al., in 2017, provided more detailed characterization of the clinical and socioeconomic factors of deaths associated with RSV in children (5). Analysing a total of 358 fatal cases children from 23 countries, 33% were from LMICs and 58% were less than six months of age. A history of prematurity was identified in 8% of the children, and a further 28% had underlying

comorbidities. The vast majority of these deaths (77%) occurred during an identifiable RSV season. While 20% of children who died were admitted to the intensive care unit (ICU) during the course of their illness, only 24% of the total deaths had access to ICU care.

A number of studies on RSV morbidity have been undertaken in South Africa highlighting different angles on RSV associated mortality within the country including in-hospital and out-of-hospital illness, and associations with underlying co-morbidities such as HIV infection. In 2003, Madhi et al. prospectively evaluated and compared RSV associated illness among high risk children and otherwise healthy children less than 24 months. The study was conducted in four provinces over two distinct time periods and enrolled 258 children (6). Overall, 53 cases of RSV associated LRTI were identified, with a higher prevalence among high risk children (30%) compared to non-high-risk children (22%). Although 'high risk" children were more likely to be hospitalized, including admission to the ICU, there were no deaths reported in this study. Venter et al. 2011 tested for fourteen viruses among children less than five years of age who either required outpatient or hospital-based care for an acute respiratory tract infection (7). The study was located in the Pretoria area and enrolled patients who sought care at one of three public hospitals. A significant portion of this population was HIV seropositive (32%). RSV was the second most common infection (second to rhinovirus) and identified in 30.1% of cases. In this study 10% (6/60) of children with RSV identified as a single organism died, and 2.9% (2/70) of children in whom RSV was identified with another respiratory virus. The study did not analyse the relationship between RSV mortality and HIV status. Moyes et al. in 2013 examined this relationship more closely among children less than five years with and without HIV infection hospitalized for acute LRTI or neonatal sepsis (8). The study was conducted at four surveillance sites and identified RSV infection in 27% (1157/4293) of tested children. HIV-infection status was known for 69% of the children with 6% (49/802) of children diagnosed as being HIV infected; only half were receiving anti-retroviral therapy (ART). Overall, the CFR among RSV cases was 1% (9/1153) during hospitalisation. The CFR was 31.1-fold higher (95% CI: 5.4–179.8, p <0.001) in children living with HIV (CLWH; 8% 4/49) than those without HIV (<1% 3/751). The CFR was 1% (2/353) in RSV associated hospitalisations among those with an unknown HIV status. The analysis was limited by a lack of data on additional parameters such as HIV-disease staging, ART and viral loads.

Finally, Cohen et al. 2018 modelled RSV associated in-hospital and out-of-hospital mortality from 2009 to 2013 for different age groups including children (9). These estimates were

classified into four ICD coded themes including: “all-causes”, “all-respiratory”, “all-circulatory”, and “pneumonia/influenza”. The estimated mean annual rate of RSV associated deaths in children varied widely based upon the type of ICD coding that used. The highest RSV associated mortality rates occurred in children less than one year of age. Among children less than one year of age, the “all-causes” rate was highest at an estimated 143.4 per 100,000 person years (95% CI: 0.0-195.4). The “all-respiratory” and “pneumonia/influenza” rates were closest to each other with 42.0 per 100,000 (95% CI: 0.0–63.9) and 30.4 per 100,000 (95% CI: 0.0–42.1) person years respectively. The lowest rate 1.5 per 100,000 person years (95% CI: 0.0–6.8) occurred when utilizing “all-circulatory” codes. RSV was estimated to be the cause of 22% - 33% deaths outside of the hospital in children less than five years.

As was mentioned earlier, much of our understanding of RSV associated mortality is derived from hospital-based studies. Global attention has now turned to understanding community-based mortality with several collaborative efforts underway including the CHAMPS and RSV GOLD projects (10, 11). These studies require painstaking efforts to find and evaluate individual cases of RSV associated deaths to better understand which children are most likely to die of an RSV infection before reaching care. While the data related to these studies are not yet publicly available there are smaller but no less important efforts underway. A recent case control study conducted in Argentina examined the rates of “at-home” mortality associated with acute respiratory infections in children less than five years from a low income background (12). The “at-home” rate of RSV associated mortality was reported as 0.26 per 100 live births. The risk of dying from any acute respiratory infection (ARI) at home was associated with several important factors including household crowding (OR 3.73), an adolescent mother (OR 4.89), no running water (OR 4.39), incomplete vaccination status (OR 3.39), (prior) admission to the ICU (OR 7.17) and no emergency medical attention (OR 72.32).

### 1.5 RSV risk factors

The vast majority of RSV hospitalisation occurs among healthy, full term infants with no identifiable risk factors other than being young or born in close proximity to the season (13) . Numerous medical, anatomical and sociodemographic risk factors have been examined in an effort to better understand why some develop severe RSV disease while others do not, leading to inconsistent results (14). Physicians who care for infants and children can easily

provide a list of high-risk conditions including prematurity, chronic lung conditions, congenital heart disease, and immune deficiency (14). The anatomical and physiologic basis for the increased risk of RSV can be explained for each of these categories. Small airway diameters, immature immune systems, lack of protective maternal antibody, abnormal respiratory and circulatory systems prevent these infants and children from responding appropriately to an infectious insult. A more complex task is to identify and disentangle a long list of non-medical factors which could contribute to disease risk, many of which are present in combination with each other as well as with the medical factors listed previously. Variations in study design, of participant bias and risk factor definitions complicate the task further.

A 2003 literature review by Simoes et al. examined twelve risk factors including race, sex, age at time of illness, absence of breast feeding, poor nutrition, birth during the RSV season, low socioeconomic status, level of maternal education, crowding, number of siblings in the bedroom, day care exposure, and household tobacco smoke exposure (14). The quality and strength of evidence were assessed based upon the type of study design. Simoes et al. concluded that male sex, young age, birth in the first half of the season, day care attendance and crowding/siblings were all independently associated with risk for severe RSV disease. There was conflicting evidence with regard to whether lack of breast feeding or passive exposure to tobacco smoke were risk factors.

A more recent systematic review and meta-analysis of 20 studies by Shi et al. published in 2015 identified eight risk factors associated with RSV disease (15). These included prematurity, low birth weight, male sex, presence of siblings, maternal smoking, lack of breastfeeding, history of atopy, and household crowding. In a smaller set of studies, other factors such as low parental education, passive smoking, crèche attendance, indoor air pollution, HIV, multiple births, malnutrition, higher altitude, previous illness and lack of plumbed water in the household were also found to be associated with RSV LRTI. In utero HIV exposure in the absence of HIV acquisition was not specifically reviewed for, but it is discussed in a later section of this introduction.

### 1.6 The seasonality of RSV disease – globally and in South Africa

In temperate climates, RSV occurs as a seasonal epidemic with a distinct onset, peak and offset that generally lasts between five and six months (16). Some countries can have shorter seasons lasting 3 or 4 months. There are 1-3 week shifts in season onset/offset which can be

observed from year to year, country to country or even within a single country if it is large enough to contain different climactic zones (16, 17). Occasionally these shifts in time can be up to four weeks. A recent global overview of seasonality across 27 countries revealed that some countries demonstrate two extremely different seasonal patterns which alternate. For example, Germany alternates yearly between an early fall or late spring season onset, both of which maintain the same duration. Finland has either an early/large epidemic or late/small epidemic that also alternates every two years. Tropical zones have more prolonged seasons lasting for as long as ten months.

Each year, RSV is believed to begin in the southern hemisphere and progress northward through a wide variety of climactic zones (16). It is important to understand how local climate impacts RSV seasonality, as was done in a 2013 descriptive analysis of seven global CDC associated surveillance sites including South Africa (18). In this study, monthly measures of temperature, humidity and precipitation were assessed alongside lab confirmed RSV disease activity. The seven countries represent tropical and subtropical climate zones as described in detail by the Köppen classification system. In this study, South Africa contributed between 3 to 6 years of data (2006 to 2012) from Soweto and Pretoria, each of which fell into different Köppen climate classifications. More specifically, both Soweto and Pretoria are described as having warm temperate climates with desert precipitation. Their classifications diverge when describing the general temperature, with Soweto designated as possessing warm summer temperatures while Pretoria, located approximately 90 km north of Soweto, possessing hot summer temperatures. In this analysis however, the South African data from the two locations were conglomerated into a "moderate hot" grouping. The descriptive results go on to show that there is a distinctive, single RSV season which could begin as early as January. This seasonal onset occurs within one month of the highest recorded rainfall and humidity. The peak occurs during the autumn period. Temperatures could fall below freezing. The authors highlight that this descriptive analysis could not account for other variables which might also impact RSV seasonality. Factors such as in-door crowding behaviours, environmental pollution, as well as host and viral characteristics could all conceivably influence transmission dynamics, season timing and severity.

### 1.7 South African surveillance and seasonality

South African seasonality has been defined by the National Institute of Communicable Diseases (NICD) which issues weekly influenza and RSV surveillance reports. These reports

summarize data from three distinct syndromic surveillance programs which fall under the purview of the Center for Respiratory Diseases and Meningitis including influenza like illness (ILI), Viral Watch and the National Syndromic Surveillance for Pneumonia (19). There are also data collected from private hospital consultations. Each of these surveillance programs began at different time points and differs somewhat in their parameters. The Viral Watch program established in 1984 is the oldest of the surveillance programs and was developed to primarily monitor outpatient influenza activity (during the anticipated influenza season) but also tests for other respiratory pathogens (20). The Viral Watch program is extensive and represents disease activity across all nine provinces through the involvement of 205 volunteer public and private clinics. Viral Watch inclusion criteria are informed by the typical clinical characteristics found in influenza patients including the presence of fever ≥38 C˚. The National Syndromic Surveillance for Pneumonia, also known as the Severe Acute Respiratory Illness (SARI) program is a younger hospital-based surveillance program founded in 2009 in response to the influenza pandemic. The program is less geographically extensive but compensates by providing detailed epidemiological data in addition to laboratory testing for multiple respiratory pathogens throughout the year (20). The SARI case definition is more flexible in order to capture a larger range of lower respiratory tract infections and does not require the presence of fever in children less than 5 years of age. All three of these surveillance programs collect and test respiratory samples using multiplex reverse transcription polymerase chain reaction (RT-PCR) from patients of all ages. Samples can be collected as oropharyngeal or nasopharyngeal swabs, nasopharyngeal aspirates, or induced or expectorated sputum.

In years past, and in some current surveillance systems found in other parts of the world including the United States (U.S.), RSV detection has traditionally been nested into influenza surveillance. The implications of this approach mean that if the surveillance is timed to the influenza season then testing, will fail to capture a significant portion of RSV burden which occurred prior to its start. Furthermore, ILI surveillance inclusion criteria require the presence of fever which does not always occur with RSV infection. Therefore, an RSV case which occurs within the influenza season timeframe without fever will be excluded from viral testing, further eclipsing the ability to capture the true burden of disease. Finally, it is important to highlight that the data come from specific surveillance sites and only reflect the burden of RSV disease among those who have access to and seek care.

In 2015, the combined NICD surveillance systems identified the RSV season onset as beginning in week 9 (approximately March 1st), peaking in week 17 (approximately April 26th) and ending in week 29 (approximately July 19th). The season timeframe for 2016 was very similar with the onset beginning in week 8, peaking in week 18 and ending week 29. These data confirm the observation noted earlier that seasons can shift between 1 to 3 weeks from year to year.

A summary of previous studies on RSV epidemiology in South Africa will be covered in detail in Objective 1.

**1.8 RSV disease presentation in infants**

RSV presentation in infants occurs along a wide clinical spectrum of respiratory disease including upper respiratory tract infection to LRTI manifested as bronchiolitis, pneumonia or occasionally croup (21). Infection with RSV begins with signs of an upper respiratory tract infection including rhinorrhoea, cough and congestion. Fever can be present but is typically low grade. Over the course of several days, approximately 40% of infants will progress to signs of lower respiratory tract infection including wheeze, crackles, and crepitations (21). Stridor occurs in cases of RSV associated croup but is otherwise not characteristic. Moderate to severe forms of LRTI are accompanied by varying levels of respiratory distress as indicated by the presence of tachypnoea, intercostal and subcostal retractions, nasal flaring, grunting, hypoxemia. Some infants can present with only apnoea before more overt signs of respiratory infection develop, but the exact frequency is difficult to determine as the literature reports a range between 1% and 24% (22). Several non-respiratory signs are also recognized as indicators of severe disease including inability to feed, irritability or lethargy. The overall course of illness lasts a total median time of 2 weeks and it is possible for caregivers to bring their infant or child to medical attention several times (23). Infants who come to medical attention typically do so between 2 to 4 days of illness when parents observe that symptoms have peaked (22). Even after an acute care episode, there may be additional outpatient, emergency room visits or hospitalisation readmissions depending upon how the disease evolves.

An important quality of RSV LRTI is the dynamic clinical presentation that occurs both between infants and within the same infant over a very short period of time (23, 24). Signs such as wheeze, crackles, respiratory rate and retractions can change as quickly as "minute to minute" depending upon whether the infant is awake or asleep, or if secretions have

accumulated or a recent coughing fit has successfully dislodged mucus from a section of small airways (23). Therefore, a proper assessment of disease severity requires serial examinations in order to account for fluctuations in physical signs (23, 24).

### 1.9 The limitations of a bedside diagnosis

The clinical picture described above is a fairly generic presentation of LRTI that could be caused by any number of viruses. Traditionally, wheeze has been considered a trademark characteristic of RSV associated LRTI. However, there is significant overlap of clinical signs and symptoms between different types viral infections, making a bedside diagnosis challenging. A systematic literature review and meta-analysis conducted by Ma et al. identified both cough (OR =2.9 95% CI:  1.8–4.6) and dyspnoea (OR 2.3 95% CI: 1.7–3.0) were most strongly associated with RSV infection (25). Wheezing was also significantly associated with RSV (OR =2.2 95% CI: 1.7–2.8) but also for rhinovirus, human metapneumovirus and human bocavirus. The same authors conducted a prospective cohort to further evaluate the association of clinical signs and symptoms with RSV versus other viral infections. Their findings revealed several additional clinical features which were strongly associated with RSV disease, including anorexia/difficulty feeding (OR =1.6 95% CI: 1.4–1.8) and apnoea (OR 1.5 95% CI: 1.1–2.1). Other clinical features such as fever, headache, myalgia, seizures, rash and sore throat were negatively associated with RSV. The study concluded that clinical characteristics were not sufficiently discriminatory to diagnosis specific viral aetiologies of respiratory disease. Rather, laboratory testing was required for confirmation.

### 1.10 Clinical management of RSV

The lack of a definitive cure for acute illness limits clinical management to supportive care interventions. Clinical management guidelines for RSV are enfolded within bronchiolitis guidelines. Florin et al. in their 2017 review of viral bronchiolitis provide a comprehensive comparison of national guidelines on bronchiolitis management in eight countries including the United Kingdom (UK), the United States (US), Canada, Scotland, Italy, Spain, Australia, and France. Overall, the guidelines are consistent in terms of recommending supportive care measures including supplemental oxygenation and hydration when deemed clinically appropriate (23). None of the guidelines recommend chest radiography, antibiotic administration, bronchodilators, epinephrine (adrenaline), corticosteroids, antiviral therapy with ribavirin, chest physiotherapy. There are some notable differences. Viral testing is only

recommended by a few countries to facilitate patient cohorting on the wards to prevent contagion. The oxygen saturation threshold for oxygen supplementation varies from 90% to 95% with some countries adding more specific instructions about relationship to infant age, measurements taken during feeds or accompanying signs of respiratory distress. Two countries (France and Spain) recommended the use of nebulized hypertonic saline while others did not recommend or did not mention the intervention. There were variable recommendations on the use of nasal suctioning with some countries not recommending the intervention at all while others specified the depth and frequency.

South Africa has published a diagnostic flow for acute viral bronchiolitis as well as guidance for the intensive care management for severe cases (26, 27). Supportive care measures are recommended in a similar fashion to those reviewed in the Florin article. The oxygen saturation threshold for supplementation is differentiated by sea level (<92%) and inland (<90%). Chest radiographs are not recommended unles:  the diagnosis is uncertain, there is high fever ≥38 ˚C, suspicion of pleural effusion or pneumothorax, severe disease or failure to improve. The diagnostic flow acknowledges that there is little value to viral testing as it does not alter clinical management but may be useful for surveillance, patient cohorting, and etiologic diagnosis in infants less than one month or clinical presentations of apnoea. The South African diagnostic flow is helpful to clinicians by providing a list of differential diagnoses to consider when evaluating a suspected case of bronchiolitis. Clinicians should consider an alternative diagnosis which could alter the clinical management of the patient including: bronchopneumonia, pertussis, foreign body, myocarditis, recurrent wheeze, cystic fibrosis, cardiac disease, HIV/tuberculosis.  In these cases, chest radiographs, antibiotics, bronchodilators, glucocorticoids, or other interventions might be needed.

Overall, a review of these variations in care practices reflects the need for further data on the disease pathophysiology, the clinical presentation that results, identifying reliable measures of disease severity and the effectiveness of the existing interventions available.

### 1.11 Laboratory testing

The lack of definitive treatment for RSV infection limits the role and utility of diagnostic testing. Several forms of testing are available including rapid diagnostics or culture techniques. Serological testing of acute and convalescent antibody is also an option. There are several types of rapid testing that have been employed over the years such as enzyme immunoassays, direct immunofluorescence, and RT-PCR. There are also several options for

respiratory sampling including nasopharyngeal swabs, oropharyngeal swabs, and nasopharyngeal aspirates. Laboratory diagnostics are not commonly employed outside the hospital setting. In some countries, such as the U.S., rapid testing is performed in an infant or child who is being admitted for LRTI in part to determine the aetiology but mostly for the purposes of appropriate cohorting of patients on the wards to prevent nosocomial spread.

### 1.12 The RSV Virion

RSV was first identified in a chimpanzee who demonstrated signs of coryza or acute inflammation of the nasal passages during an outbreak of respiratory disease amongst its colony (28). The same virus was soon thereafter discovered in several infants presenting with similar respiratory symptoms which initiated further investigations and characterization of its virological nature (29). RSV is a member of the *Pneumovirinae* subfamily, which links up to a larger viral order *mononegavirales* which is characterized by the possession of a linear, negative-sense, single stranded RSV molecule (30). RSV virions can take on round or filamentous forms ,both of which are capable of infection (30). The RSV genome, which codes for a total of eleven proteins, sits within a viral envelope formed from the infected host cell plasma membrane. Each of these proteins serves a distinct role in support of the infectious cycle, including attachment and fusion to the host cell, transcription and replication, encapsidation and interference with the host immune system (30).

RSV has two antigenic types or subgroups identified as A and B. These antigenic groups were originally identified through testing with polyclonal animal serum and monoclonal antibodies (31). The largest degree of genetic variability between the two subtypes is found within the G protein. Testing of prototype strains demonstrated a 47% difference in nucleotide sequence between subgroups (31). Genetic variability also exists within each subtype ,which allows for identification of multiple genotypes, also known as clades or lineages. Over time new genotypes evolve, while others disappear, and to date a total of 11 RSV A and 23 RSV B genotypes have been identified (32). In spite of variable genotype nomenclature, the analysis of nucleotide sequences has enabled global comparisons of circulating strains. RSV A and B subtypes and multiple genotypes for each subtype co-circulate within the same season. Attempts have been made in the past to discern meaningful patterns in subtype distributions as they relate to age, disease severity, season year and month (32, 33). Some studies suggest that subtype A predominates and causes more severe disease (33-36), although some studies report subtype B to be associated with more severe disease

(37, 38), no such association has been observed by others (39-41). Cyclical patterns of subtype dominance over a period of years have been observed suggesting the population immunity to specific subtypes waxes and wanes (32, 34). Ultimately, it is difficult to draw conclusions as such heterogeneous data are likely to be the result of different study designs, age groups, geographic settings and season years.

Two proteins located on the viral surface, F and G, are recognized as the main targets of the host immune system, resulting in the production of neutralizing antibodies (42). Protein G facilitates initial attachment of the virus to the host respiratory epithelial cells, after which the F protein facilitates fusion between the virus and host cell. The fusion of multiple host cells into an aggregated form is called a syncytia. It is this characteristic appearance that has been aptly captured in the name of the disease *respiratory syncytial virus.*

The F protein can take a pre or post fusion form, each with important immunological ramifications. Viral fusion and thereby infection can only occur while the F protein is in its pre-fusion state (43). The pre-fusion state is highly unstable and easily triggered into a post-fusion form where it can no longer fuse with the host cell. The most highly neutralizing antibodies are directed at epitopes identified in the pre-fusion F form, which directly inhibits fusion with host cells and interrupt the infectious process. Recent advances in the crystallization of the F protein have revealed that specific epitope sites located on pre-fusion form of F can produce a spectrum of neutralizing potency (43).

The presence of neutralizing antibody against RSV has been associated with protection against disease (13, 44). Young RSV naïve infants may acquire neutralizing antibody against RSV from their mothers in utero (45, 46). While antibodies are believed to be correlates of protection against severe disease, a specific threshold of protection has not been identified. Furthermore, several studies have failed to detect a protective relationship suggesting there are other aspects of the immune response to RSV which may play an important role (47) (48). The relationship between antibodies and protection against disease will be summarized in greater detail in Chapter 3 “Exploring for an RSV sero-correlate of protection”.

### 1.13 Justification and objectives:

This introduction has set the context by establishing that RSV is a seasonal infection known to be a leading cause of respiratory related disease and hospitalisation in children less than five years of age around the world. Studies from multiple countries have demonstrated that

the bulk of RSV associated hospitalisations occur within the first six months of life and peak between 1 and 2 months of age. The majority of disease is believed to occur in LMIC settings, but additional data are needed. South African efforts across the past several decades have already established important epidemiological patterns through a variety of prospective and retrospective studies. However, there is little granular data on the burden of RSV hospitalisations stratified by month of age in the first year of life. Furthermore, the characterization of the clinical presentation, hospital course and outcomes also lack these age specific details. Certain groups of children are known to be at higher risk for RSV including children living with HIV infection. The success of the prevention-of-mother-to-child-transmission (PMTCT) program in South Africa has resulted in a growing population of HIV exposed uninfected infants (HEU) who appear to be at higher risk of infectious and non-infectious morbidity and mortality for reasons that remain to be fully elucidated. The risk for severe RSV disease in HEU infants also requires further inquiry with equal granularity.

This dissertation aims to provide additional epidemiological and sero-epidemiological data to further our understanding of the burden of severe RSV disease among HIV exposed and unexposed infants in their first year of life. There are three primary objectives through which this will be achieved:

1. To measure the incidence of RSV associated hospitalisations in HIV exposed and HIV unexposed infants under 12 months of age in Soweto, South Africa
2. To compare the level of RSV neutralizing antibody in women living with HIV and those without HIV at the time of delivery and the ratio of transplacental transfer of antibody to their newborns.
3. To explore for a sero-correlate of protection against RSV associated hospitalisation during the first six months of life through an RSV neutralization assay.

## 1.14 Materials and Methods

### 1.14.1 Study Population

This epidemiological and sero-epidemiological study occurred at the Chris Hani Baragwanath Academic Hospital (CHBAH) located in Soweto, South Africa. The bulk of the study population, infants with RSV and their mothers who brought them to CHBAH for care, comes from the historically, socially and economically complex setting of Soweto. The name

Soweto is a geographic acronym meant to describe its locale as a township within the southwest corner of Johannesburg. In the present day, Soweto is now identified as region D, one of seven administrative regions belonging to the city at large (49). There are approximately 1.4 million inhabitants living in this urban setting who occupy a total area of 75 square kilometres. This densely populated area resulted from the conglomeration of multiple, race-based townships developed through the course of apartheid history. While apartheid is now officially in the past, the social and economic consequence of forcing black South Africans to live on the periphery of city life has resulted in an urban sprawl that cannot be easily understood through a simple study of a map. The history of Soweto has shaped the lives of its residents on multiple levels including access to the basic necessities of life such as water, sanitation, electricity, transportation, employment and healthcare.

It is extremely challenging to find recent or reliably sourced documentation on specific Sowetan demographics, and much of the detail provided hereafter is based on documents for the City of Johannesburg (CoJ) which combines data from all of its seven regions. It is more than likely that the unique qualities of Sowetan demographics are lost or diluted when combined with those of other city regions; however, these reports can provide an oblique view into the reality of the infants included within study population. Wherever possible, Sowetan specific details will be added to ensure that the closest approximation of this reality is drawn. The main source of information came from three documents: the 2008 Johannesburg Poverty and Livelihoods Study (JPLS) (49), the 2018 CoJ Draft Integrated Development Plan (50), and the 2018 Wong et al. publication on 2012 healthcare seeking behaviours in Soweto and Klerksdorp (51).

The majority of the Sowetan community is black-African with many of the South African ethnic groups and languages spoken. This cultural diversity reflects both past politico-historical “forced resettlement” and current economically motivated migration patterns in and out of the area (52). A publicly available planning document for the CoJ estimates that 25% of city population comes from outside Gauteng and 10% outside of South Africa with over 3000 people moving into the city each month (50). The 2008 JPLS study conducted by the University of Johannesburg looked at eight of the most deprived wards located in the CoJ and selected Ward 15 Phiri/Senoane as the representative community from region D (49). According to the JPLS, 89% of Phiri/Senoane inhabitants are South African and 11% are non-South African with the majority of those inhabitants speaking IsiZulu (61%) followed by

Sesotho (17%) and IsiXhosa (6%). Living conditions and pressures are immensely challenging for most Sowetans. Families are heavily reliant upon their own household members and sometimes other households. Housing may be small, poorly constructed, densely packed and far away from jobs located in other parts of the city. Employment does not preclude the need for government grants and the loss of a family member in their prime earning years can place additional stressors on household needs. Illness and food insecurity only add to the pressures to maintain the needs of the family. All of these factors play an important role in the health outcomes of a community.

The Respiratory and Meningeal Pathogens Research Unit (RMPRU) has made progress to electronically capture the total live births and stillbirths occurring at Chris Hani Baragwanath Academic Hospital (CHBAH), Bheki Malangeni District Hospital (BMDH) and surrounding midwife operated units (MOUs). This bottom-up assessment of the population denominator attempts to ensure that incidence and risk of an RSV hospitalisation can be calculated from the time point of birth itself. It is important to note that this denominator is vulnerable to migration in and out of Soweto.

### 1.14.2 Paediatric Care within Soweto

CHBAH provides free, secondary and tertiary level care to the surrounding public. The hospital is affiliated with the University of the Witwatersrand and provides both acute inpatient and non-acute outpatient care to all age groups. There are approximately 3200 total beds of which 408 are paediatric beds. The Department of Paediatrics provides emergency care twenty-four hours per day with the ability to admit to short stay, general medical surgical, intensive care and several subspecialty wards. Each year, there are an average of 4200 admissions for infants less than 12 months of age. Patients who do not require tertiary level care can be transferred to Selby Hospital. In 2014, the 300 bed BMDH was opened to relieve a small portion of the patient volume at CHBAH by providing level one care to the Northern and Western Sowetan communities.

### 1.14.3 Obstetrical care within Soweto

CHBAH provides tertiary obstetrical care to the surrounding area with a total of 18,282 and 18,763 live births recorded for the years 2015 and 2016 (unpublished RMPRU data). An additional seven surrounding maternal obstetrical units (MOUs) provide additional delivery services to low risk pregnancies leading to a total of 29,264 and 28,302 annual live Sowetan births.

### 1.14.4 Health care seeking behaviour within Soweto

Patterns of health care seeking behaviours are an important variable to consider when analysing epidemiologic data. The proportion of people within a community that seeks care at the site or sites of surveillance will directly impact both the numerator and the denominator. Both CHBAH and BMDH should be viewed as parts of a larger formal network of healthcare including 16 local clinics and 14 provincial clinics. Sowetan residents can also access alternative forms of healthcare including : pharmacies, private clinics, traditional and religious healers. A 2012 survey of healthcare utilization among households in Soweto and Klerksdorp assessed such patterns in relationship to different infectious disease syndromes including: pneumonia, ILI, chronic febrile respiratory illness, diarrhoea and meningitis. This study revealed that most children less than five years of age were brought to public clinics for pneumonia, ILI and diarrheal syndromes (51). Individuals who initially presented to a clinic but required a higher level of care due to illness severity most commonly transitioned to a public hospital. Most individuals who sought care at a public facility for pneumonia came from households with low monthly incomes and/or identified as having HIV infection compared to those who sought care in private facilities. The survey identified 24% of deaths occurred outside of the hospital

### 1.14.5 Maternal HIV infection and the HEU infant

Maternal HIV infection in South Africa has been the focus of intense public health efforts since 2001 with the goal of eliminating transmission to infants during pregnancy (53). The prevention of mother to child transmission (PMTCT) guidelines has evolved considerably since this time. The initial recommendations guided healthcare workers to provide a single dose of nevirapine (NVP) at the start of labour and for their HIV exposed infants to also receive treatment within the first 72 hours of life. As of 2015, the PMTCT guidelines now direct healthcare workers to provide all HIV positive pregnant or lactating women with life-

long triple ART. In addition, their HIV exposed infants are to be tested at birth in order to facilitate early identification and treatment. The success of these policy changes is reflected in the comparison of transmission rates over time. In 2010, approximately 32% of pregnant women were HIV infected and the vertical transmission rate was 3.5% (54). A recent national level review now estimates that 29.7% of pregnant women are living with HIV and the vertical transmission rate has been reduced to approximately 0.9% (54). Of note, these rates do vary across provinces and districts but those for Gauteng are similar with an antenatal prevalence rate of 28.6% and transmission rate of 0.9%.

The success of the South African PMTCT program has resulted in a growing population of HIV exposed uninfected infants (HEU). Their lack of infection however does not preclude them from clinical complications. Numerous studies have suggested that HEU infants suffer from increased rates of morbidity and mortality as compared to their unexposed uninfected counterparts (55-58). The HEU infant gained attention during the pre-ART era when clinical management options were limited. During this time studies conducted in high burden African countries measured outcomes in HEU infants, but varying study designs makes the comparison of results difficult (59-61). The most frequently cited is the ZVITAMBO study of over 14,000 Zimbabwean infants who were followed through their first two years of life. ZVITAMBO demonstrated that the two-year mortality in HEU infants was 9.2% compared to 2.9% in HUU infants (62). In this same study there were higher rates of all-cause sick clinic visits at all age intervals and higher rates of LRTI-associated visits specifically during the first six months of life (63).

### 1.15 Methods for objective 1 – Ward surveillance for RSV hospitalisations

A detailed description of the study design, screening, enrolment, data collection and storage, sample collection and testing is provided in Chapter 2 “RSV Hospitalisation incidence, prevalence, seasonality and subtype distributions in HIV-exposed and HIV-unexposed infants”.

### 1.16 Methods for Objectives 2 and 3 – Transplacental transfer ratios and RSV sero-correlates of protection

#### 1.16.1 Study design, screening and enrolment

Research staff positioned in the labour and delivery wards at CHBAH reviewed the maternity registry on a daily basis to identify potential mother-infant pairs for screening and enrolment

as part of a larger sero-epidemiological surveillance study identified as “28OB” on infant Group B Streptococcal (GBS) disease (HREC approval number 140203). At the time of this doctoral proposal and approval (October 2014) the goal enrolment was 30,000 mother-infant pairs. Since this time the sample size had been increased to a total of 38,000 mother-infant pairs. Actively labouring women who were ≥18 years and able to understand and comply with the planned study procedures as well as provide written, informed consent were enrolled.

### 1.16.2 Patient data collection and storage

A medical questionnaire was verbally administered to collect relevant historical, sociological and medical information on both the mother and infant. Additional details were collected from both the mother and infant medical charts. Data were manually captured into an electronically secure database owned and managed by the RMPRU. On a monthly basis, the total daily births were tabulated from the labour and delivery registry logs and then compared to the number of births captured on the RMPRU database. An additional tabulation at the end of the year was conducted to ensure that the total live births recorded were within the expected range based upon previous yearly counts.

### 1.16.3 Patient sample collection and storage

Maternal serum and infant cord blood were collected and stored in RMPRU laboratory freezer facilities. Monitoring for subsequent hospitalisation due to invasive GBS disease in any of the 28OB infants at CHBAH occurred on a daily basis. If and when an infant with invasive GBS disease was identified, then the respective maternal serum and cord blood samples were retrieved for 28OB study analysis. These maternal serum and cord blood samples were then unavailable to other studies nested within the 28OB cohort. The remaining (and majority) of the maternal-infant samples were available for the RSV sero-epidemiological objectives conducted within the RMPRU.

### 1.16.4 Patient sample testing

Specific details related to the testing of maternal serum and infant cord blood samples will be described in detail within the relevant objective chapters.

## 1.17 Statistical analysis for Objectives 1 - 3

The data analyses for Objectives 1 and 3 were generated using SAS software 9.4 (SAS Institute, Carey NC). The details of the statistical analyses will be shared within the respective objective chapters. The data analyses for Objective 2 were generated using Excel and Stata14 (Stata Corp LP, Texas, USA).

### 1.18 Ethics

This dissertation proposal was approved by the Human Research Ethics Committee (HREC) on 3 October 2014 and assigned the clearance certificate no. M140985. The document is located in the Appendix section.

A single written informed consent was obtained from the infant's mother for objective 1. A separate written informed consent from the labouring mother was obtained for objective 2 and 3. These documents are available in the Appendix section.

### 1.19 Funding

Funding for the RSV study was provided by the Medical Research Council, Respiratory and Meningeal Pathogens Research Unit and Department of Science and Technology/National Research Foundation: South African Research Chair in Vaccine Preventable Diseases. Funding for the larger GBS sero-epidemiological surveillance study was provided by Novartis and GSK.

## Chapter 2 RSV hospitalisation incidence, prevalence, seasonality and subtype distributions in HIV-exposed and HIV-unexposed infants

### 2.1 Introduction to South African RSV epidemiology

The review of global RSV epidemiology in the introduction section has established several important conclusions about the RSV burden of disease. RSV is a seasonal disease with a distinct onset, peak and offset. Seasonal timeframes can shift from year to year and place to place. RSV prevalence during its seasonal timeframe can account for a significant percentage of acute respiratory infections. RSV disease disproportionately affects infants in their first six months of life, most of whom are born as healthy, full term infants. Risk factors and risk groups have been identified including those who are born prematurely or with congenital lung and heart disease or immunocompromised. The majority of RSV related severe morbidity and mortality occur in LMICs but the data are sparse. A historical recount of the key South African publications will help to define the context for this doctoral research.

RSV disease has been examined in South Africa since the 1970's (64). Earlier studies conducted in the 2000s examined risk factors for severe RSV disease such as HIV infection and prematurity. A 2000 study by Madhi et al. observed through a prospective surveillance study an increased burden of hospitalised LRTI among children between two months and five years infected with human immunodeficiency virus-1 (HIV) (65). While RSV was more prevalent among HIV-uninfected children hospitalised for LRTI, the incidence was higher in HIV-infected children (among whom RSV-associated LRTI hospitalisation was perennial). In a similar study conducted in 2001 within the same surveillance program, HIV infection was observed to predispose children to more severe RSV associated LRTI after six months of age. In addition, HIV infection was more strongly associated with a clinical presentation of pneumonia, bacterial co-infection and mortality (66). Finally, a study published by Madhi et al in 2006 observed a 2.5 fold higher incidence among HIV infected children and 4.9 fold higher incidence among infants born less than 36 weeks gestation (67). Nevertheless, while the risk was greater for these two specific subpopulations, the majority (>80%) of RSV LRTI hospitalisations occurred among healthy, full term infants. The season was noted to begin at the end of the rainy season and peaked with the coldest temperatures.

Venter et al in 2011 described the prevalence of different viral aetiologies of hospitalized lower respiratory tract infections (LRTI) in children under five years (7). These data were derived from two years of prospective surveillance from three public hospitals and

emergency rooms in the Pretoria area. RSV illness mainly occurred between April and May (autumn) and was second most common virus in all children; associated with 30.1% of LRTI hospitalisations. Although, rhinovirus was the most commonly identified virus, its role in the pathogenesis of LRTI is uncertain (68). Respiratory distress was the most frequently identified clinical characteristic and occurred in 63.3% of cases. Hospitalisations were diagnosed mainly as bronchiolitis, bronchopneumonia or pneumonia in roughly equal proportions. The majority of these hospitalisations (61.7%) occurred in males and in infants between one and three months of age. RSV occurred largely as a single infection and led to a higher percentage of cases requiring ICU level care. Ten percent of cases died; and outcome that is likely associated with a sicker (ICU based) study population. The study by Venter et al. showed that RSV was responsible for a significant portion of respiratory related hospitalisation in children less than five years who sought care at public hospitals. The authors noted a HIV sero-prevalence rate of 32.2% among enrolled children however, 59% of participants had an unknown HIV status. Therefore, the impact of HIV infection upon the prevalence and outcome of RSV hospitalisation could not be assessed adequately. The relationship between HIV infection and RSV disease would be explored in several other studies.

Kyeyagalire et al modelled age based trends in paediatric RSV hospitalisation incidence rates. Hospital administrative data from a private hospital network were grouped into respiratory, circulatory or pneumonia/influenza coded diagnoses, and then linked to NHLS RSV and influenza testing between the years of 2007 to 2011 (69). The highest incidence was reported for children less than one year of age with an annual rate of 7601 per 100,000 person years (95% CI: 4312–10817). The rate declined to 1182 per 100,000 person years (95% CI: 704–1643) for children between one and four years of age. HIV sero-prevalence was not available for this population. There are several important caveats when interpreting these modelled data. In this study, all the included hospitalisations could only be linked to RSV or influenza but not to other circulating respiratory pathogens. Therefore, by excluding the option of considering other infectious aetiologies there may have been an over-estimation of RSV incidence. Furthermore, data from a private health care system may not represent the majority of the national population (80%) whom attend public clinics (70). In spite of these important limitations, the Kyeyagalire data do confirm an age based trend seen on the global scale (1, 2, 71).

The SARI surveillance program which was described in detail within the introductory chapter has provided nationally representative data epidemiologic and virological data for children <five years located in four provinces (20) (72, 73). Cohen et al utilized SARI surveillance to demonstrate that the majority (64%) of virally associated LRTI hospitalisations occurred in children less than 12 months of age between 2009 and 2012 (72). RSV was the second most prevalent virus identified (26%) after rhinovirus (37%). This prevalence rate was generally maintained across different age groups with the highest prevalence (33%) in the 0–3 month age group and the lowest prevalence (17%) occurring in 24–59 month old children.

SARI surveillance has also demonstrated that HIV infection in children less than five years is associated with higher LRTI incidence rates, disease severity and mortality in South African children (72). In the same analysis, Cohen shows the risk of all-cause LRTI hospitalisation was between 1.1 and 3.0 fold higher in HIV infected children as compared to HIV uninfected children were more likely to experience severe disease as indicated by the need for oxygen supplementation (OR 1.3 95% CI: 1.1–1.7). The case fatality risk for HIV infected children with all-cause LRTI was high (OR 4.2 95% CI: 2.6–6.8) as compared to HIV uninfected children. However, the authors did note that the case fatality ratio was lower for those children with RSV infection (1%) compared to children without RSV infection (2%). There have also been several recent studies on RSV infection within HIV exposed infants and these data will be reviewed in the discussion section in conjunction with the results of this thesis (73, 74).

In addition to understanding the size of the RSV disease burden, it is also important to understand what RSV disease looks like clinically. RSV disease can manifest as an upper or lower respiratory tract infection (LRTI). Typically, approximately 1% to 3% of RSV infection results in hospitalisation of infants in their first year of life (75). Lower tract disease can present one of several respiratory syndromes including bronchiolitis, bronchitis, pneumonia and croup (21). In some infants there are no obvious signs of respiratory disease apart from an initial episode of apnoea or cessation of breathing. Reported rates of apnoea in infants with bronchiolitis have ranged between 1% and 24% (22). The earliest phases of RSV disease presents as an upper respiratory tract infection with signs of congestion, rhinorrhoea and occasionally low grade fever (21). In less than 40% of children, the disease can progress to the lower respiratory tract over several days (21). At this stage, clinicians can observe clinical signs indicative of respiratory distress including a cough, expiratory wheezing, crackles, rhonchi and varying degrees of respiratory distress as indicated by

tachypnoea, grunting, abdominal breathing, chest wall retractions, nasal flaring, head bobbing, and hypoxemia (21, 22) (23). Many of these symptoms are dynamic and change rapidly over a short period of time; necessitating frequent evaluations to capture the full clinical picture. In the most severe cases, respiratory failure and death can occur. Young infants who are obligate nasal breathers can present with dehydration secondary to difficulty feeding that results from congestion, respiratory distress and post-tussive emesis. Finally, non- respiratory symptoms may be observed such as irritability and lethargy. Overall, the symptomatic period for RSV lasts a median of two weeks (23).

In high income settings, most infants and children who are hospitalized with RSV are discharged home. The majority of RSV deaths in these setting occur in infants and children with complex medical conditions or life-threatening conditions such as sepsis (5, 76). In the US, mortality rates are approximately 3 to 4 per 10,000 admissions (76). Global in-hospital mortality has recently been estimated for children less than five years (1). In-hospital case fatality ratios (hCFR) and number of deaths were estimated for different World Bank Income regions and age groupings. The 2015 hCFR for upper middle-income countries in 0-5 month olds was 1.8 (95% CI: 1.2–2.6) with an estimated 7200 deaths (95% CI: 42,000–12,300). For infants between 6 and 11 months the hCFR was 2.4 (95% CI: 1.1–5.4) with an estimated 8000 deaths (95% CI: 2800–22,100). Previously reported case fatality rates in South Africa have ranged widely between 0 and 61.5 per 1000 children depending upon the study design, population, study year and age group (6, 7, 72, 77). Most recently, Rha et al reported in a national prospective surveillance of acute LRTI hospitalisations in children less than five that 18/2677 or 0.7% of all children with RSV died (78). There are currently no South African estimates for RSV deaths which occur in the community.

The South African data on RSV in children available to date have generally concurred with what has been reported in the global literature, however, gaps in knowledge remain. The overarching goal of this dissertation is to provide a deeper look into the nature and pattern of RSV disease in Sowetan children less than one year of age. In this second objective specifically the RSV seasonality, prevalence and incidence will be described in detail.

**Table 2.0** Summary of selected South African studies on paediatric RSV epidemiology

| **Author / publication and data years** | **Study design** | **Surveillance case definition** | **RSV testing** | **Study population and sample size** | **Results** |
|---|---|---|---|---|---|
| **Madhi (2000)**<br><br>Data years:<br>1 year (March 1997 – March 1998) | Prospective, hospital based surveillance<br><br>Measured bacterial and viral associated severe LRTI (SLRTI) including prevalence, incidence and relative risk of RSV associated severe LRTI (SLRTI) | SLRTI based on clinical criteria from WHO complemented with pulse oximetry | Nasopharyngeal aspirates<br><br>Direct pooled immunofluorescent test for respiratory viruses<br><br>If sample positive then tested for RSV with mouse anti-RSV monoclonal fluorescent antibody<br><br>If sample negative then cultured with shell viral culture technique followed by specific monoclonal fluorescein conjugated antibodies | Children 2 months to 5 years<br><br>HIV infected and uninfected<br><br>Soweto / CHBAH<br><br>N =990 | RSV was the predominant pathogen identified among HIV uninfected children (18.1%) but not in HIV infected children (5.3%)<br><br>RSV incidence in children 2–23 months for:<br><br>HIV infected<br>1,444 per 100,000<br><br>HIV uninfected<br>309 per 100,000<br><br>Relative risk 1.92 (1.29–2.83) |

| Author / publication and data years | Study design | Surveillance case definition | RSV testing | Study population and sample size | Results |
|---|---|---|---|---|---|
| **Madhi (2001)**<br><br>Data years: 2 years (March 1997 – March 1999) | Prospective, hospital based surveillance<br><br>Measured clinical characteristics and risk factors associated with RSV associated severe LRTI (SLRTI) | SLRTI based on clinical criteria from WHO complemented with pulse oximetry | Nasopharyngeal aspirates<br><br>Direct pooled immunofluorescent test for respiratory viruses<br><br>Is sample positive then tested for RSV with mouse anti-RSV monoclonal fluorescent antibody<br><br>RT-PCR for identification of RSV subgroups | Children 2 months to 5 years (Year 1)<br><br>All children <5 years (Year 2)<br><br>HIV infected and uninfected<br><br>Soweto / CHBAH<br><br>N =281 | HIV infected children with RSV associated SLRTI more likely to:<br><br>• Present with pneumonia<br>• Present with a concurrent bacteraemia<br><br>Risk for RSV associated SLRTI in HIV infected children persists beyond first six months of life<br><br>The case fatality rate was higher for HIV infected children (RR 4.40) |

| Author / publication and data years | Study design | Surveillance case definition | RSV testing | Study population and sample size | Results |
|---|---|---|---|---|---|
| **Madhi (2006)**<br><br>Data years: 5 years (March 1998 – Dec 31, 2004) | Prospective surveillance of children enrolled into a double blind randomized efficacy trial of PCV9<br><br>Measure the impact of gestational age upon hospitalised RSV LRTI incidence | Physician diagnosed LRTI | Nasopharyngeal aspirates<br><br>Immunofluorescence assay | Children <5 years<br><br>Soweto / CHBAH<br><br>N =39,836 | Incidence of RSV hospitalisation:<br><br>HIV uninfected 19.4 per 1000 children<br><br>HIV infected 45 per 1000 children<br><br>Incidence of RSV LRTI 4.9 fold greater in children born <36 weeks |
| **Venter (2011)**<br><br>Data years:<br>2 years (2006-2007) | Measured prevalence of RSV among patients seeking medical attention or hospitalized for acute respiratory infections.<br><br>Testing occurred in the emergency room or hospitalisation. Healthy controls in vaccine clinic | Not explicitly defined. Text refers to "all patients suffering from acute respiratory tract infections" | Nasopharyngeal aspirates<br><br>RT-PCR | Children <5 years<br><br>HIV sero-positive and sero-negative<br><br>Pretoria area<br>3 public hospitals<br><br>N = 1702 | RSV identified in 30.1% of children <5 years hospitalized for respiratory infections |

| Author / publication and data years | Study design | Surveillance case definition | RSV testing | Study population and sample size | Results |
|---|---|---|---|---|---|
| **Kyeyagalire (2014)**<br><br>Data years: 2007-2012 | Estimated annual age specific rates for RSV hospitalisation<br><br>Calculated prevalence among total hospitalisations<br><br>Applied monthly regression models to administrative hospitalisation data<br><br>Used RSV surveillance data as a covariate | Used ICD codes for 3 general categories of: all-respiratory, pneumonia and influenza, all-circulatory | Virologic data obtained from the NHLS | Infants <1 year<br><br>Private hospital group estimated to have 30% of market share of privately insured population<br><br>Used countrywide annual population estimates and applied a calculated proportion of the population with private health insurance<br><br>N =39,529 | all-respiratory codes:<br><br>7601 per 100,000 person-years in children <1 year (95% CI: 4312–10,817)<br><br>Pneumonia and influenza codes:<br><br>3055 per 100,000 (95% CI: 1732–4345)<br><br>36.2% of total hospitalisation (95% CI: 25.3–47.1) |

| Author / publication and data years | Study design | Surveillance case definition | RSV testing | Study population and sample size | Results |
|---|---|---|---|---|---|
| **Moyes (2013)**<br><br>Data years:<br>2 years (2010–2011) | Prospective, hospital based surveillance<br><br>Estimated hospitalisation incidence rates<br><br>Estimated hospital prevalence<br><br>SARI surveillance program conducted at 6 hospitals within 4 surveillance sites<br><br>RSV incidence calculated for CHBAH only | Physician diagnosed ARLTI in children <5 years<br><br>In infants 2 weeks to 3 months with neonatal sepsis or ALRTI | Nasopharyngeal aspirates<br><br>RT-PCR | Children <5 years<br><br>HIV infected and uninfected<br><br>Urban, peri-urban and rural settings<br><br>Gauteng, Mpumalanga, KwaZulu-Natal and Northwest provinces<br><br>N =4489 | Prevalence of RSV 27%<br><br>2010:<br><br>HIV uninfected:<br>24 per 1000 population<br>(95% CI: 22–26)<br><br>HIV infected:<br>128 per 1000 population (95% CI: 104–157)<br><br>2011:<br><br>HIV uninfected:<br>32 per 1000 population (95%CI: 29–34)<br><br>HIV infected:<br>93 per 1000 population (95% CI: 73–118) |

| Author / publication and data years | Study design | Surveillance case definition | RSV testing | Study population and sample size | Results |
|---|---|---|---|---|---|
| **Cohen (2016)**<br><br>Data years: 2010–2013 | SARI surveillance program – active prospective hospital based surveillance<br><br>Compared incidence of hospitalized LRTI by viral aetiology including RSV<br><br>Adjust incidence rates for non-enrolments on weekends and through refusals<br><br>Etiologic fraction used to calculate incidence rates for specific age/HIV exposure status groups<br><br>Incidence rates calculated for CHBAH only | LRTI<br><br>Hospitalized infants ≤7 days of symptoms meeting age appropriate clinical case definitions<br><br>2 days to <3 months with physician diagnosed sepsis or LRTI<br><br>3-6 months physician diagnosed bronchitis, bronchiolitis, pneumonia, and/or pleural effusion | Nasopharyngeal aspirates<br><br>RT-PCR | Infants <6 months<br><br>HIV unexposed and exposed infants<br><br>N =3537 | HUU:<br>3507 per 100,000 population (3244–3787)<br><br>HEU:<br>5003 per 100,000 population (4505–5541)<br><br>HIV infected:<br>6709 per 100,000 population (4589–9471) |

| Author / publication and data years | Study design | Surveillance case definition | RSV testing | Study population and sample size | Results |
|---|---|---|---|---|---|
| **McMorrow (2019)**<br><br>Data years: 2011–2016 | Prospective surveillance for SRI<br><br>Adjust incidence rates for non-enrolments on weekends and through refusals<br><br>Etiologic fraction used to calculate incidence rates for specific age/HIV exposure status groups | 2 days to <3 months with physician diagnosed sepsis or LRTI<br><br>3-6 months physician diagnosed bronchitis, bronchiolitis, pneumonia, and/or pleural effusion | Nasopharyngeal aspirates<br><br>RT-PCR | Children <5 years<br><br>3 hospitals in 3 provinces:<br><br>Mpumalanga, KwaZulu-Natal and Northwest provinces.<br><br>N =3650 | 0-5 months<br><br>HUU:<br>4488 per 100,000 (3852–5148)<br><br>HEU<br>6419 per 100,000 (5335–7604)<br><br>HIV infected<br>8760 per 100,000 (0–21,339)<br><br>0-11 months<br><br>HUU<br>2687 per 100,000 (2272–3129)<br><br>HEU<br>2933 per 100,000 (2403–3521)<br><br>HIV infected<br>6864 per 100,000 (715–15,266) |

## 2.2 Methods:

### 2.2.1 Study design, screening and enrolment

This report is based on active surveillance conducted at the CHBAH paediatric medical wards including the short stay ward between January 2015 and December 2016. The surgical ward and burn unit were excluded. Enrolment of LRTI cases occurred seven days per week from 8 am until 4 pm. Infants admitted overnight were screened and enrolled the next day. In August of 2016 ward surveillance was reduced to five days per week from Monday through Friday. Any patient who was admitted over the weekend and still present on Monday morning was screened for possible inclusion in the study. Each morning research staff reviewed the admission log to identify infants whose admission diagnosis matched the inclusion criteria of the study. The surveillance case definition included any infants <3 months of age who was diagnosed with suspected sepsis or physician diagnosed LRTI and infants between 3 months and <11 months who had a physician diagnosed LRTI including bronchiolitis, pneumonia, bronchitis and pleural effusion. Short stay ward patients were screened first in order to ensure that they could be enrolled prior to discharge given the restricted time frame for their hospital stay. Patient caregivers were approached by research staff and provided a background on the purpose and process of the study. Upon consent the infant was enrolled into the study.

### 2.2.2 Patient data collection and storage

Upon caregiver consent the infant's demographic, clinical and laboratory data were collected on a paper base case report form (CRF). Research staff verbally administered a study questionnaire in addition to reviewing the patient chart. CRFs captured the required clinical data that were available at the time of enrolment. Photocopies of the patient chart were added to the case report form for auditing and quality check purposes. After enrolment, patient logs were periodically reviewed to capture any changes to the level of care including admission to the intensive care unit. Additional laboratory data which may not have been available at the time of enrolment was accessed through the National Health Laboratory System (NHLS) and collated to the patient case report form. Discharge outcome data were by necessity collected separately and then merged with the patient case report form at a later point in time. RMPRU viral testing results for study participants were recorded and stored on a separate laboratory database but linked to the main CRF through a common numerical identifier. All patient case report forms were filled out by hand and delivered to the research unit on a daily basis for

auditing. After auditing by a research clinician for accuracy and completeness, the CRFs were then uploaded by hand to a centralized and secure electronic database system at the RMPRU. Data entry was managed by a database manager.

### 2.2.3 Sample collection and testing

A commercially available nasal swab was used to collect mid-turbinate respiratory secretions from infants at the time of enrolment (FLOQS, Copan Flock Technologies, Brescia, Italy). Prior to collection, a label with the patient's study ID was placed on the collection tube. Details of the date, time and name of the sampler were filled out in the patient's CRF. For the specimen collection itself, a clean swab was placed into the nostril and gently advanced towards the direction of the infant's ear. The swab was advanced until it was estimated to be at the mid-turbinate point or halfway between the opening of the nostril and the ear. The swab was then rotated several times, gently removed and placed into a collection tube with universal transport medium (UTM). Samples were placed on ice and transported to the RMPRU laboratories for processing and analysis. A multi-plex real-time PCR assay was used to detect the presence of RSV within the nasopharyngeal samples. A cycle threshold (Ct) value of <37 was used as a cut off for identifying positive samples.

### 2.2.4 RSV case and non-case definitions

An RSV case was defined as any infant who met the surveillance case definition and had a PCR positive test result for RSV.

A non-RSV case was defined as any child who met the surveillance case definition and but had a negative PCR test result for RSV.

### 2.2.5 Data analysis

**Figures 2.0a and 2.0b** provide a flowchart of study enrolment, data collection and source for the incidence and prevalence calculations.

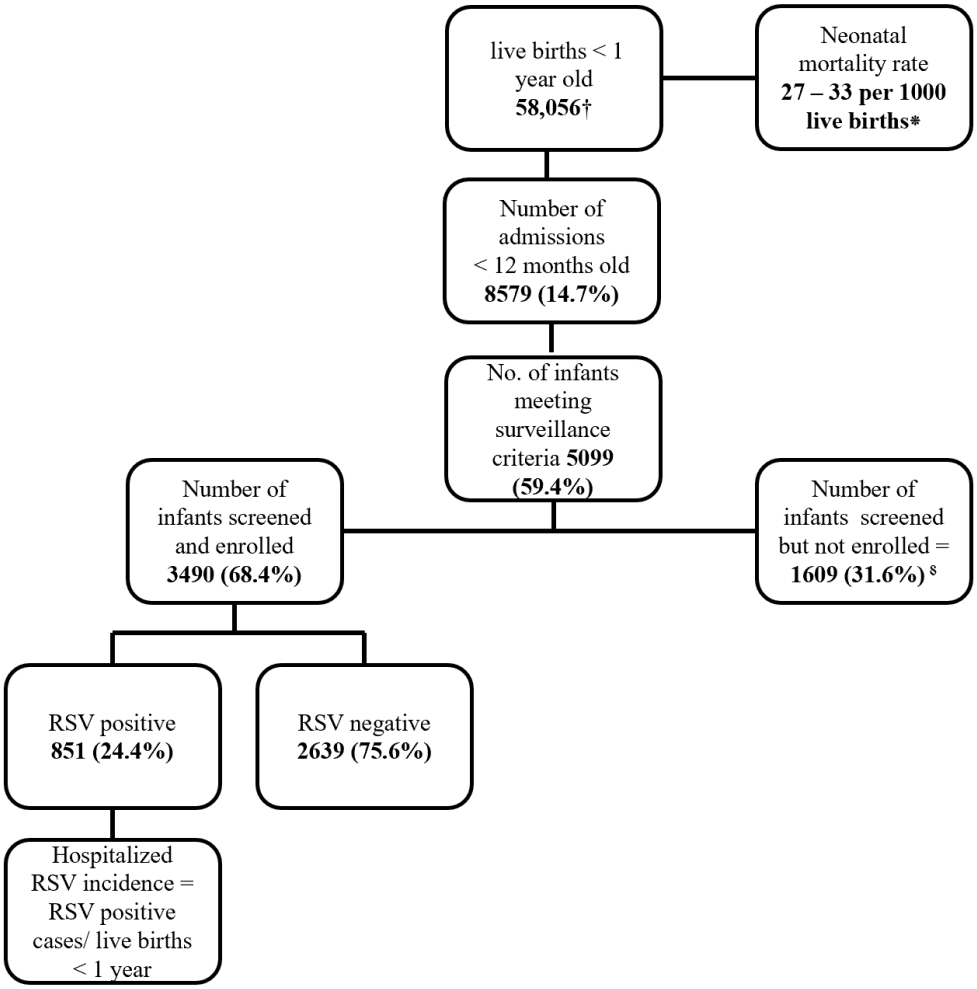


**Figure 2.0a** Flowchart of study enrolment and data collection in infants for January 1st, 2015 to 31st December 2016.

† RMPRU live births register 58,056 (2015 and 2016)
* *South African Medical Journal* 2018;108(3a):s25-s32.
§ During times when enrollment staff were not present - enrolment of occurred seven days per week from 8 am until 4 pm. Infants admitted overnight were screened and enrolled the next day. In August of 2016 ward surveillance was reduced to five days per week from Monday through Friday

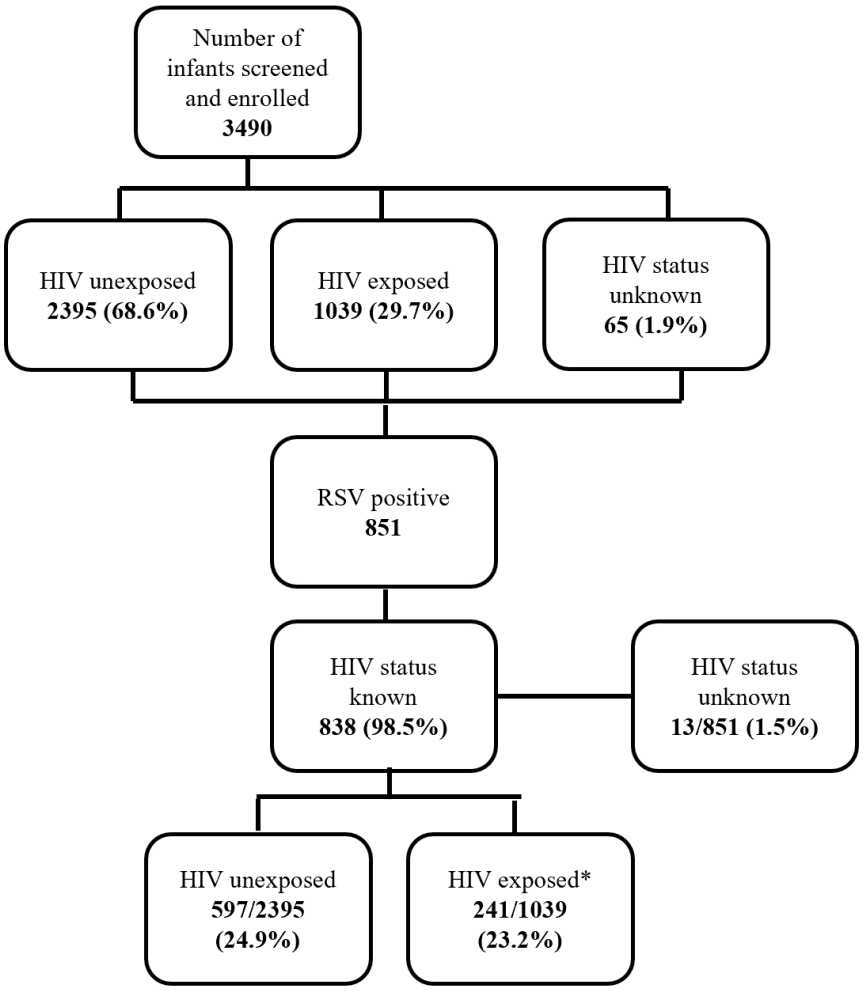


**Figure 2.0b** Flowchart of study enrolment and data collection for determining HIV exposure status.

* 3/241 (1%) of RSV positive HIV exposed infants were identified as children living with HIV (CLWH) on the surveillance case report forms

The month of the RSV season onset was determined when more than 10% of total respiratory related hospitalisations tested positive for RSV for two weeks in a row. Descriptive statistics were used to characterize RSV associated hospitalisations in the study population from multiple angles. The prevalence and incidence of RSV hospitalisations were calculated for infants less ≤12 months of age by month of hospitalisation, by month of age, by RSV subtype, and by HIV exposure status. These incidence calculations were then adjusted for the 1609 non-enrolled infants by assuming the infants who were not enrolled were a random sample and had the same prevalence rate and distribution of disease by month of age as observed for enrolled infants. 95% Confidence intervals (95% CI) were calculated using the

normal approximation for a binomial distribution and it was assumed that overlapping CI limits implied that results were not statistically significant.

Adjustments were calculated as an upper limit for all RSV cases, as well as for HIV unexposed and HIV exposed infants separately. Prevalence calculations were reported as percentages. Incidence were reported as per 1000 live births in Soweto.

The denominator for incidence was taken from the total number of live births recorded in 2015 and 2016 by RMPRU surveillance at CHBAH and the surrounding midwife operated units (MOUs). The socioeconomic conditions in Soweto (high unemployment and low rates of private insurance) are such that the majority of births occur in government health facilities. It is estimated that 90% of Sowetan births occur in government health facilities with approximately 75% of annual births occurring at CHBAH (79). In 2015 there were 29,728 live births recorded. In 2016 there were 28,328 live births recorded.

Baseline characteristics of mothers and infants were presented as percentages with associated p values. Maternal age, parity birth weight and birth length were presented as medians with interquartile ranges. Term gestation was defined as a birth that occurred ≥37 weeks. Low birth weight was defined as an infant who was born weighing <2500 grams.

Descriptive statistics were used to characterize the prevalence (as a frequency and percentage) of clinical signs, hospitalisation interventions, outcomes and discharge diagnoses in infants <12 months of age. The denominator used for each analysis was provided within each table underneath the designated group. Missing values were noted where appropriate. Crude risk ratios with 95% confidence intervals and p values were calculated for select clinical signs comparing RSV to non-RSV cases.

All the data analyses were generated using SAS software 9.4 (SAS Institute, Carey NC).

### 2.3 Results

The burden of RSV disease among infants was described in several ways including seasonality, prevalence, incidence, clinical characteristics and outcomes.

Over the two-year surveillance period there were a total of 8579 infants less than 12 months admitted to the hospital of which 5099 (59%) met the surveillance criteria for LRTI (**Figure 2.0a**). A total of 3490 (68%) were screened and enrolled into the surveillance study. Of these, a total of 851 (24%) participants were PCR positive for RSV. The number of RSV cases was

fairly consistent between the two reporting years with a total of 448 cases identified in 2015 and 403 cases identified in 2016. There were a total 3490 respiratory related hospitalisations of which 2395 (68.6%) were identified as HIV unexposed infants and 1039 (29.7%) as HIV exposed infants less than one year of age (**Figure 2.0b**). Of these total respiratory related hospitalisations there were 65 (1.9%) respiratory hospitalisations whose HIV status was missing. Of the total of 851 RSV cases identified there were 13 (1.5%) cases whose HIV exposure status was missing. The data described below are combined for both surveillance years unless otherwise noted.

The baseline characteristics of RSV and non-RSV cases were similar with the exception of gestational age and birth weight (**Table 2.1**). There was a higher percentage of term infants among RSV cases compared to non-RSV cases (81% vs 77%, p =0.0243). The median birth weight was higher in RSV cases than non-RSV cases (3015 grams vs 2995 grams, p =0.0411); an indirect reflection of the higher percentage of term infants. There were no significant differences between RSV and non-RSV cases when examining maternal age or maternal HIV infection status. The median maternal age was 27 years (IQR 23, 32). The HIV sero-prevalence rate among mothers was 29.5%. Unfortunately, there were significant amounts of missing data for several maternal variables including parity (58%), mode of delivery (53%), maternal alcohol use during pregnancy (35%), and smoking during pregnancy (28%); these data were not generally available from the birth registry. The percentages of missing data were similar between mothers of RSV cases and non-cases with the exception of maternal alcohol use during pregnancy for which there was a higher percentage of missing data among RSV cases (47% vs 31%).

**Table 2.1** Baseline maternal and infant characteristics of study participants

| | **Total** | **RSV** | **Non-RSV** | **p - value** |
|---|---|---|---|---|
| | *n = 3490* | n = 851 | n = 2639 | |
| ***Maternal*** | ***n (%)*** | ***n (%)*** | ***n (%)*** | |
| **Maternal Age** | 27 | 26 | 27 | 0.0596 |
| Median [IQR] | (23,32) | (22, 31) | (23, 32) | |
| Missing | 61 | 14 | 47 | |
| **Parity** | 1 | 1 | 1 | 0.4951 |
| Median | [1,2] | [1,2] | [1,2] | |
| Missing | 2024 | 521 | 1503 | |
| **Mode of delivery** | | | | 0.0825 |
| Vaginal | 895 | 190 (51) | 705 (56) | |
| C-section | 732 | 182 (49) | 550 (44) | |
| Missing | 1863 | 479 | 1384 | |
| **HIV status** | | | | 0.3399 |
| Positive | 1030 | 241 (29) | 789 (31) | |
| Negative | 2395 | 597 (71) | 1798 (69) | |
| Missing | 65 | 13 | 52 | |
| **Alcohol use in pregnancy** | | | | 0.0543 |
| Yes | 66 | 7 (2) | 59 (3) | |
| No | 2192 | 443 (98) | 1749 (97) | |
| Missing | 1232 | 401 | 831 | |
| **Smoking in pregnancy** | | | | 0.0914 |
| Yes | 111 | 20 (3) | 91 (5) | |
| No | 2387 | 599 (97) | 1788 (95) | |
| Missing | 992 | 232 | 760 | |
| ***Infant*** | ***n (%)*** | ***n (%)*** | ***n (%)*** | |
| **Sex** | | | | 0.5414 |
| Male | 1983 | 476 (56) | 1507 (57) | |
| Female | 1506 | 375 (44) | 1131 (43) | |
| Ambiguous | 1 | 0 | 1 | |
| **Gestational age** | | | | 0.0243 |
| Term | 2420 | 602 (81) | 1818 (77) | |
| Preterm (< 37 weeks) | 672 | 139 (19) | 533 (23) | |
| Missing | 398 | 110 | 288 | |
| **Birth weight** | 3000 | 3015 | 2995 | 0.0411 |
| Median [IQR] | [2590, 3320] | [2650, 3345] | [2560, 3315] | |
| Missing | 293 | 80 | 213 | |
| **Low birth weight < 2500 g** | | | | 0.0075 |
| Yes | 628 | 138 (18) | 544 (22) | |
| No | 2515 | 633 (82) | 1882 (78) | |
| Missing | 293 | 80 | 213 | |

*RSV Seasonality*

The bulk of RSV hospitalisations occurred within a distinct seasonal timeframe. The number of RSV positive and negative tests were reported by epidemiological calendar week as outlined by the U.S. based Centres for Disease Control (80). The seasonal onset and offset were determined when the number of RSV cases were ≥ and ≤10 percent of the total respiratory hospitalisation respectively (bolded within the table). As is observed in **Figure 2.1** the seasonal time differed slightly between the two years. In 2015, the season occurred at weeks 9 and week 31. In 2016, the season onset and offset occurred at weeks 14 and 36. The 2015 season had a bimodal peak. There was little to no disease outside the seasonal timeframe for both years. A table of weekly counts of RSV negative and positive results can be found within the Appendix 3.

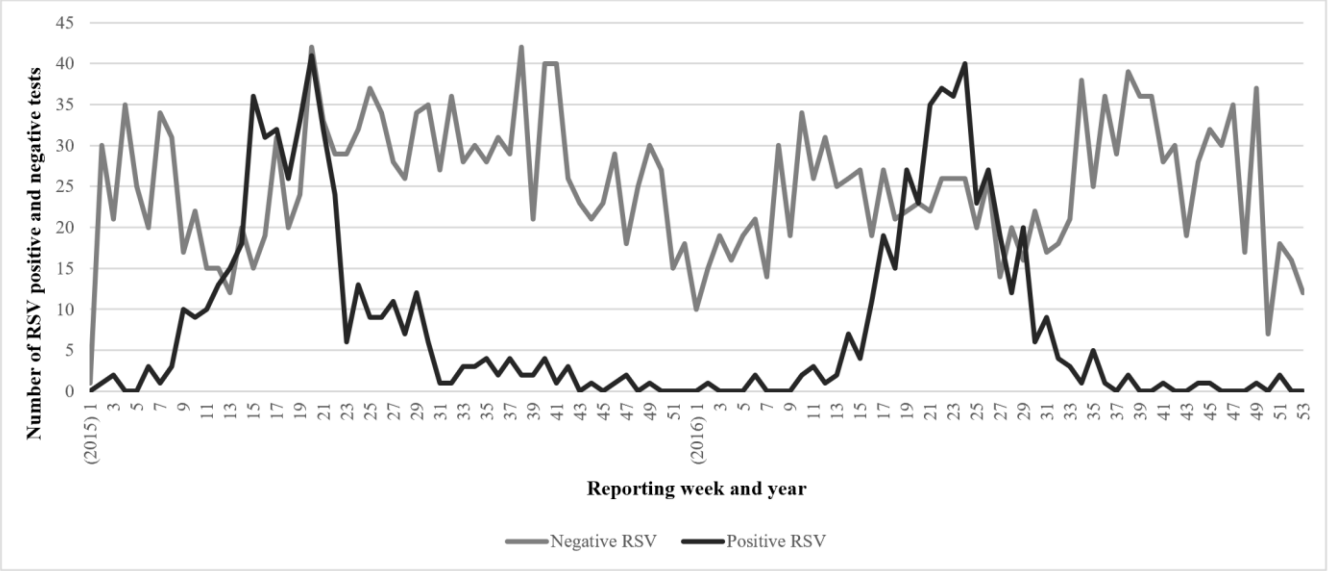


**Figure 2.1** RSV seasonality reported by week, 2015 and 2016

*RSV subtype distribution*

RSV subtype A dominated the circulation in both season years. The prevalence of subtype A infection ranged between 61.6% and 93.9% between the months of March and August when both season years were combined (**Table 2.2)** . There were few subtype A and B co-infections with a total of five cases over the two years. Subtype A was responsible for the majority of disease across age groups within the first year of life (**Table 2.3)**.

**Table 2.2** RSV subtype by month of admission, 2015 and 2016 seasons combined

| Month of admission | A | | B | | AB | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| **March** | 45 | 61.6 | 28 | 38.3 | 1 | 0 |
| **April** | 125 | 69.8 | 54 | 30.1 | 1 | 0 |
| **May** | 206 | 76.6 | 62 | 23.0 | 2 | 0 |
| **June** | 125 | 76.7 | 38 | 23.3 | 0 | 0 |
| **July** | 59 | 70.0 | 25 | 29.7 | 1 | 0 |
| **August** | 19 | 76.0 | 6 | 24.0 | 0 | 0 |
| **Total** | **579** | | **213** | | **5** | |

**Table 2.3** RSV subtype by month of age, 2015 and 2016 seasons combined

| Month of age | A | | B | | Total |
|---|---|---|---|---|---|
| | n | % | n | % | |
| 0 | 62 | 75.6 | 20 | 24.3 | 82 |
| 1 | 97 | 72.3 | 37 | 27.6 | 134 |
| 2 | 84 | 77.0 | 25 | 22.9 | 109 |
| 3 | 93 | 77.5 | 27 | 22.5 | 120 |
| 4 | 60 | 70.5 | 25 | 29.4 | 85 |
| 5 | 41 | 71.9 | 16 | 28.0 | 57 |
| 6 | 47 | 65.2 | 25 | 34.7 | 72 |
| 7 | 39 | 69.6 | 17 | 30.3 | 56 |
| 8 | 27 | 61.3 | 17 | 38.6 | 44 |
| 9 | 28 | 77.7 | 8 | 22.2 | 36 |
| 10 | 18 | 72.0 | 7 | 28.0 | 25 |
| 11 | 18 | 69.2 | 8 | 30.7 | 26 |
| **Total** | **614** | | **232** | | **846** |

*RSV prevalence*

The prevalence of RSV hospitalisations was reported by month of admission, month of age and by HIV exposure status. Seasonally, RSV accounted for a large proportion of respiratory admissions between March and July with a peak prevalence of 53.2% occurring in May

(**Table 2.4**) Annually, RSV accounted for 24% of total respiratory hospitalisations among children less than one year of age.

**Table 2.4** Prevalence of RSV hospitalisation by month of admission, 2015 and 2016

| Month of Admission | Total | RSV | % |
|---|---|---|---|
| **January** | 187 | 5 | 2.7 |
| **February** | 215 | 18 | 8.4 |
| **March** | 278 | 74 | 26.6 |
| **April** | 368 | 180 | 49 |
| **May** | 507 | 270 | 53.2 |
| **June** | 402 | 163 | 40.5 |
| **July** | 305 | 85 | 27.9 |
| **August** | 277 | 25 | 9 |
| **September** | 311 | 15 | 4.8 |
| **October** | 241 | 8 | 3.3 |
| **November** | 244 | 5 | 2 |
| **December** | 156 | 3 | 1.9 |
| **Total** | **3491** | **851** | **24.4** |

The prevalence of RSV hospitalisation for all children regardless of HIV exposure status was reported by month of age (**Appendix 3**). In each of the surveillance years there was an identical pattern of disease prevalence rising dramatically between zero and one month of age. Over the two surveillance years the majority of RSV associated hospitalisations (69.4%) occurred within the first six months of life. Annual RSV prevalence did not differ greatly between HIV unexposed and exposed infants in the first year of life; 24.9% vs 23.4% of total respiratory hospitalisations respectively (**Table 2.5**). However, there was a higher prevalence of RSV in the 0 month age group among HIV exposed infants (14.8%) as compared to HIV-unexposed infants (8.7%) hospitalised for LRTI. Children living with HIV (n=11) are included within the HIV exposed category but only constituted 1% of RSV cases (3/241) and non-RSV cases (8/789). However, there were 65 infants who were missing HIV status of which 13 were RSV cases.

**Table 2.5** Percentage of hospitalisations due to RSV by HIV exposure status, 2015 and 2016

| | HIV Unexposed | | | | | HIV Exposed | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Age in months** | **Total respiratory hospitalisations** | **RSV Cases (n)** | **RSV Cases (%)** | **95% CI** | **p-value** | **Total respiratory hospitalisations** | **RSV Case (n)** | **RSV cases (%)** | **95% CI** | **p-value** |
| **0** | 588 | 51 | 8.7 | 6.4 - 10.9 | <.0001 | 210 | 31 | 14.8 | 10.0 - 19.6 | <.0001 |
| **1** | 355 | 94 | 26.5 | 21.9 - 31.0 | <.0001 | 175 | 38 | 21.7 | 15.6 - 27.8 | <.0001 |
| **2** | 236 | 77 | 32.6 | 26.7 - 38.6 | <.0001 | 107 | 32 | 29.9 | 21.2 - 38.6 | <.0001 |
| **3** | 205 | 87 | 42.4 | 35.7 - 49.2 | 0.0304 | 101 | 34 | 33.7 | 24.5 - 42.9 | 0.001 |
| **4** | 169 | 66 | 39.0 | 31.7 - 46.4 | 0.0044 | 70 | 20 | 28.6 | 17.99 - 39.2 | 0.0003 |
| **5** | 141 | 36 | 25.5 | 18.3 - 32.7 | <.0001 | 67 | 18 | 26.9 | 16.3 - 37.5 | 0.0002 |
| **6** | 147 | 54 | 36.7 | 28.9 - 44.5 | 0.0013 | 61 | 18 | 29.5 | 18.1 - 41.0 | 0.0014 |
| **7** | 132 | 44 | 33.3 | 25.3 - 41.4 | 0.0001 | 51 | 12 | 23.5 | 11.9 - 35.2 | 0.0002 |
| **8** | 128 | 33 | 25.8 | 18.2 - 33.4 | <.0001 | 58 | 11 | 19.0 | 8.9 - 29.1 | <.0001 |
| **9** | 97 | 22 | 22.7 | 14.4 - 31.0 | <.0001 | 53 | 10 | 18.9 | 8.33 - 29.4 | <.0001 |
| **10** | 88 | 15 | 17.0 | 9.2 - 24.9 | <.0001 | 42 | 10 | 23.8 | 10.9 - 36.7 | 0.0007 |
| **11** | 109 | 18 | 16.5 | 9.5 - 23.5 | <.0001 | 35 | 7 | 20.0 | 6.8 - 33.3 | 0.0004 |
| **Total** | **2395** | **597** | **24.9** | | | **1030** | **241** | **23.4** | | |

Missing HIV exposure status: Total 65, RSV 13

*RSV incidence*

Unadjusted and adjusted incidence of RSV hospitalisation was calculated by month of age for all children and by HIV exposure status. The annual, unadjusted incidence for all infants, regardless of HIV exposure status was 14.56 per 1000 live births (95% CI: 13.68 - 15.63) (**Table 2.6**). The adjusted (i.e. adjusting for non-enrolment of eligible cases hospitalised for LRTI as noted in the methods section) incidence for RSV hospitalisation for all infants was 21.37 per 1000 live births (95% CI: 20.19–22.55) (**Table 2.6**). Similar sets of unadjusted (**Table 2.7**) and adjusted calculations (**Table 2.8)** were done for infants according to their HIV exposure status. The adjusted incidence for HIV unexposed and HIV exposed infants were 21.45 and 20.15 per 1000 live births respectively (**Table 2.8**). For both HIV unexposed and HIV exposed infants the incidence peaked at one month of age; 3.37 vs 3.21 per 1000 live births respectively. The incidence rates by month of age in both HIV unexposed and HIV exposed infants thereafter fluctuated minimally through the first six months of life and then trended downward through to 12 months of age.

**Table 2.6** Unadjusted and adjusted hospitalisation incidence by month of age for *all* children, 2015 and 2016

| **Unadjusted Incidence*** | | | | | | **Adjusted Incidence†** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age at admission** | **Total** | **RSV** | **IR** | **Lower Limit** | **Upper Limit** | **Age at admission** | **Total** | **RSV** | **IR** | **Lower Limit** | **Upper Limit** |
| **0 months** | 807 | 82 | 1.41243 | 1.106931 | 1.717928 | **0 months** | 1179 | 120 | 2.06697 | 1.697524 | 2.436415 |
| **1 month** | 541 | 134 | 2.30812 | 1.917762 | 2.698471 | **1 month** | 790 | 196 | 3.37605 | 2.904202 | 3.847899 |
| **2 months** | 349 | 110 | 1.89472 | 1.540975 | 2.24847 | **2 months** | 510 | 161 | 2.77318 | 2.345406 | 3.200963 |
| **3 months** | 313 | 122 | 2.10142 | 1.728914 | 2.473924 | **3 months** | 457 | 178 | 3.06601 | 2.616275 | 3.515736 |
| **4 months** | 244 | 86 | 1.48133 | 1.168478 | 1.794179 | **4 months** | 356 | 125 | 2.15309 | 1.776046 | 2.530141 |
| **5 months** | 216 | 57 | 0.98181 | 0.72705 | 1.236572 | **5 months** | 316 | 83 | 1.42965 | 1.122301 | 1.737007 |
| **6 months** | 209 | 72 | 1.24018 | 0.953892 | 1.526472 | **6 months** | 305 | 105 | 1.8086 | 1.462969 | 2.154228 |
| **7 months** | 185 | 57 | 0.98181 | 0.72705 | 1.236572 | **7 months** | 270 | 83 | 1.42965 | 1.122301 | 1.737007 |
| **8 months** | 189 | 44 | 0.75789 | 0.534032 | 0.981746 | **8 months** | 276 | 64 | 1.10238 | 0.832449 | 1.372319 |
| **9 months** | 155 | 36 | 0.62009 | 0.417591 | 0.822591 | **9 months** | 226 | 52 | 0.89569 | 0.652346 | 1.139028 |
| **10 months** | 135 | 25 | 0.43062 | 0.261853 | 0.599385 | **10 months** | 197 | 36 | 0.62009 | 0.417591 | 0.822591 |
| **11 months** | 147 | 26 | 0.44784 | 0.275737 | 0.61995 | **11 months** | 215 | 38 | 0.65454 | 0.446495 | 0.862586 |
| **Total** | **3490** | **851** | **14.65826** | **13.68065** | **15.63587** | **Total** | **5097** | **1241** | **21.37591** | **20.19938** | **22.55244** |

Live births denominator 58056

IR: Incidence rate defined as cases per 1000 live births

* Unadjusted - only includes infants who were enrolled

† Adjusted - includes both enrolled and unenrolled infants

**Table 2.7** Unadjusted hospitalisation incidence rates for HIV unexposed and HIV exposed infants, 2015/2016

| | HIV Unexposed | | | | | HIV Exposed | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Age at Admission** | **Total** | **RSV** | **IR*** | **Lower Limit** | **Upper Limit** | **Total** | **RSV** | **IR*** | **Lower Limit** | **Upper Limit** |
| **0 months** | 588 | 51 | 1.25492 | 0.9107182 | 1.5991244 | 210 | 31 | 1.77997 | 1.1539331 | 2.4060118 |
| **1 month** | 355 | 94 | 2.31299 | 1.8459420 | 2.7800422 | 175 | 38 | 2.1819 | 1.4889146 | 2.8748888 |
| **2 months** | 236 | 77 | 1.89469 | 1.4718841 | 2.3174860 | 107 | 32 | 1.83739 | 1.2013526 | 2.4734292 |
| **3 months** | 205 | 87 | 2.14075 | 1.6913857 | 2.5901103 | 101 | 34 | 1.95223 | 1.2966522 | 2.6078034 |
| **4 months** | 169 | 66 | 1.62402 | 1.2325251 | 2.0155064 | 70 | 20 | 1.14837 | 0.6453633 | 1.6513753 |
| **5 months** | 141 | 36 | 0.88583 | 0.5965849 | 1.1750687 | 67 | 18 | 1.03353 | 0.5563116 | 1.5107532 |
| **6 months** | 147 | 54 | 1.32874 | 0.9745712 | 1.6829091 | 61 | 18 | 1.03353 | 0.5563116 | 1.5107532 |
| **7 months** | 132 | 44 | 1.08268 | 0.7629397 | 1.4024146 | 51 | 12 | 0.68902 | 0.2993053 | 1.0787379 |
| **8 months** | 128 | 33 | 0.81201 | 0.5350696 | 1.0889461 | 58 | 11 | 0.6316 | 0.2584674 | 1.0047388 |
| **9 months** | 97 | 22 | 0.54134 | 0.3151888 | 0.7674883 | 53 | 10 | 0.57418 | 0.2184035 | 0.9299658 |
| **10 months** | 88 | 15 | 0.36909 | 0.1823414 | 0.5558476 | 42 | 10 | 0.57418 | 0.2184035 | 0.9299658 |
| **11 months** | 109 | 18 | 0.44291 | 0.2383432 | 0.6474836 | 35 | 7 | 0.40193 | 0.1042357 | 0.6996228 |
| **Total** | **2395** | **597** | **14.68996** | **13.5202566** | **15.8596647** | **1030** | **241** | **13.83785** | **13.0913682** | **14.5843323** |

HIV unexposed live birth denominator 40,640
HIV exposed live birth denominator 17,416
Missing – 13 RSV case infants missing HIV exposure status and excluded from analysis
IR: Incidence rate defined as cases per 1000 live births

**Table 2.8** Adjusted hospitalisation incidence rates for HIV unexposed and HIV exposed infants, 2015/2016

| | HIV Unexposed | | | | | HIV Exposed | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Age at Admission** | **Total** | **RSV** | **IR*** | **Lower Limit** | **Upper Limit** | **Total** | **RSV** | **IR*** | **Lower Limit** | **Upper Limit** |
| **0 months** | 859 | 75 | 1.84547 | 1.4281883 | 2.2627566 | 307 | 45 | 2.58383 | 1.8298643 | 3.3377976 |
| **1 month** | 518 | 137 | 3.37106 | 2.8075170 | 3.9346090 | 256 | 56 | 3.21543 | 2.3746155 | 4.0562527 |
| **2 months** | 345 | 113 | 2.78051 | 2.2685507 | 3.2924729 | 156 | 47 | 2.69867 | 1.9281730 | 3.4691628 |
| **3 months** | 299 | 127 | 3.125 | 2.5823440 | 3.6676560 | 147 | 49 | 2.8135 | 2.0268325 | 3.6001772 |
| **4 months** | 247 | 96 | 2.3622 | 1.8902238 | 2.8341856 | 102 | 29 | 1.66514 | 1.0595928 | 2.2706782 |
| **5 months** | 206 | 53 | 1.30413 | 0.9532552 | 1.6550125 | 98 | 26 | 1.49288 | 0.9194640 | 2.0662962 |
| **6 months** | 215 | 79 | 1.9439 | 1.5156516 | 2.3721437 | 89 | 26 | 1.49288 | 0.9194640 | 2.0662962 |
| **7 months** | 193 | 64 | 1.5748 | 1.1892803 | 1.9603260 | 74 | 17 | 0.97611 | 0.5123253 | 1.4399025 |
| **8 months** | 187 | 48 | 1.1811 | 0.8471640 | 1.5150408 | 85 | 16 | 0.9187 | 0.4687415 | 1.3686494 |
| **9 months** | 141 | 32 | 0.7874 | 0.5146883 | 1.0601149 | 77 | 15 | 0.86128 | 0.4255984 | 1.2969555 |
| **10 months** | 128 | 22 | 0.54134 | 0.3151888 | 0.7674883 | 61 | 15 | 0.86128 | 0.4255984 | 1.2969555 |
| **11 months** | 159 | 26 | 0.63976 | 0.3939252 | 0.8856024 | 51 | 10 | 0.57418 | 0.2184035 | 0.9299658 |
| **Totals** | **3497** | **872** | **21.45669** | **20.0478887** | **22.8654971** | **1503** | **351** | **20.15388** | **18.0667964** | **22.2409666** |

HIV unexposed live birth denominator 40,640
HIV exposed live birth denominator 17,416
Missing – 22 infants missing HIV exposure status and excluded from analysis
IR: Incidence rate defined as cases per 1000 live birth

*Clinical characteristics and outcomes*

There were five clinical signs which occurred in 50% or more of RSV cases at the time of admission; cough (94%), chest wall indrawing (78%), tachypnoea (68%), crepitations/crackles (57%), and wheezing (50%) (**Table 2.9**). Each of these signs occurred at higher frequencies as compared to non-RSV LRTI cases. Chest wall indrawing and tachypnoea were the only two IMCI danger signs which occurred in the majority of RSV patients. The clinical sign most strongly associated with RSV cases was wheezing with a crude RR of 1.94 (95% CI: 1.76, 2.13, p <0.0001). Hypoxemia occurred in only 11% of RSV patients and fever occurred in only 18% of RSV patients.

**Table 2.9** Clinical characteristics of hospitalized RSV in children <1 year, 2015/2016

| **Sign or symptom** | **RSV n =851** | | **non-RSV n =2639** | | **P value** |
|---|---|---|---|---|---|
| | n | (%) | n | (%) | |
| Cough | 786 | 94 | 1691 | 65 | <.0001 |
| Chest wall indrawing | 659 | 78 | 1359 | 52 | <.0001 |
| Tachypnoea* | 499 | 68 | 1060 | 49 | <.0001 |
| Crepitations/crackles | 480 | 57 | 865 | 33 | <.0001 |
| Wheezing | 424 | 50 | 677 | 26 | <.0001 |
| Nasal flaring | 296 | 35 | 651 | 25 | <.0001 |
| Runny nose | 296 | 35 | 542 | 21 | <.0001 |
| Fever >37.5 C | 134 | 18 | 520 | 22 | .0175 |
| Irritability | 122 | 15 | 602 | 23 | <.0001 |
| Feeding intolerance/vomiting | 104 | 12 | 192 | 7 | <.0001 |
| Hypoxemia† | 76 | 11 | 221 | 10 | 0.6699 |
| Diarrhoea | 83 | 10 | 346 | 13 | 0.0088 |
| Lethargy or unconscious | 53 | 6 | 216 | 8 | 0.0527 |
| Poor feeding after having fed well | 41 | 5 | 77 | 3 | 0.0079 |
| Expiratory grunting | 33 | 4 | 94 | 4 | 0.7091 |
| Apnoea | 23 | 3 | 89 | 3 | 0.3027 |
| Central cyanosis | 13 | 2 | 58 | 2 | 0.2153 |
| Stridor | 14 | 2 | 108 | 4 | 0.0006 |
| Seizures | 6 | 1 | 75 | 3 | 0.0003 |
| Sunken eyes | 8 | 1 | 53 | 2 | 0.0379 |
| Sunken fontanelle | 8 | 1 | 93 | 4 | <.0001 |
| Decreased skin turgor | 4 | <1 | 56 | 2 | 0.0013 |
| Mottled skin | 2 | <1 | 12 | <1 | 0.3515 |
| Poor capillary refill | 4 | <1 | 25 | 1 | 0.1717 |

* age based cut-offs for tachypnea: ≥60 in children <2 months, ≥50 in children ≥2 months
† hypoxemia defined as pulse oximetry <90

The hospital course and outcomes of RSV and non-RSV cases were compared (**Table 2.10**). The median length of hospital stay was slightly longer in non-RSV cases (3 days vs 2 days). The percentage of ICU admission was slightly higher in non-RSV cases (3% vs 1%). Of note there were approximately 50% of ICU admission values were missing for each group. However, it was assumed that if there was no ICU admission recorded on the patient's CRF then it was unlikely that an ICU admission truly occurred. There was a higher percentage of oxygen supplementation among RSV cases compared to non-RSV cases (32% vs 23%, p <.0001). There were high rates of antibiotic usage in both RSV and non-RSV cases (69% vs 79%). In hospital CFR were infrequent in RSV (0.24%) and non-RSV (0.68%) cases.

**Table 2.10** Hospital course and outcomes in RSV and non-RSV cases

| | RSV n =851 n (%) | non-RSV n =2639 n (%) | P value |
|---|---|---|---|
| **Hospital course** | | | |
| **Length of stay, median [IQR]** | 2 [1,5] | 3 [1,7] | <.0001 |
| **ICU admission** | 10 (1.2) | 66 (2.5) | 0.0373 |
| **Oxygen supplementation** | 273 (0.32) | 617 (0.23) | <.0001 |
| **Mechanical ventilation** | 3 (0.35) | 17 (0.64) | 0.5938 |
| **Antibiotics** | 583 (68.5) | 2076 (78.7) | <.0001 |
| **Inotrope medication** | 4 (0.47) | 15 (0.57) | 1 |
| **Intravenous fluids** | 13 (1.5) | 111 (4.2) | 0.0004 |
| **Steroid medications** | 24 (2.8) | 132 (5.0) | 0.0081 |
| **Hospital outcomes** | | | 0.1087 |
| **Death in hospital** | 2 (0.24) | 18 (0.68) | |
| **Transfer to step-down** | 7 (0.82) | 50 (1.9) | |
| **Refused treatment** | 0 (0) | 2 (0.08) | |
| **Discharged home** | 834 (98.0) | 2546 (96.5) | |
| **Missing** | 8 | 23 | |

## 2.4 Discussion

RSV is a significant, seasonal contributor to respiratory related hospitalisations in children less than one year in Soweto. RSV accounted for approximately one quarter (24.4%) of the total 3,490 annual hospitalisations in this age group as captured through the surveillance study. The majority of cases were due to RSV subtype A with no distinct differences in

season month or age-based distributions. Despite a slight shift in the seasonal timeframes, the number of RSV cases identified in each of the two season years was consistent.

During peak season months the prevalence of RSV hospitalisations accounted for more than half of the respiratory admissions. The majority (69%) of these admissions occurred among infants in their first six months of life with a peak of 39% occurring at three months of age. These prevalence data effectively communicate that the RSV burden of disease is concentrated among the youngest infants. Bont et al in their 2016 systematic review of RSV epidemiology in western countries concluded that between 44% and 83% of infants hospitalized were less than 6 months (71). Parikh et al in their aggregate analysis of several key US studies and national datasets report that 75% of RSV hospitalisations occurred in those less 6 months of age (3). The Parikh data also confirmed that the distribution patterns were consistent between term and preterm infants.

The adjusted incidence for RSV LRTI hospitalisation was 21.4 per 1000 live births. This was in the middle of the 2016 global systematic review estimate on incidence of RSV LRTI hospitalisation which ranged between 8.4 and 42.7 per 1000 infants (71). This wide global range likely reflects differences in study design, study denominators, and cultural differences in care seeking behaviours, and clinical management.

Most recently, the South African SARI surveillance program has published data estimating the mean annual RSV and influenza specific hospitalisations rates in HUU and HEU children less than five years of age (Table 2.0, McMorrow data)(81). Data from three hospitals located in Pietermaritzburg and Klerksdorp between the years 2011 and 2016 were utilized. The mean estimated annual rate of RSV hospitalisation for HUU infants 0-11 months was 26.87 per 1000 population (95% CI: 22.72–31.29). Both SARI and this study utilized similar surveillance case definitions and observed similar distributions of disease concentrated within the first six months of life. The study by McMorrow et al estimated RSV hospitalisations by multiplying a number of serious respiratory hospitalisations (SRI) by an age-specific proportion of positive RSV tests. These crude rates were then adjusted upward to compensate for an assumed number of non-enrolments (i.e. not attending hospital) secondary to healthcare seeking behaviours. While the exact degree of adjustment was not identified in the publication, this approach likely led to higher point-estimate of disease. The authors acknowledge that their estimates are more than twice that of rates calculated for infants <6 months in a prior South African study conducted in 2010 and 2011 (77). While this study

calculated adjusted rates for infants who were admitted but not enrolled, it is still possible that these rates remain an underestimate of disease due to the inability to account for cases which did not present for hospital care altogether.

This study demonstrates that in Soweto, the overall distribution of disease, including a peak at one month of age, is similar to studies conducted elsewhere(2, 3, 82). It is challenging to interpret the clinical significance of a wide range of incidence rates reported from different countries and for different age ranges. As was noted earlier, differences in study design, study populations, study years, testing approaches may partially explain these divergent results. In addition, differences in healthcare seeking behaviour and clinical practice (i.e. the threshold for admission decisions) in high income countries may be lower or more wide ranging; allowing for a wide spectrum of disease severity. In Soweto, the threshold for admission is likely higher given that only infants with evidence of severe LRTI as determined by the WHO criteria would be considered.

The nearly identical hospital incidence rates found in HIV unexposed and exposed infants in our study (14.69 vs 13.84 per 1000 live births) was in contrast to earlier reports which showed higher rates of disease (74, 81). Prior studies would suggest that HIV exposure would lead to a higher risk for RSV hospitalisation (55, 83, 84). As summarized in Table 2, Cohen et al reported RSV hospitalisation incidence rates and incidence rate ratios for all infants <6 months of age who were HIV unexposed and exposed (74). In this study, rates were calculated using cases identified at CHBAH and Sowetan population denominators from the years 2010 and 2011 using a related dataset and methodology to the McMorrow publication described earlier (81). Cohen et al reported an RSV hospitalisation incidence of 35 and 50 per 1000 population for HUU and HEU infants <6 months of age. As with the McMorrow data, the authors of this study adjusted their rates upward to account for non-enrolments (on weekends and refusals), healthcare seeking behaviour and the attributable fraction of infection to illness; an approach which may have led to an overestimation. The resulting incidence rate ratio for RSV hospitalisation in HEU infants in the Cohen study was calculated to be 1.4 (95% CI: 1.3–1.6). The McMorrow SRI data described earlier also reported a similarly high rate of RSV disease in HEU infants <6 months at 64 per 1000 population as well as a rate for infants between 0–11 months at 29 per 1000 infants.

The surveillance case definition is a major determinant of how much disease a surveillance program is able to capture. A strength of this study was the utilization of age specific case

definitions in an effort to capture the spectrum of RSV disease. These case definitions were identical to those used in both the SARI and SRI surveillance programs. The ability to monitor the wards intensively is an important consideration when the average length of stay for RSV hospitalisations at CBHAH has not been well characterized. If the majority of RSV cases only require short (observational) stays then daily coverage would be imperative. Another strength of the study was the extent of RMPRU surveillance which occurred on all paediatric medical wards including the short stay ward. Screening and enrolment occurred seven day per week between the hours of 8 am and 4 pm from 2015 through August 2016. In August 2016 ward coverage was then reduced to five days per week. Overnight admissions were screened for enrolment the following morning. It is therefore possible that this reduction to five days per week led to missed cases. However previous RSV surveillance conducted by the NICD indicates that the RSV season was essentially complete by this point in the year suggesting that the number of missed cases would have been minimal (19).

The study may have suffered from a form of selection bias if the infants of caregivers who refused enrolment were fundamentally different from those who did enrol. Although both the surveillance case definition, ward coverage were robust neither of these surveillance elements can account or compensate for the community's care seeking behaviour. A caregiver's decision is what ultimately determines who presents to the hospital for evaluation enabling a surveillance program the opportunity to case capture. Of note, the Zola-Jabulani hospital was opened in Soweto in May of 2014 in an effort to decompress the large patient volume at CHBAH. It is possible that this 300 bed hospital which includes a paediatric ward may have diverted a portion of RSV cases away from the CHBAH surveillance program leading to a smaller case count.

RSV case identification is reliant upon sensitive virological testing. In this project, samples were obtained with nasopharyngeal swabs and tested for RSV through RT-PCR. There were, however, no differences in the sample type, manner of collection, processing or laboratory techniques between the SARI, SRI and RMPRU surveillance programs (personal communication Professor Madhi, February 22, 2019).

This thesis collected data for two season years and may not have captured the seasonal and epidemic variability that can be observed over longer periods of time (16). It has been observed in prior studies that both the size and timing of an RSV epidemic can fluctuate. For example, Finland is noted to have a two year cycle whereby a "small" epidemic occurs on an

odd-numbered year which is then followed by a "major" epidemic during an even year (85). The Cohen data was collected over two seasons (2010–2011) but at different years which may have been "major" epidemic years. The study by McMorrow et al. was done over five years (2011–2016) and likely captured more variability. It may be that the either or both of the two seasons captured through this thesis project were "small epidemic" relative to years past.

There are virus and population specific changes which could have occurred over time and account for differences in rate calculations. RSV strain evolution and a resulting population level immunity could account for a less virulent form of disease over time; accounting for rate differences between 2010-2011 and 2015-2016. The population health and disease susceptibility of the infant population could have also changed over time. More specifically, changes in infant vaccination practices and management of maternal HIV infection may have indirectly reduced the risk of RSV disease. The 2009 introduction of PCV7 to the public immunization plan which was then followed by PCV13 in 2011 (including a catchup campaign) had drastically reduced pneumococcal disease in the population. Several publications have noted the relationship between pneumococcal and RSV disease suggesting that a reduction in the former could also impact the latter (86, 87). Equally important, the national PMTCT and ART guidelines have changed significantly over time since the initial rollout in 2002 (88). In 2013, the guidelines were changed to allow all pregnant women to be eligible for HAART irrespective of their CD4 count. Additionally, the guidelines advised for their infants to receive nevirapine prophylaxis for six weeks. This important change to the clinical management of maternal HIV infection has reduced the national rate of MTCT from 3.5% in 2010 to 1.1% in 2015/2016 (89). While the study by McMorrow et al, incidence rates for HEU infant data included more recent data it is important to note that these data were derived from population surveillance in Pietermaritzburg and Klerksdorp; which may not be representative of (urban and rural) other South African populations.

The selection of a population denominator is a critical element in an incidence calculation. This thesis utilized a live birth denominator collected by the RMPRU at CHBAH and the surrounding MOUs. The denominator for each surveillance year ranged between 28,000 and 29,000 live births. Adjustments were not made for private health insurance usage or infant mortality rates, both of which may have reduced the total number of infants seeking care at CHBAH. Additionally, the live birth data cannot account for population movement over time

and it is possible that infants entered and exited the catchment area over the course of time altering the true number of infants who should have been included within the denominator.

The results summarized in this chapter communicate the significance of the RSV burden among the Sowetan community who came to CHBAH for care. RSV disease is concentrated among the youngest infants and during its seasonal peak accounts for more than half of respiratory related hospitalisations. These observations are only made more urgent by the fact that the standard of care is limited in what it can provide to alleviate the problem. Multiple vaccines and anti-virals are currently in development to address this issue. Until safe, effective and easily accessible forms of prevention and treatment are available to the global community it is imperative to better characterize the disease burden so that we can be prepared to operationalize those solutions.

## Chapter 3 The transplacental transfer of RSV neutralizing antibody in HIV exposed and unexposed infants

### 3.1 Introduction

Infants passively acquire their earliest forms of immunity against infectious disease from their mothers during pregnancy (90). Pregnant women have a wide array of antibodies against different antigens through either natural exposure or vaccination. Antigen specific IgG antibody can then be actively transferred across the placenta to the developing foetus with the aid of the Fc neonatal receptor FcRn (90, 91). The amount of antibody that an infant has at birth depends upon several factors, including the level of circulating antibody in the mother, the efficiency of transplacental transfer, gestational age at birth, and underlying health status of mother and the pregnancy itself (90).

Baseline maternal antibody levels rely upon exposure to the antigen. RSV exposure and illness have been observed in pregnant women, however, the data are limited (92). This is because when RSV testing in pregnancy occurs, it is often tied to influenza surveillance periods and case definitions thereby leading to likely under-estimation. One international multi-site study of 13,694 respiratory hospitalisation during pregnancy demonstrated that only 6% of women were tested for RSV (93). There is evidence of wider RSV exposure during pregnancy through serological studies of both maternal serum and infant cord blood (13, 94-96). The current data suggests that RSV mainly manifest as a mild illness in pregnant women, with limited evidence for moderate to severe disease (97-99). A study conducted in South Africa calculated an incidence rate of RSV among pregnant women to be 5.3 per 1000 person-months (97), whilst lower incidence were calculated in two other studies from Nepal (3.9 per 1000 person-years) and Mongolia (0.3 per 1000 person-days) (98, 99).

Transplacental transfer of IgG begins as early as the first trimester, albeit at very low rates (100). Transfer efficiency increases with each successive week of gestation reaching their peak transfer in the third trimester (90, 100). There are multiple subclasses of IgG, of which IgG1 is transferred most efficiently (101). Infant antibody levels measured at birth can exceed maternal levels due to the active transport mechanism mentioned earlier (102). Shorter gestation periods which result in premature delivery effectively reduces the time window for transfer; the resulting lower titre levels increases the susceptibility to infection (103, 104).

There is also evidence to suggest that transfer rates can be limited by saturation of the FcRn receptors by maternal conditions such as hypergammaglobulinemia (90).

Additional factors can negatively impact transfer efficiency including maternal infections, chronic conditions (malnutrition, diabetes mellitus) and other pregnancy-related conditions (gestational hypertension) (90).

The transplacental transfer of RSV neutralizing antibody has been measured in different populations. Transfer ratios have generally been highly efficient with foetal (cord) : maternal ratios >1.0 across different populations and subtypes as summarized in **Table 4.0** (45, 46, 105-107). The earliest study was conducted by Suara et al which compared the transfer rates of RSV neutralizing antibody between Gambian and American (USA) mother-infant pairs over an identical period of time (105). Cord maternal ratios (CMR) were calculated separately for RSV subtypes A and B. The titre levels between mother and infants were strongly correlated and efficient transfer ratios were seen in both populations for both subtypes. Transfer ratio were slightly lower among Gambian (CMR: 0.99 for both subtypes) compared to USA (CMR: 1.02 subtype A and 1.03 subtype B) women. Differences in subtype circulation within the two populations were directly reflected in maternal and infant subtype specific GMT titres. This supports the notion that subtype specific maternal exposure results in transfer of subtype specific IgG to their infants. Gambian women had higher titres to subtype B than women from USA, and vice versa for subtype A. Maternal age did not impact the GMTs in either Gambian or USA maternal populations. Gambian mothers with a lower parity (<3) had higher GMTs to both subgroups, however, this difference was not reflected in infant GMTs; and parity had no effect on USA maternal GMTs.

Later studies conducted in Bangladeshi mother-infant pairs also identified efficient transplacental transfer of RSV IgG, with a CMR of >1.0 (95% 0.99–1.03) (45). There was no impact of different covariates upon this ratio, including type of delivery, infant sex, parity, birth weight, maternal age and maternal education. A similar analysis from Nepal, reported an overall CMR of 1.03 (95% CI: 0.89–1.19); and also did not identify any association with maternal age, maternal education, parity, breastfeeding, smoking in the household, preterm, low birthweight or small for gestational age (46). Male gender was associated with a modestly lower CMR compared to female counterparts (1.02 versus 1.05, p =0.0017).

Finally, there are two recently published studies from Brazil and Botswana with similar objectives to this dissertation chapter. Each of these studies measured the impact of maternal

HIV infection upon CMR and observed efficient (>1.0) but reduced transplacental transfer rates in infants born to women living with HIV. In the Brazilian study the CMR was 1.8 vs 1.3 in women living with HIV vs. not living with HIV respectively. In the Botswana study the CMR was 1.15 vs 1.02 in women living with HIV vs. not living with HIV respectively (56, 108). In Brazil, HEU infants had higher RSV geometric mean titres compared to HUU counterparts (77.4 vs 49.6 EU/ml, p =0.002), despite a lower CMR (1.3 vs 1.8; p =0.05. This suggests there was higher RSV exposure within households where women with HIV live, resulting in them developing higher titres which offset the lower CMR. The Botswana study corroborated the observations from Brazil and provided additional insight on maternal and infant predictors of transplacental transfer efficiency. An undetectable maternal HIV viral load was associated with higher transfer rates among women living with HIV. Those in whom HIV-1viral load was <400 copies/ml had significantly higher CMRs than those with viral loads >400 copies/ml (1.06 vs. 0.55, p =0.01).

This objective of this study was to explore the transplacental transfer efficiency (CMR) of RSV specific neutralizing antibody in infants born to Sowetan women living with and without HIV infection.

**Table 3.0 Summary of studies on RSV transplacental transfer**

| Author/publications and data years | Study design and/or description | Study population | Sample size | Testing method | CMR results |
|---|---|---|---|---|---|
| **Suaya (1996)**<br><br>Data collection years:<br>May 1992 – Feb 1993 | Measured prevalence of RSV A and B antibodies in maternal venous and cord blood samples in two geographic populations | The Gambia<br>U.S.A. | The Gambia:<br>75 maternal<br>90 infant samples<br>of which 50 were maternal-infant pairs<br><br>U.S.A.:<br>110 maternal / 107 infant samples<br>107 were maternal-infant pairs | Microneutralization assay for RSV subtypes A and B – titres expressed as a GMT $log_2$ | The Gambia:<br>0.99 subtype A<br>0.99 subtype B<br><br>U.S.A.:<br>1.02 subtype A<br>1.03 subtype B |
| **Chu (2014)**<br><br>Data collection years:<br>Aug 2004 – Dec 2005 | Nested within a maternal influenza immunization trial | Bangladesh | 149 maternal-infant pairs | Microneutralization assay – expressed as $log_2$ titres | 1.01 (95% CI: 0.99 – 1.03) |
| Chu (2017)<br><br>Data collection years:<br>Mar 2012 – Oct 2013 | Nested within a maternal influenza immunization trial | Nepal | 310 maternal-infant pairs | Microneutralization assay – expressed as $log_2$ titres | 1.03 (95% CI: 0.88 – 1.19) |
| **Weinberg (2017)**<br><br>Data collection years:<br>2011 – 2013 | Prospective cohort study of adverse pregnancy, birth and childhood outcomes in HIV infected women | Brazil | 335 maternal-infant pairs<br>247 HIV infected mothers<br>88 HIV uninfected mothers | ELISA assay | 1.8 HIV uninfected<br>1.3 HIV infected |
| **Patel (2019)**<br><br>Data collection years:<br>2015 – 2016 | Cross sectional study of HIV infected and HIV uninfected women | Botswana | 316 maternal-infant pairs | Microneutralization assay* - expressed as $log_2$ titres | 1.15 HIV uninfected<br>1.02 HIV infected |

### 3.2 Methods

Cord maternal ratios were determined by measuring RSV neutralizing antibody titres in both maternal serum and infant cord blood taken at birth. These samples were collected from pregnant women ≥18 years enrolled into a larger surveillance study exploring for a correlate of protection against invasive Group B streptococcal disease at Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, South Africa (HREC approval number 140203). Only women who delivered full term, singleton births were considered for inclusion. Maternal serum was collected either at birth or within 24 hours of delivery and paired with their infant's cord blood samples. Women were matched by HIV status, age, their infants' gestational age and date of birth. A total sample size of 240 matched mother-infant pairs were randomly selected in order to provide an 80% power to detect at least a 0.16 difference in CMR between women living with and without HIV infection. Between January and December 2015, 10 mother-infant pairs for each arm were randomly selected monthly. This sample size calculation was based upon the starting assumption that the CMR of RSV neutralizing antibodies among women without HIV would be 0.9(109, 110).

#### 3.2.1 Laboratory methods

The paired maternal and infant samples were analysed at the Respiratory and Meningeal Pathogens Research Unit for levels of RSV neutralizing antibody using a microneutralization assay. The assay was performed using 96-well flat bottom, micro-titre plates using HEP2 cells (ATCC CCL-81) and the long strain of human RSV (ATCC VR-26). Each 96-well plate contained four samples (or two maternal infant pairs) tested in duplicate using 12 two-fold dilutions and paired to a positive/negative control plate. Plates were incubated at 37 °C and 5% $CO_2$ for 144 hours (6 days) with daily checks for virus-specific cytopathic effect. After the six-day incubation period the plates were: decanted, overlaid with crystal violet, and left to stain for 24 hours. After the 24-hour staining period the plates were gently decanted, rinsed and allowed to dry upside down before reading the wells. Readings were performed immediately after drying and then a second time two days later to ensure accuracy. Titres were determined by the last well with at least an 80% fully protected HEP2 cell monolayer. The final titre for each sample was the average of its duplicate pair. The Reed and Meunch method was used to calculate both the end dose (ED) 50 per ml. Hypergammaglobulinemia

was defined as ≥15 g/L and determined by measuring total IgG using a commercially-available assay (Tina-quant IgG Gen.2, Roche diagnostics, Switzerland) (90).

### 3.2.2 Statistical analysis

The geometric mean titres (GMT) of neutralizing antibody in maternal and infant samples were determined individually. The cord to maternal ratio (CMR) was the result of dividing the cord titre by the maternal titre. GMTs were compared using multiple regression on $\log_{10}$ transformed data. Confounding variables were analysed as covariates. Chi square or Fisher's exact test were used to compare categorical values expressed as percentages. Pearson's correlation test was used for determining the association between maternal infant titres, total IgG and neutralizing titres and transplacental transfer ratios. 95% confidence intervals were calculated. A p-value of <0.05 indicated statistical significance. Data analysis was performed using Excel and Stata14 (Stata Corp LP, Texas, USA). P values were adjusted for maternal HIV status, race, parity, mean upper arm circumference (MUAC), haemoglobin levels, hypertension, alcohol use and hypergammaglobulinemia between women living with and without HIV. P values were further adjusted for CD4 counts and viral loads among women living with HIV.

### 3.2.3 Ethics

This protocol was approved by the Human Research Ethics Committee of the University of the Witwatersrand (HREC no: 140203) as part of a larger study on invasive Group B Streptococcal disease in newborns and infants. The informed consent provided and signed by participants allowed for additional testing of samples including those performed in this study.

### 3.2.4 Other items

Additional materials can be found in **Appendix 4 and 5** including the co-authorship agreement form and the RSV antibody assay protocol.

## 3.3 Results

A total of 240 maternal-infant sample pairs were analysed for this study. Baseline maternal characteristics were compared between women living with and without HIV (**Table 3.1**). The groups were similar, except for higher rate of alcohol use (9.4% vs 1.1%, p =0.020) and higher percentage with hypergammaglobulinemia (89.7% vs 7.3%, p <0.0001) in women living with HIV. Overall, 67% of women were ≥25 years age, and 65% were of <3 parity in women living with and without HIV. Ninety-four percent of women living with HIV were on

anti-retroviral treatment (ART), including 92.8% on Atroiza (Efanvirenz, Emtricibatine and Tenofovir). CD4+ lymphocyte counts were available for 94.4% of women living with HIV, with a median CD4+ count of 413.5 cells/mL (20–998). HIV-1 viral load data were available for 50.2% of women, in whom the mean HIV-1 viral load was $10^{3.2}$ log copies/mL. There were no significant differences in the baseline characteristics between infants born to women living with and without HIV (**Table 4.1, Table 4.2**).

The geometric mean titres (GMT) were compared between mother-newborn dyads in each group (**Table 4.3**), and association between GMTs and maternal and infant characteristics included analyses in relation to maternal age, mid-upper arm circumference (as a proxy for nutritional status), parity and low birth weight.

## Table 3.1 Baseline characteristics of mothers

| | Overall | HIV uninfected | HIV infected | p value |
|---|---|---|---|---|
| **Number of women** | 240 | 119 (49.6%) | 121 (50.4%) | n/a |
| **Median maternal age, in years (range)** | 27.8 (18.1 - 35.5) | 27.6 (18.5 - 35.5) | 27.9 (18.1 - 34.8) | 0.643 |
| **Maternal age, in years** | | | | 0.999 |
| **< 25 years** | 79 (32.9%) | 39 (32.8%) | 40 (33.1%) | |
| **≥ 25 years** | 161 (67.1%) | 80 (67.2%) | 81 (66.9%) | |
| **Race - Black African decent** | 237 (98.8%) | 116 (97.5%) | 121 (100%) | 0.12 |
| **Median gravidity (range)** | 2 (1-6) | 2 (1-5) | 2 (1-6) | 0.349 |
| **Median parity (range)** | 2 (1-6) | 2 (1-5) | 2 (1-6) | 0.167 |
| **< 3** | 156 (65.0%) | 84 (70.6%) | 72 (59.5%) | 0.08 |
| **≥ 3** | 84 (35.0%) | 35 (29.4%) | 49 (40.5%) | |
| **Mean mid-upper arm circumfrence (SD)** | 29.2 (4.2) | 29.7 (4.4) | 28.7 (4.0) | 0.073 |
| **≤ 23.5 cm** | 17 (7.3%) | 8 (7.0%) | 9 (7.7%) | 0.999 |
| **> 23.5 cm** | 215 (92.7%) | 107 (93.0%) | 108 (92.3%) | |
| **Maternal medical history** | | | | |
| **Urinary tract infection** | 1 (0.48%) | 1/101 (1.0%) | 0/106 | 0.49 |
| **Hemoglobin < 10 mg/dl** | 46 (21.0%) | 19/118 (16.1%) | 27/115 (23.5%) | 0.189 |
| **Gestational diabetes** | 1 (0.92%) | 0/59 | 1/50 (2%) | 0.464 |
| **Maternal hypertension** | 15 (13.6%) | 11/59 (18.6%) | 4/51 (7.8%) | 0.176 |
| **PROM* ≥ 18 hours at delivery** | 4 (3.7%) | 2/59 (3.4%) | 2/50 (4%) | 0.999 |
| **Meconium stained liquor** | 16 (7.7%) | 8/95 (8.4%) | 8/111 (7.2%) | 0.273 |
| **Hemorrhage** | 6 (5.5%) | 3/59 (5.1%) | 3/50 (4%) | 0.999 |
| **Anemia** | 13 (11.9%) | 5/59 (8.5%) | 8/50 (16%) | 0.381 |
| **Syphilis** | 6 (2.8%) | 1/110 (0.91%) | 5/107 (4.7%) | 0.212 |
| **Smoking** | 4 (2.1%) | 2/94 (2.1%) | 2/96 (2.1%) | 0.999 |
| **Alcohol** | 10 (5.3%) | 1/94 (1.1%) | 9/96 (9.4%) | 0.02 |
| **Total IgG** | 234 (97.5%) | 117/119 (98.3%) | 117/121 (96.7%) | 0.999 |
| **GMT of total IgG in g/L** | 12.4 (11.9 - 13.0) | 10.5 (10.0 - 11.0) | 14.7 (13.9 - 15.6) | <0.001 |
| **Hypergammaglobulinemia** | 58 (24.8%) | 6/58 (10.3%) | 52/58 (89.7%) | <0.001 |
| **Antiretroviral therapy** | 97 (94.2%) | NA | 97/103 (94.2%) | NA |
| **Atroiza** | | NA | 90/97 (92.8%) | NA |
| **CD4+ lymphocytic count available** | 90 (94.4%) | NA | 90 (94.4%) | NA |
| **Median CD4 count** | | NA | 413.5 (20 - 998) | NA |
| **Mean CD4** | | NA | 22.6 (95) | NA |
| **Viral load data available** | 62 (51.2%) | NA | 59 (48.8%) | NA |
| **Mean viral load in copies/ml (range)** | | NA | 0 (0-405736) | NA |
| **Mean log viral load copies/ml (SD)** | | NA | 3.2 (1.2) | NA |

***Prolonged rupture of membranes**

**Table 3.1 (continued)** Baseline characteristics of infants

| | Overall | HIV uninfected | HIV infected | p value |
|---|---|---|---|---|
| **Number of infants** | 240 | 119 | 121 | NA |
| **Female gender, n (%)** | 122 | 60 (50.4%) | 62 (51.2%) | 0.899 |
| **Race - Black African descent,n (%)** | 238 (99.2%) | 117 (98.3%) | 121 (110%) | 0.245 |
| **Mean Birth weight in g, (SD)** | 3174.1 (421.4) | 3199.5 (413.9) | 3149.1 (429.0) | 0.356 |
| **Low birth weight (<2500 g), n (%)** | 11 (4.6%) | 7 (5.9%) | 4 93.3%) | 0.373 |
| **Median APGAR 5 minutes (range)** | 10 (6-10) | 10 (8-10) | 10 (6-10) | 0.026 |
| **Median Placenta weight (range)** | 613 (349 - 1069) | 613.5 (349 - 1069) | 611.5 (349 - 986) | 0.325 |

### 3.3.1 Maternal titres were similar between exposure groups

The RSV MN GMTs were similar in women living with and without HIV (1449 ED50/ml vs 1560 ED50/ml, p =0.467). GMTs did not differ by maternal age, parity mean mid-upper arm circumference. GMTs among either group of women. In women living with HIV, there was no association between RSV GMTs and maternal CD4+ count, mean HIV-1 viral load, or use of anti-retrovirals. Maternal RSV GMTs did not correlate with total IgG ($r^2$ = -0.002, p =0.56).

### 3.3.2 Infant titres are lower than maternal titres

Overall mean infant titres were lower than maternal titres (1113.5 versus 1503.6, p <0.001) but strongly correlated ($r^2$ =0.66, p ≤0.001). The strong correlation between maternal and infant titres was present in both women living with s ($r^2$ =0.72, p <0.001) and without HIV ($r^2$ =0.61, p <0.001). Infants born to women living with HIV had lower GMTs (966.7) compared to those born to women without HIV (1285, p =0.009).

**Table 3.2** Geometric mean titres and cord-maternal ratios of anti-RSV antibodies

| | | maternal serum | | | Cord serum | | | CMR | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | GMT (95% CI) | p value | p adj | GMT (95% CI) | p value | p adj | GMT (95% CI) | p value | p adj |
| **Overall** | | 1503.6 (1361.7 - 1660.3) | | | 1113.5 (999.4 - 1240.7) | | | 0.74 (0.69 - 0.79) | | |
| **Maternal HIV** | | | | | | | | | | |
| **negative** | 119 | 1560.3 (1367.1 - 1780.9) | | | 1285.6 (1106.3 - 1493.9) | | | 0.82 (0.75 - 0.91) | | |
| **positive** | 121 | 1449.8 (1249.0 - 1683.0) | 0.467 | 0.467 | 966.7 (829.2 - 1127.1) | 0.009 | 0.009 | 0.67 (0.61 - 0.72) | 0.001 | 0.122 |
| **Maternal CD4** | | | | | | | | | | |
| **<200 cells/µl** | 21 | 1666.8 (1147.9 - 2420.4) | | | 1140.4 (759.6 - 1712.0) | | | 0.69 (0.58 - 0.81) | | |
| **200-500 cells/µl** | 36 | 1395.9 (1139.6 - 1709.8) | | | 949.7 (765.5-1178.2) | | | 0.68 (0.57-0.81) | | |
| **>500 cells/µl** | 33 | 1280 (950.3 - 1724.1) | 0.45 | 0.216 | 814.9 (612.4 - 1084.3) | 0.292 | 0.116 | 0.64 (0.54 - 0.74) | 0.709 | 0.715 |
| **Maternal Viral load** | | | | | | | | | | |
| **<500 copies/mL** | 46 | 1454 (1181.1 - 1792.2) | 0.557 | 0.557 | 1126.1 (892.0 - 1421.7) | 0.347 | 0.347 | 0.77 (0.67 - 0.89) | 0.011 | 0.074 |
| **≥ 500 copies/mL** | 16 | 1660.0 (1004.4 - 2743.3) | | | 905.1 (584.7 - 1401.2) | | | 0.55 (0.43-0.70) | | |
| **Antiretroviral therapy** | | | | | | | | | | |
| **none** | 6 | 1140.4 (519.8-2501.9) | | | 905.1 (382.7-2140.4) | | | 0.79 (0.44-1.44) | | |
| **triple therapy** | 97 | 1422.3 (1206.2 -1677.1) | 0.369 | 0.214 | 954.9 (805.4-1132.2) | 0.019 | 0.005 | 0.67(0.61-0.74) | 0.016 | 0.151 |
| **Hypergammaglobulinemia (total IgG>15 g/L)** | | | | | | | | | | |
| **No** | 176 | 1477.9 (1327.4 - 1645.4) | 0.94 | 0.94 | 1184.2 (1050.7-1334.7) | 0.013 | 0.013 | 0.80 (0.74 - 0.86) | <0.000 | <0.001 |
| **Yes** | 58 | 1490.7 (1186.3 - 1873.2) | | | 868.0 (690.8 - 1090.8) | | | 0.58 (0.52 - 0.65) | | |
| **Maternal age (yrs.)** | | | | | | | | | | |
| **<25** | 79 | 1422.1 (1203.5 - 1680.5) | | | 1112.4 (920.7 - 1344.0) | | | 0.8 (0.7-0.9) | | |
| **≥25** | 161 | 1545.3 (1365.1-1749.3) | 0.439 | 0.439 | 1114.1(975.1-1272.9) | 0.99 | 0.81 | 0.7(0.7-0.8) | 0.242 | 0.358 |
| **Mid-Upper Arm Circumfrence (MUAC)** | | | | | | | | | | |
| **≤23.5 cms** | 17 | 1446.6 (1043.1-2006.1) | | | 1191.9 (838.0-1695.2) | | | 0.8(0.7-1.0) | | |
| **>23.5 cms** | 215 | 1510.0 (1358.4-1678.5) | 0.827 | 0.827 | 1117.9 (997.2-1253.2) | 0.762 | 0.877 | 0.7(0.7-0.8) | 0.4 | 0.617 |
| **Parity** | | | | | | | | | | |
| **<3** | 156 | 1535.8(1358.2-1736.6) | | | 1193.5(1038.5-1371.7) | | | 0.78(0.72-0.84) | | |
| **≥3** | 84 | 1445.7(1218.5-1715.2) | 0.568 | 0.568 | 978.9(826.1-1160) | 0.085 | 0.24 | 0.68(0.61-0.75) | 0.044 | 0.164 |
| **Low birth weight (<2500g)** | | | | | | | | | | |
| **No** | 229 | 1490.3(1346.0-1650.1) | | | 1104.4(987.6-1235.1) | | | 0.7(0.7-0.8) | | |
| **Yes** | 11 | 1810.2(1110.8-2950.1) | 0.42 | 0.42 | 1321.0(844.6-2066.1) | 0.496 | 0.423 | 0.7(0.5-1.1) | 0.981 | 0.96 |
| **Infant Sex** | | | | | | | | | | |
| **Male** | 118 | 1484.7(1261.3-1747.5) | 0.805 | 0.805 | 1124.8(949.7-1332.3) | 0.857 | 0.987 | 0.76(0.69-0.83) | 0.492 | 0.321 |
| **Female** | 122 | 1522.2(1353.9-1711.4) | | | 1102.7(960.2-1266.2) | | | 0.72 (0.66-0.80) | | |

**padj:** p-value after adjusting for maternal HIV status, race, parity, MUAC, hemoglobin, hypertension, alcohol and hypergammaglobulinemia between HIV-infected and uninfected women. Among HIV-infected women additional adjustment for CD4 count and viral loads

Lower GMTs were also observed among infants born to women living with HIV whose mothers were not on ART as compared to those who were on ART (1140.4 vs 1422.3, p =0.005). Lower infant GMTs were also observed in infants born to women with hypergammaglobulinemia than those born to women without hypergammaglobulinemia (868.0 vs 1184.2, p =0.013). As noted earlier, the majority of women living with HIV infection had hypergammaglobulinemia (89.7%).

### 3.3.3 Cord maternal ratios

The overall CMR was 0.74 (95% CI: 0.69–0.79). The CMR was not significantly different in newborns born to women living without HIV (0.82) than those living with HIV (0.67, $p_{adj}$ =0.122). Among women living with HIV, HIV-1 viral loads ≥500 copies were associated (non-significantly) with a lower CMR as compared to those with viral load below 500 copies (0.77 vs 0.55, $p_{adj}$ =0.074 ). There was a weak negative correlation between total IgG and CMR ($r^2$ = -0.09, p = <0.001). This weak negative correlation remained consistent for women living with HIV ($r^2$ = -0.05, p =0.012), and in those without HIV ($r^2$ = -0.05, p =0.17) albeit not-significant. Higher maternal parity (≥3 vs. <3) lost statistical significance after adjustment for confounders (0.68 vs 0.78, $p_{adj}$ =0.164). Maternal age and nutritional status (indicated by MUAC) were also not associated with CMR, and neither were infant birth weight and gender.

## 3.4 Discussion

The overall GMTs of RSV neutralizing antibody in maternal serum were not significantly different between women living with and without HIV. The association of lower titres in infants born to mothers living with HIV, however, was not independent of maternal hypergammaglobulinemia Among women living with HIV, factors such as CD4+ counts, HIV-1 viral load and ART were not associated with RSV microneutralization GMTs. These analyses were, however, limited by the fact that most (94%) of women living with HIV were on ART. Additionally, data on HIV-1 viral loads were only available for half of these women which also limits the interpretation of the data. Of those for whom HIV-1 viral loads were available, most had viral loads <500 suggesting effective control of HIV-1 replication . The impact of HIV infection upon maternal titres appears to be both study specific and antigen specific (111). For example, different studies on maternally derived measles immunity have

shown maternal HIV infection resulting in higher or lower or no difference in CMRs for the same antigen (112-115). A study conducted in Cape Town, reductions in transplacental transfer rates were antigen specific (116). CMR reductions ranged from 15% for pneumococcus (not statistically significant), 23% for Haemophilus influenzae type b (HiB), 27% for tetanus toxoid and 40% for pertussis. The mechanism underlying this antigen-specific interference is poorly understood. It is theorized that an upregulated state of inflammation in both mother and foetus plays an important role in the pathophysiology (111).

The overall CMR in our study was lower than reported in other studies. Prior studies conducted in Bangladesh, Nepal, The Gambia and the United States have reported CMRs of approximately 1.0 (46, 105). In this study population, maternal hypergammaglobulinemia was a major factor in reduced transplacental transfer of RSV neutralizing antibody. Maternal hypergammaglobulinemia reduced both infant titres and CMR before and after adjustment for covariates. The effect of hypergammaglobulinemia upon transfer is not surprising when one considers the mechanism. The active transport mediated by the FcRn receptors are sensitive to saturation. High levels of IgG antibody within the maternal circulation eventually achieve a saturation point that in turn prevents binding of additional antibody. These high levels of circulating antibody are not necessarily RSV specific. In fact, in this study maternal titres did not correlate with total IgG ($r^2$ = -0.002, p =0.56). This lack of correlation suggests there was a degree of competition created between RSV and non-RSV antibodies for receptor binding. The negative effects of maternal hypergammaglobulinemia on transplacental transfer of RSV antibody has been observed in other studies. In The Gambia, maternal hypergammaglobulinemia reduced the transplacental transfer of RSV antibody by 90%(117).

This study has several limitations which must be noted. First, this study was not designed to relate titre or transplacental transfer rates to a clinical outcome of RSV hospitalisation in either HUU or HEU infants. This would have been a valuable analysis given that the majority of the paediatric RSV burden is believed to occur in LMIC settings (1). The reason for this outsized LMIC burden is multi-factorial but lower levels of maternally derived antibody may be a contributor. In this study, South African CMRs were lower compared to other LMIC studies summarized in **Table 3.0.** This would suggest that RSV hospitalisation incidence would be higher among all infants compared to other populations. However, the RSV hospitalisation incidence rates reported in **Chapter 2** do not suggest that the Sowetan burden outweighs what is seen in high income settings. Furthermore, hospitalisation rates were

nearly identical between HUU and HEU infants (14 vs 13 per 1000 live births). Both of these findings are were in contrast to previous reports which estimated much higher South African disease burden in general and among HEU infants compared to HUU infants (73, 74). One cannot directly connect the data between the two chapters, however, the data analyses borrow from the same patient population and surveillance timeframe which does allow for further hypothesis generation. In this population, access to quality healthcare could make the difference for both mothers and their infants. For mothers, this is reflected in a high percentage of mothers receiving ART with low viral loads (although there were missing data). For infants, this is reflected in the majority of RSV hospitalisations resolving in discharge to home as was reported in **Chapter 2**.

Another important limitation was that samples were collected over a single year with 10 maternal-infant pairs for each arm selected by month. RSV is a seasonal disease that fluctuates in size from year to year. The data may have looked different had sample collection occurred over more than one season or concentrated within the seasonal timeframe when exposure is known to result in higher titres (96).

Lastly, data on viral loads were available for only half the women living with HIV. It would have been useful to have a more complete dataset to be able to better characterize the relationship between HIV-1 viral replication control and transplacental transfer as was demonstrated in the Botswana study referred to earlier (110).

Transplacental transfer of protective antibody is a complex biological process. Comparing titre levels in maternal and cord blood samples is a gross measure at best. Systems serology is a newer approach currently being explored to better understand the mechanisms that underpin transfer; down to the level of antibody glycosylation patterns (118). Recent research in this area suggest that the placenta behaves like a sieve, selecting antibodies with specific glycosylation patterns to induce downstream immune functions within the infant that are most appropriate for a particular antigen exposure. In this sense, the quality of antibody rather than the quantity may prove to be the more important measure of maternally derived immunity.

## Chapter 4 Exploring for an RSV sero-correlate of protection

### 4.1 Introduction

Maternally derived RSV neutralizing antibody has been inversely associated with infant RSV lower respiratory tract infection (LRTI) in some studies, however, a correlate of protection against RSV-LRTI remains elusive (13, 44, 96). Further, few studies did not observe any association between overall and F-protein epitope specific RSV neutralizing antibody and protection against RSV disease (47, 48). Palivizumab, a monoclonal antibody directed at the site II epitope on the pre-fusion F protein, has provided proof of concept that RSV specific neutralizing antibody can reduce severe RSV-LRTI (119, 120). The pivotal "IMpact" RSV trial upon which palivizumab licensure was based, demonstrated a 55% reduction in RSV associated hospitalisation in high risk preterm-birth infants who received the monoclonal (121).

The immune response to RSV infection is complex and evolves with age and repeat infections. There are time dependent orchestrations between innate/adaptive immunity and humoral/cellular responses (122-127). Although antibody directed to F-protein epitopes plays an important role in the reduction of disease severity, this role may be synergistic with other aspects of the immune response rather than centrally defining. Additionally, there are other physiological factors, such as infant airway diameter, which contribute to the risk of developing severe disease. While the clinical interpretation of neutralizing RSV titres can be challenging, a better understanding of their role in protection against disease remains an important endeavour; particularly as it relates to vaccine design (128, 129). There are several studies which have examined the protective role of RSV neutralizing antibody in infants, **Table 4.0**. Importantly, comparisons across studies are limited due to differences in study design, testing methods and criteria used for investigating for RSV LRTI, and RSV immunological assay methodologies. Nevertheless, there are important patterns which can be highlighted. This review will depict the evolution of the field's understanding of an RSV antibody as sero-correlate of protection.

#### 4.1.1 Higher maternally derived titres are associated with less infant disease

Glezen et al examined the relationship between levels of neutralizing RSV antibody and RSV disease via prospective surveillance of infants from low income setting in the United States (13). The study demonstrated through the evaluation of cord sera in cases (n=68) and randomly selected non-matched controls (n=575), that the geometric mean titre (GMT) were

significantly lower in cases (92 vs. 138, p <0.01); **Figure 4.0** is a graphical display of the distribution (13: p 710, table 3).

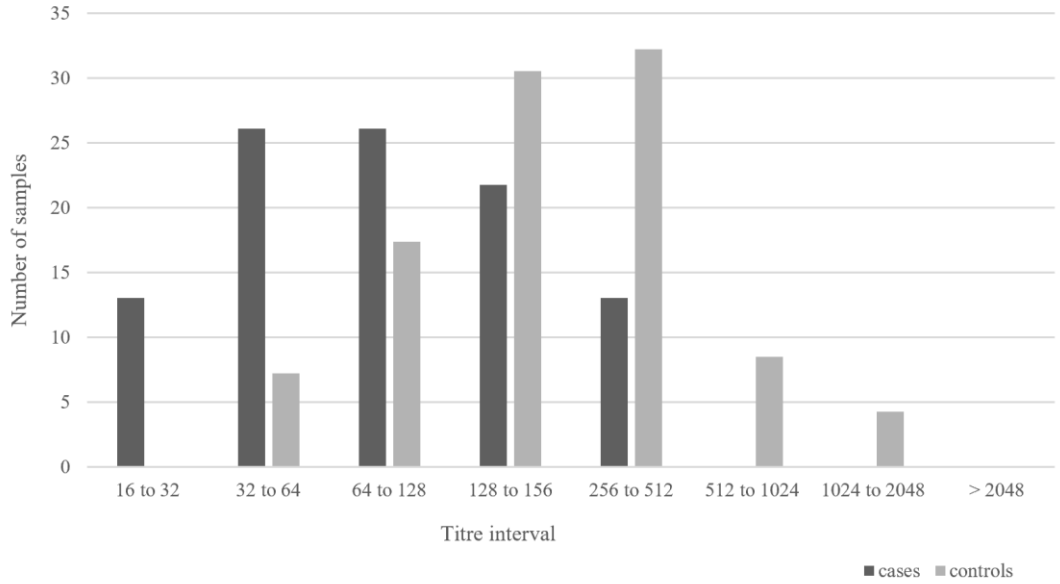


**Figure 4.0** Titre distributions of cases and controls during RSV epidemic period (13)

The relationship between higher RSV IgG titres and protection from disease was also observed by Olgivie et al. in the United Kingdom (130), in infants part of a prospective birth cohort representative from multiple socioeconomic backgrounds. All families who were screened for participation, however, had a member with asthma and the authors did not provide further detail on the rationale behind this selection process. In contrast to Glezen et al., the Olgivie study relied mostly upon maternal serum and did not measure neutralizing capacity of antibody. IgG titres in mothers varied considerably and likely reflected variable amounts of natural exposure to RSV. Despite this variability, mothers of RSV hospitalized cases (7.43 $\log_2 \pm 0.19$) and RSV community cases (7.63 $\log_2 \pm 0.31$) had lower GMTs of RSV IgG than the birth cohort (community) controls (9.35 $\log_2 \pm 0.23$; p <0.001).

### 4.1.2 The seasonal timing of maternal infectious exposure effects the titre level

The study by Glezen et al, further analysed RSV neutralizing GMTs in infants <6 months looking closely at birth timing relative to the RSV season; defined as either epidemic or pre-

epidemic periods. Amongst  cases, infants born during the epidemic period were younger and had lower GMTs compared to those born in the pre-epidemic period. Conversely among the controls, infants born during the epidemic-period had higher GMTs compared to those born during the pre-epidemic period. The majority of cases were infants born during or just prior to the epidemic period and were less than three months of age at the time of infection. Consequently,  Glezen concluded that the timing of maternal exposure to RSV played an important role in determining the amount of protective antibody that could be trans-placentally transferred to their infants. Infants who were born in the earlier part of the season received less antibody. This is due to a combination of factors within the mother including: waning RSV antibody from the prior season, less natural exposure to the RSV virus in the current season, less boosting of their immune memory to RSV, and thereby less transplacental transfer of neutralizing RSV antibody.  The concept of how seasonality links to both the amount of maternally derived antibody and consequent risk for RSV disease was corroborated almost three decades later in a Danish study by Stensballe et al (96).

The study by Stensballe et al. conducted a correlation analysis linking datasets on RSV cord blood titres to nationally reported rates of RSV hospitalisations in infants less than six months. The RSV seasonal timeframe was captured through the changing RSV hospitalisation incidence per 100,000 infants less than six months. The mean neutralizing titre measured in 459 randomly selected infants rises and falls in relationship to the season. Titres reach their nadir just prior to or during the peak of RSV hospitalisations.  As the epidemic progressed and maternal exposure increased, the cord titres rose and  peaked just as infant RSV hospitalisations declined; **Figure 4.1** (97: p. 297).

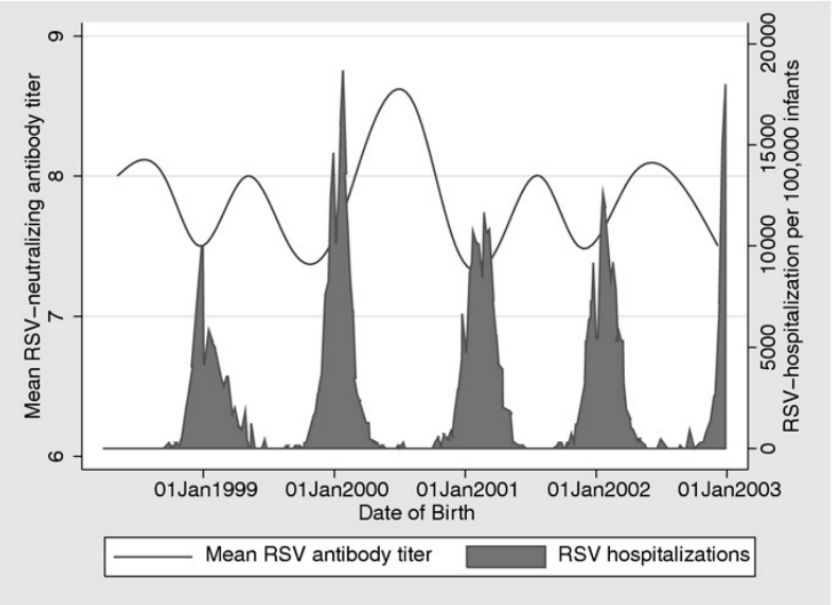


**Figure 4.1** Temporal relationship between RSV cord blood titres and RSV hospitalisation [96]

### 4.1.3 The inverse relationship between RSV microneutralization titre and RSV-LRTI

The study by Piedra et al examined the relationship between RSV neutralizing titres in acute serum and hospitalized RSV disease (44). Neutralizing titres to RSV subtypes A, B and surface protein F were measured for different age strata. For infants less than one year of age, the geometric mean serum neutralising titres against RSV subtype A, B and F protein were 4.1, 5.5 and 10.9, respectively. Utilizing logistic regression to describe the relationship between a single unit ($log_2$) fold rise and a reduction in the likelihood of hospitalisation, across all age groups for each unit $log_2$ increase in titre there was a 22% to 25% (depending upon the assay) reduction in likelihood of being hospitalized. A similar relationship between a unit fold rise and reduction in hospitalisation risk is described in several other populations (47, 95). In Native American infants, every $log_2$ increase in neutralising cord blood titre corresponded to a 30% decrease in RSV LRTI hospitalisation in infants <6 months (OR 0.69, p =0.0003) (95). In Denmark, every $log_2$ increase in neutralising titre corresponded to a 26% reduction in RSV hospitalisation in infants <6 months (IRR 0.74, 95% CI: 0.62–0.87) (131). In Kenya, every $log_2$ increase in neutralising cord blood titre corresponded to a 33% reduction in hospitalisation for infants less than 3 months of age, albeit non-significant (OR 0.67, 95% CI: 0.33–1.38, p =0.2) (47). In the Kenyan study, cases and age matched controls had similar mean concentrations of RSV neutralizing antibody (10.65 versus 10.8 $log_2$ , p =0.04); and multiple sub-analyses stratified by age at time of illness (including less than three or six months and 3-6 months age failed to demonstrate difference between cases and

controls. The authors speculated that there may be other factors which contribute to disease risk and that neutralising antibodies alone cannot overcome, and that protection against severe hospitalized RSV disease may require titres higher than the what natural exposure can induce.

The case control study conducted by Eick et al examined the role of maternally derived neutralizing RSV antibody and protection against RSV hospitalisation in Native American infants from the Navajo and White Mountain Apache tribes (95). Prior epidemiological studies had demonstrated a significantly increased risk of RSV hospitalisation in these two groups; two and five fold higher than the general U.S. population (132, 133). Eick hypothesized that the increased incidence and severity among Native American tribes might be related to sub-optimal levels of maternally derived neutralizing antibody. The study explored this relationship from several angles by measuring baseline maternal titres, transplacental transfer rates, and antibody half-life. Cases had lower titres than their matched controls (392.1 vs 538) with non-overlapping 95% CI. Among these, there was a higher proportion of controls with a titre above 500 (OR 0.61, p =0.034). The transplacental transfer ratio was 1.19. Among cases, there was no association between titre levels and disease severity.

### 4.1.4 The quantity of antibody can impact disease severity in some infants

Three studies provide insight into the relationship between neutralising RSV titres and disease severity. The case control study by Ogilvie et al, also compared RSV IgG titres between hospitalized and community cases (130). The GMTs reported for hospitalized (7.43 $\log_2 \pm 0.19$) and community cases (7.63 $\log_2 \pm 0.31$) were lower than in controls (9.35 $\log_2 \pm 0.23$), but similar to one each other. This poses the question regarding the extent to which neutralizing antibody can mitigate disease severity once infection has occurred. Notably, the study by Ogilvie et al mainly analysed IgG GMT in maternal serum as opposed to cord blood, however, the transplacental transfer ratio was generally $\geq 1.0$ which suggests that cord blood GMTs would be similar (46).

In a more recent but different study design by Walsh et al, anti-F protein titre values were measured in acutely ill infants using an enzyme immunoassay (EIA) and then correlated to a clinical severity score (as opposed to correlating to risk for hospitalisation) (134). These sera were collected from three separate group of RSV-infected children from outpatient, emergency room and hospitalized settings. There were no controls included. Amongst all

infants from all acute care settings, each 2-fold rise in anti-F titre was associated with a 0.56 unit decline in global respiratory severity score or GRSS (p =0.009). When the analysis was limited to infants during their first two months of life, the 2-fold increase correlated to a 0.9 unit decline in the GRSS, suggesting that the maternally derived RSV antibody impart a greater degree of protection against severe disease in the youngest infants when both titre and risk for disease are at their highest levels.

### 4.1.5 The quality of maternally derived antibody also matters

The study by Freitas et al evaluated both RSV titre levels and avidity in the acute sera of children hospitalized for RSV (135). Avidity refers to the ability of a single antibody to bind to multiple epitopes located upon the antigen and suggests a higher quality antibody response. In this study, avidity was defined as low, intermediate or high based on predetermined, arbitrary cut offs applied to an avidity index. This study conducted in Brazilian children identified significantly different percentages of high and low avidity RSV neutralizing antibody in infant less than three months of age. Infant cases had lower percentages of high-avidity antibody compared to controls (9.5% vs 42.1%, p =0.0055). A near identical pattern was observed for IgG1; a subclass of antibody preferentially transported across the placenta to the foetus (4.9% vs 42.1%, p =0.0009). Conversely, a higher percentage of low avidity antibody was measured in the cases compared to controls (51.2% vs 10.5%, p =0.0037), suggesting that infants less than three months are less able to neutralize RSV due to mainly having low avidity antibody. Similar findings were observed in a study from The Netherlands in infants less than 3 months of age (48).

The studies reviewed measured the relationship between antibody levels and protection against disease differently which makes comparisons difficult. Some studies measured anti-F IgG while others measured neutralizing antibody and or epitope specific neutralizing antibody. Despite differences in methods, anti-RSV antibody levels were generally higher in controls even amongst vulnerable populations (13, 96).

This objective of this study was to investigate the association between RSV/A IgG neutralizing antibody and hospitalisation for RSV-associated LRTI in a setting with a high prevalence of maternal HIV-infection. Furthermore, we explore for a sero-correlate of protection against RSVA/B LRTI hospitalisation.

**Table 4.0** Summary of the key literature exploring the protective role of RSV antibody

| **Author / publication and data years** | **Study design** | **Study population and Sample Size** | **Samples and testing** | **Results** |
|---|---|---|---|---|
| **Glezen (13)**<br><br>**1981**<br><br>Data collection years: 1975 - 1979 | Described the distribution of RSV neutralizing antibody in cord sera from RSV cases and controls within a prospective hospital/outpatient respiratory and sepsis surveillance program. .<br><br>Cord sera from cases were matched to controls <6 months who were "similarly aged" and born during the same pre-epidemic and epidemic periods. Cases were both hospitalized and outpatient. | U.S.A - one city, low income communities from one city<br><br>68 cases / 575 controls | nasal wash for viral culture<br><br>cord blood for RSV IgG<br><br>neutralizing antibody assay | Protection against RSV disease is correlated to the level of maternally derived antibody. Higher levels are correlated to less severe disease and a delay in onset to primary infection within the first six months of life.<br><br>Cases GMT: 92<br><br>Controls GMT: 138<br><br>$p < 0.01$ |
| **Olgivie** **(130)**<br><br>**1981**<br><br>Data collection years: n/a | Examination of RSV specific IgG antibody levels in infants and their mothers via a prospective birth cohort in infants <1 year with prospective surveillance for RSV hospitalisation. | U.K. - one city, all social classes<br><br>48 cases / 31 controls | NPA for virus isolation and/or immune-fluorescence, plus one serologically identified case<br><br>Maternal serum* for RSV IgG indirect | There are variable RSV titre levels in mothers. There were significant lower IgG RSV titre levels measured in infant cases versus controls. |

| Author / publication and data years | Study design | Study population and Sample Size | Samples and testing | Results |
|---|---|---|---|---|
| | Controls were uninfected infants from same cohort but no details on specific matching criteria were provided<br><br>Note - infants were selected from families with one asthmatic member. | | membrane immunofluorescence<br><br>*Only 3 cord blood samples were available | Cases (Hospitalized) 7.43 $\log_2 \pm 0.19$<br>Cases (Community) 7.63 $\log_2 \pm 0.31$<br>Controls 9.35 $\log_2 \pm 0.23$<br><br>$p < 0.001$ in each case |
| **Piedra** (44)<br><br>**2003**<br><br>Data collection years: 1991 - 1993 | Measured RSV neutralizing titres in patients between 1 month and 89 years who were hospitalized for an acute LRTI. Patients were part of a larger prospective epidemiological study of respiratory infections.<br><br>Controls were non-RSV LRTI hospitalisation but do not explicitly describe matching criteria.<br><br>Utilized logistic regression to calculate a fold rise relative to hospitalisation. Age, RSV season, RSV specific titres were used in the model. | U.S.A – one city<br><br>included 28 infants | Nasal wash and/or throat swab for viral culture<br><br>Acute and convalescence serum for microneutralization to RSV A and B | Inverse relationship between level of RSV antibody at time of acute infection and RSV hospitalisation across all ages.<br><br>GMT of infants <1 year for<br><br>RSV A: 4.1 ± 2.0<br><br>RSV B: 5.5 ± 2.5<br><br>ELISA F: 10.9 ± 2.1<br><br>RSV/A of $\geq 6.0 \log_2$ : 3.5 times more likely to not be hospitalised (95% CI: 1.4–9.1, p =0.008) |

| Author / publication and data years | Study design | Study population and Sample Size | Samples and testing | Results |
|---|---|---|---|---|
| | | | | RSV/B ≥8.0 $log_2$, : 2.9 times more likely to not be hospitalised (95% CI: 1.1–7.7. p =0.03)<br><br>No threshold titre identified for ELISA-F binding antibodies |
| **Eick** (95)<br><br>**2008**<br><br>Data collection years: 1997 - 2000 | Explored the relationship between neutralizing RSV antibodies and protection against RSV disease.<br><br>Utilized logistic regression to calculate the difference in titres between cases and control infants <6 months via prospective population-based hospital surveillance<br><br>Controls matched by date of birth and geographic location | U.S.A -<br><br>2 Native American tribes<br><br>169 cases / 248 controls | NPA for<br><br>EIA<br><br>Cord blood for RSV IgG neutralizing assay 60% neutralization | Inverse relationship between level of maternally derived RSV antibody and RSV hospitalisation.<br><br>Cases 392.1 (95% CI: 336.9 - 456.4), Controls 538 (95% CI: 470.9 - 609.2)<br><br>Severe cases: 385.6<br><br>Non-severe cases: 386.1<br><br>For every $log_2$ increase in titre of cord blood titre, subjects were 30% less likely to be hospitalized. OR 0.69, p =0.0003. |

| Author / publication and data years | Study design | Study population and Sample Size | Samples and testing | Results |
|---|---|---|---|---|
| **Stensballe** (96)<br><br>**2009**<br><br>Data collection years: 1998 - 2003 | Prospectively examined the correlation between levels of RSV antibody in cord blood with RSV seasonality in infants by linking two data sets via correlation analysis.<br><br>Data set 1: cord blood RSV titres<br><br>Data set 2: RSV hospitalisations per 100,000 infants <6 months<br><br>No controls. | Denmark - (national data)<br><br>459 random cord samples | Not specified - utilized national database on RSV hospitalisations<br><br>Cord blood for RSV IgG microneutralization assay | Inverse relationship between level of maternally derived RSV antibody and RSV hospitalisation.<br><br>Population level GMTs < 7.5 $\log_2$ or 1: 181 is correlated to a steep rise in RSV hospitalisations for 3 months |
| **Freitas** (135)<br><br>**2011**<br><br>Data collection years: 2000 - 2009 | Measured the levels and avidity of RSV total and isotype specific antibodies via an age-matched case control of children <5 years hospitalized for respiratory disease.<br><br>Additionally, explored relationship between avidity and RSV disease severity (upper versus lower respiratory tract infection) | Brazil – one city<br><br>104 cases / 66 controls | NPA for IFA<br><br>Serum collected at time of illness (for indirect ELISA to measure levels of RSV IgG1, IgG2, IgG3, IgG4. Avidity assay for levels of IgG1 and IgG3 | Infants < 3 months had high levels of RSV antibody. Antibody avidity was lower in RSV cases compared to age matched controls. Higher levels of high avidity antibodies were associated with less severe disease in children >24 months.<br><br>High avidity RSV total IgG < 3 months:<br>Controls: 42.1% (8/19) |

| Author / publication and data years | Study design | Study population and Sample Size | Samples and testing | Results |
|---|---|---|---|---|
| | Age matched controls. | | | Cases 9.5% (4/42)<br>p =0.0055<br><br>High avidity RSV IgG1 <3 months:<br>Controls: 42.1% (8/19)<br>Cases: 4.9% (2/41)<br>p =0.0009<br><br>Low avidity RSV IgG1 <3 months:<br>Controls: 10.5% (2/19)<br>Cases: 51.2% (21/41)<br>p =0.0037 |
| **Nyiro** (47)<br><br>**2016**<br><br>Data collection years: 2002 - 2007 | Measured levels of maternally derived RSV antibody at birth in order to identify a protective threshold against hospitalized, severe pneumonia or LRTI in infants <6 months.<br><br>Aged matched case controls | Kenya - Rural, coastal community<br><br>30 cases / 60 controls | IFAT<br><br>Cord blood for RSV plaque reduction neutralizing titres 50% neutralization | Unable to identify titre threshold of protection against severe RSV disease. Titres between cases and controls overlapped. Natural exposure may not induce high enough titres to protect against severe disease.<br><br>Overall:<br>Cases mean titre 10.65 log2 (10.3–11.0)<br>Controls mean titre 10.8 log2 (10.6–11.1)<br>p =0.04<br><br><6 months age group:<br>Cases mean titre: 10.7 (10.3–11.0)<br>Controls mean titre: 10.8 (10.6–11.1) |

| Author / publication and data years | Study design | Study population and Sample Size | Samples and testing | Results |
|---|---|---|---|---|
| | | | | p =0.4<br><br><3 month age group<br>Cases mean titre: 10.3 (9.6–10.9)<br>Controls mean titre:<br>10.7 (10.2–11.1)<br>p =0.3<br><br>3-6 month age group:<br>Cases mean titre 3:<br>11.0 (10.6–11.3)<br>Controls mean titre:<br>11.0 (10.6 –11.4)<br>p =0.9 |
| **Walsh** (134)<br><br>**2018**<br><br>Data collection years: 2012 - 2015 | Measure RSV antibody levels associated with severe RSV illness in healthy full term infants during primary RSV infection.<br><br>No controls.<br><br>Mild to severe RSV cases recruited from 3 cohorts:<br><br>Birth cohort followed development of RSV infection via active and passive surveillance.<br><br>Supplemental cohort enrolled at time of respiratory infection from outpatient and emergency rooms. | U.S.A – one city | RT-PCR<br><br>Acute serum collected at time of illness<br><br>Correlated each titre measurement to a global respiratory severity score (GRSS)<br><br>All infections:<br>Anti-F<br>Anti-Ga<br>Anti-Gb<br><br>Neutralizing titres:<br>Subtype A | Multivariate analysis adjusting for age at time of infection demonstrated an inverse relationship between titre level and disease severity. Higher titres were associated with a delay in time to infection.<br><br>All infants:<br>Each 2 fold increase in anti-F titre associated with a 0.56 unit decline in GRSS (p =0.009)<br><br>Infants ≤2 months: |

| Author / publication and data years | Study design | Study population and Sample Size | Samples and testing | Results |
|---|---|---|---|---|
| | Hospitalized cohort enrolled at time of RSV verified admission. | | Subtype B | Each 2-fold increase in anti-F titre associated with a 0.9 unit decline in GRSS (p =0.13) |

## 4.2 Methods

We undertook a matched case-control study in Sowetan infants in order to examine the association of maternally derived RSV/A neutralizing antibody in cord blood and risk of RSVA/B LRTI hospitalisation. Cases and controls were selected from an existing birth cohort of 38,223 infants (28OB study HREC approval number 140203). All cases were infants less than six months of age at the time of hospitalisation; identified through established surveillance described in chapter 2. A cross referenced list was used to confirm that an RSV hospitalized infants also had a cord blood sample available from the birth cohort. Cases were then individually matched to non-RSV controls from the same birth cohort. Cord blood RSV/A IgG neutralizing antibody titres were measured in both cases and controls. The null hypothesis assumed that there would be no difference in levels of maternally derived RSV neutralizing antibody between hospitalized RSV-LRTI cases and matched-controls. The alternative hypothesis would be accepted if a significant difference between hospitalized RSV cases and control infants was detected.

Case and controls (1 to 4 ratio) were matched on six variables including infant sex, infant date of birth, term/preterm status, birth weight category, maternal age, maternal HIV status. Date of birth was matched within one week. A term birth was defined as gestational age ≥37 weeks and preterm birth as gestational age <37 weeks. There were four birth weight categories including <2500, 2500-3000, 3001-3499 and ≥3500 grams. Maternal age was matched within five years of age.

The initial matching process resulted in the identification of 221 cases and 837 controls. A total of 143 cases (63.5%) were matched to four controls. The remaining cases could not be matched based on the four birth categories. To facilitate matching for the remaining 82 cases, the original four birth weight categories were compressed into two (i.e. <2500 and ≥2500 grams), following which 55 cases (24.4%) were matched to three controls, 15 (6.7%) to two controls, 6 (2.7%) to one control, whilst no controls were identifiable for 6 cases (2.7%) who were excluded from further analyses. From the original case control match list, there were 2 cases and 20 controls who did not have sera available to analyse. The final analysis had 219 cases and 817 controls available for analysis in infants <6 months of age.

Additionally, three exploratory analyses were performed:

The first exploratory analysis re-ran the full set of statistical tests using the ED80, to determine if a higher threshold of virus neutralisation unmasked effects not observed with the ED50.

In the second exploratory analysis, statistical testing was limited to infant cases and controls less than 3 months of age. This was done in order to explore for a stronger protective effect of neutralizing antibody against severe LRTI in the first few months of life; a pattern which was observed in a study by Walsh et al. (134).

The third exploratory analysis re-ran the full set of statistical tests after removing any control infants with a history of all-cause LRTI or sepsis admission during the first six months of life. This was done by examining RMPRU paediatric surveillance registry from which cases had been identified. Any controls hospitalized with an admission diagnosis that could have been associated with RSV were removed from the analysis. These admission codes included: LRTI, bronchiolitis, pneumonia, sepsis and urinary tract infections and resulted in exclusion of 43 controls. After the removal of these controls it was confirmed that every case had at least one control.

### 4.2.1 Laboratory methods

Cord blood serum samples were analysed at the Respiratory and Meningeal Pathogens Research Unit for RSV/A neutralizing antibody using a microneutralization assay described earlier (131, 136). The assay was performed in 96-well flat bottom, micro-titre plates using HEP2 cells (ATCC CCL-81) and the long strain of human RSV (ATCC VR-26). Each 96-well plate contained four patient samples tested in duplicate using 12 two-fold dilutions. For each assay, the following controls were included: an Intravenous immunoglobulin (IVIG) control, which was run the same as a test plate, but using IVIG instead of patient serum at four different dilutions; and another control plate with 2 rows of negative controls (no virus and no serum), 2 rows of positive controls (virus but no serum), and 4 rows to back-titrate the virus. Paired maternal and cord sera were tested on the same microtiter plate. Plates were incubated at 37 °C and 5% $CO_2$ for 144 hours (6 days) with daily checks for virus-specific cytopathic effect. After the six-day incubation period the plates were: decanted, overlaid with crystal violet, and left to stain for 24 hours. After the 24-hour staining period the plates were gently decanted, rinsed and allowed to dry upside down before reading the wells. Readings were performed immediately after drying and then a second time two days later to ensure accuracy. Titres were determined by the last well with at least 50% fully protected HEP2 cell

monolayer. The final titre for each sample was the average of its duplicate pair. The Reed and Meunch method was used to calculate the end dose (ED50 and ED80).

### 4.2.2 Statistical analysis

The titre distributions (GMT) were analysed using the frequency procedure. An accompanying chi square test was used to determine if those distributions were significantly different from one another. The equal (pooled) variance independent T-test procedure was used to evaluate for a significant difference between mean titres ($log_2$). The 95% confidence level for the means were provided. P-values were set to less than 0.05. A conditional (multivariable) logistic regression model was used to estimate the odds of RSV hospitalisation looking at different risk factors including unit titre ($log_2$), seasonality of birth, smoking during pregnancy, alcohol use during pregnancy and the presence of < or >3 siblings within the household.

## 4.3 Results

Expectantly, mothers of cases and controls were similar in mean age, HIV infection, smoking and alcohol usage during pregnancy and parity categories (**Table 4.1**).

### 4.3.1 Comparing ED50 titres distribution and values in RSV case and control infants <6 months of age

The comparative titre distribution demonstrated that the majority (52.63%) of infant controls were above a value of 1024 compared to less than half (41.55%) of cases (**Table 4.2).** The titres for controls ranged between 1130 and 1263 with a mean GMT of 1195 whereas cases had a significantly lower range with a mean GMT of 950 ($p < 0.0002$). The comparative mean $log_2$ titres for cases and controls were different with statistical significance; 9.89 vs 10.22, $p < 0.0002$ (**Table 4.3**).

**Table 4.1** Baseline demographic and clinical characteristics of mothers and infants with (cases) and without (controls) RSV lower respiratory tract infection hospitalisation

| | Overall | Cases | Controls |
|---|---|---|---|
| **No of mothers** | **1036** | **219** | **817** |
| **Mean Maternal age** | 27.05 (17.0 - 45.0) | 26.98 (17.0 - 44.0) | 27.07 (18.0 - 45) |
| **Parity** | | | |
| <3 | 819 (79.1) | 169 (77.2) | 650 (79.6) |
| ≥3 | 94 (9.1) | 28 (12.8) | 66 (8.1) |
| **HIV status** | | | |
| HIV uninfected | 761 (73.5) | 161 (73.5) | 600 (73.4) |
| **Smoking during pregnancy** | | | |
| **No** | 808 (96.4) | 168 (96.6) | 640 (96.4) |
| **Alcohol during pregnancy** | | | |
| **No** | 772 (92.9) | 160 (91.4) | 612 (93.3) |
| **No of infants** | **1036** | **219** | **817** |
| **Female** | 612 (59.1) | 128 (58.5) | 484 (59.2) |
| **Gestational age** | | | |
| **>37 weeks** | 873 (84.3) | 181 (82.7) | 692 (84.7) |
| **<37 weeks** | 163 (15.7) | 38 (17.4) | 125 (15.3) |
| **Mean birth weight in grams** | | | |
| **(SD)** | 3040 (635 - 5800) | 2995.3 (905.0 - 5800) | 3052.8 (635.0 - 4810.0) |

Missing values for maternal smoking status 198, for maternal alcohol status 205

**Table 4.2** RSV Geometric mean titre distribution for all cases and controls

| ED50 GMTs | 64 to <128 | 128 to <256 | 256 to < 512 | 512 to <1024 | >1024 | Total |
|---|---|---|---|---|---|---|
| **Infants <6 months** | | | | | | |
| **Cases** | 0 (0) | 7 (3.20) | 53 (24.20) | 68 (31.05) | 91 (41.55) | **219** |
| **Controls** | 2 (0.24) | 17 (2.08) | 103 (12.61) | 265 (32.44) | 430 (52.63) | **817** |
| **Infants <3 months** | | | | | | |
| **Cases** | 0 (0) | 2 (2.94) | 14 (20.59) | 27 (39.71) | 25 (36.76) | **68** |
| **Controls** | 2 (0.82) | 7 (2.87) | 29 (11.89) | 84 (34.43) | 122 (50.00) | **253** |

| ED80 GMTs | 32 to <64 | 64 to <128 | 128 to <256 | 256 to <512 | 512 to <1024 | >1024 | Total |
|---|---|---|---|---|---|---|---|
| **Infants <6 months** | | | | | | | |
| **All cases** | 0 (0) | 2 (0.91) | 31 (14.16) | 62 (28.31) | 64 (29.22) | 60 (27.40) | **219** |
| **All controls** | 1 (0.12) | 4 (0.49) | 45 (5.51) | 184 (22.52) | 279 (34.15) | 304 (37.21) | **817** |
| **Infants <3 months** | | | | | | | |
| **Cases** | 0 (0) | 0 (0) | 10 (14.71) | 21 (30.88) | 21 (30.88) | 16 (23.53) | **68** |
| **Controls** | 1 (0.40) | 4 (1.58) | 12 (4.74) | 50 (19.76) | 99 (39.13) | 87 (34.39) | **253** |

Missing values ED50 and ED80 =20 (2 cases, 18 controls)

**Table 4.3** Geometric mean titres and $\log_2$ titres for all cases and controls

| | | 95% confidence limit | | | 95% confidence limit | | | |
|---|---|---|---|---|---|---|---|---|
| **ED50** | **GMT** | **lower** | **upper** | **mean titre ($\log_2$)** | **lower** | **upper** | **p-value** | **Total** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Infants <6 months** | | | | | | | | |
| All cases | 950.61 | 847.36 | 1066.43 | 9.89 | 9.73 | 10.06 | 0.0002 | **219** |
| All controls | 1195.25 | 1130.92 | 1263.23 | 10.22 | 10.14 | 10.30 | | **817** |
| **Infants <3 months** | | | | | | | | |
| Cases | 923.74 | 756.11 | 1128.53 | 9.85 | 9.56 | 10.14 | 0.0615 | **68** |
| Controls | 1129.80 | 1027.59 | 1242.18 | 10.14 | 10.01 | 10.28 | | **253** |

| | | **95% confidence limit** | | **mean titre** | **95% confidence limit** | | | |
|---|---|---|---|---|---|---|---|---|
| **ED80** | **GMT** | **lower** | **upper** | **($log_2$)** | **lower** | **upper** | | **Total** |
| **Infants <6 months** | | | | | | | | |
| All cases | 689.42 | 613.66 | 774.53 | 9.43 | 9.26 | 9.60 | 0.0001 | **219** |
| All controls | 878.19 | 830.74 | 928.35 | 9.78 | 9.7 | 9.86 | | **817** |
| **Infants <3 months** | | | | | | | | |
| Cases | 661.9 | 541.14 | 809.6 | 9.37 | 9.08 | 9.66 | 0.0357 | **68** |
| Controls | 829.35 | 754.01 | 912.23 | 9.70 | 9.56 | 9.83 | | **253** |

Missing values ED50 and ED80 =20 (2 cases, 18 controls)

### 4.3.2 Risk factors for RSV hospitalisation in infants <6 months of age

A multivariate conditional logistic regression was used to analyse the effect of select variables upon an outcome of RSV hospitalisation in infants less than six months of age using ED50 values (**Table 4.4**). These factors included: cord blood titre, seasonality, smoking and alcohol usage during pregnancy and presence of siblings (<3 vs >3) in the home. The results indicated that only an increase of infant's cord blood titre (as a unit $\log_2$) was significantly associated with an OR of 1.43 (95% CI 1.193–1.715, p <.0001). The association between cord blood titre and RSV hospitalisation can be summarized as follows: in infants who were less than six months of age, every unit of $\log_2$ titre increase resulted in a 43% reduced odds of RSV hospitalisation.

**Table 4.4** Risk factor analysis for RSV hospitalisation in infants *

| Variable | ED50 <6 months OR (95% CI) | p-value | ED50 <3 months OR (95% CI) | p-value |
|---|---|---|---|---|
| **Cord blood titre (log2)** | 1.431 (1.193-1.715) | 0.0001 | 1.390 (0.946-2.044) | 0.0934 |
| **Seasonality** | 0.494 (0.060 - 4.092) | 0.5134 | <0.001 (<0.001->999.999) | 0.9935 |
| **Smoking** | 1.028 (0.354–2.986) | 0.9596 | <0.001 (<0.001->999.999) | 0.9943 |
| **Alcohol** | 0.766 (0.366–1.604) | 0.4793 | >999.999 (<0.001->999.999) | 0.9947 |
| **Siblings <3 vs >3** | 1.511 (0.811–2.817) | 0.1936 | 3.346 (0.796-14.068) | 0.0993 |

| | ED80 <6 months | | ED80 <3 months | |
|---|---|---|---|---|
| | **OR (95% CI)** | **p-value** | **OR (95% CI, p-value)** | **p-value** |
| **Cord blood titre (log2)** | 1.422 (1.192-1.696) | <0.0001 | 1.440 (0.979-2.119) | 0.0642 |
| **Seasonality** | 0.493 (0.059-4.111) | 0.5135 | <0.001 (<0.001->999.999) | 0.9936 |
| **Smoking** | 1.019 (0.351-2.963) | 0.9718 | <0.001 (<0.001->999.999) | 0.9943 |
| **Alcohol** | 0.758 (0.362-1.590) | 0.4642 | >999.999 (<0.001->999.999) | 0.9948 |
| **Siblings <3 vs >3** | 1.515 (0.813-2.823) | 0.1913 | 3.501 (0.820-14.949) | 0.0907 |

* ORs are adjusted via multivariate regression

### 4.3.3 ED80 threshold and association with RSV LRTI hospitalisation <6 months of age

The titre distributions were assessed using an ED80 threshold to further explore for titre differences between cases and controls that may have been otherwise masked by an ED50 threshold (**Figure 4.2**). ED50 and ED80 values correlated strongly (R =0.9456). Overall, the qualitative or directional relationship between cases and controls was maintained using either ED50 or ED80. The overall impact of using ED80 instead of ED50 was that it lowered the mean titre values in both case and controls while maintaining a small but significant difference between the two groups (9.43 vs 9.78, p <0.0001) (**Table 4.3**)

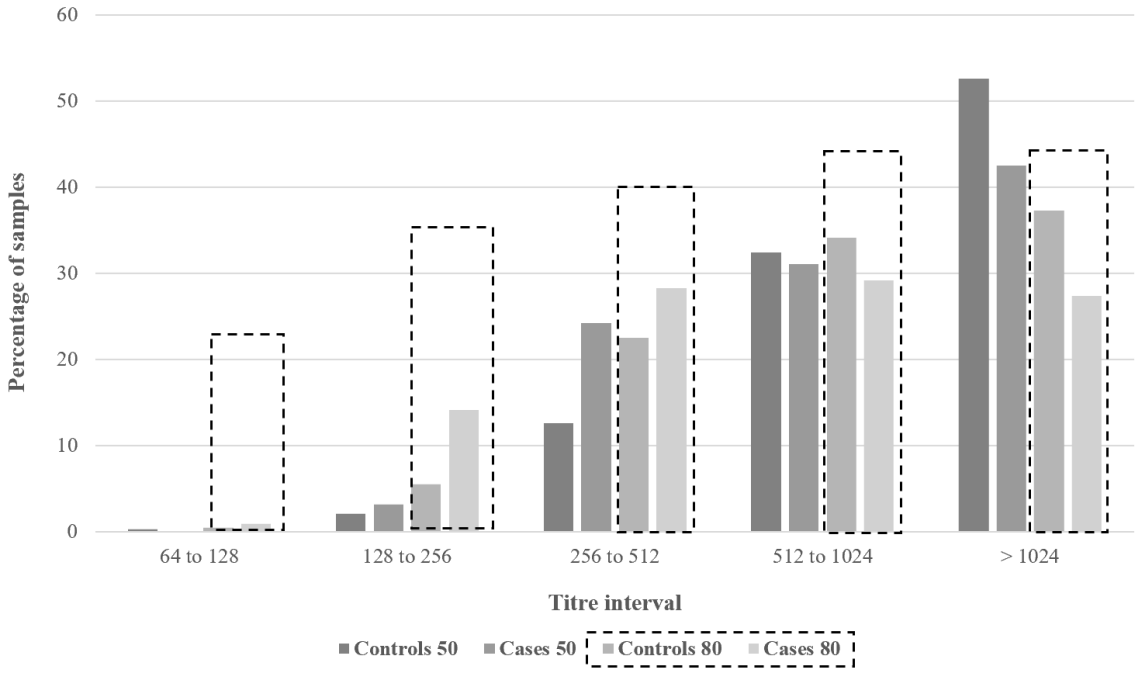


**Figure 4.2** Comparative titre distributions of all cases and controls, ED50 vs ED80

The risk factor analysis using ED80 in infants <6 months was directionally similar to the ED50 in the same cohort (**Table 4.4**). In the ED80 analysis, the infant cord blood titre remained strongly correlated to a statistically significant reduction in odds for RSV hospitalisation (OR =1.425 95% CI: 1.195 -1.699, p <0.0001).

### 4.3.4 Limiting the analysis to the first 3 months of life, ED50 and ED80

By restricting the analysis to infants less than 3 months of age, the titre distributions and comparative GMTs/$log_2$ titres were overall lower but remained directionally consistent with those observed in infants <6 months of age (**Tables 4.2 and 4.3**). In the risk factor analyses, cord blood titres maintained similar OR values but lost their statistical significance.

### 4.3.5 Excluding controls with a history of respiratory or sepsis related hospitalisation in the six months of life

The third sensitivity analysis re-ran the full set of statistical tests in infants <6 months after removing select control infants (n =43) with a history of LRTI, bronchiolitis, pneumonia, sepsis or urinary tract infection admission during the first six months of life. The statistical testing of the remaining controls did not result in dramatic differences in case/control titre distributions (**Table 4.6**) or when comparing GMTs/$log_2$ titres between cases and controls (**Table 4.7**).

**Table 4.6** RSV Geometric mean titre distribution for all cases and controls <6 months, *with select controls removed* *

| **ED50 GMTs** | **64 to <128** | **128 to <256** | **256 to <512** | **512 to <1024** | **>1024** | **Total** |
|---|---|---|---|---|---|---|
| **Infants <6 months** | | | | | | |
| **Cases** | 0 (0) | 7 (3.23) | 53 (24.42) | 68 (31.34) | 89 (41.01) | **217** |
| **Controls** | 2 (0.26) | 17 (2.19) | 98 (12.65) | 250 (32.26) | 408 (52.65) | **775** |

| **ED80 GMTs** | **32 to <64** | **64 to <128** | **128 to <256** | **256 to <512** | **512 to <1024** | **>1024** | **Total** |
|---|---|---|---|---|---|---|---|
| **Infants <6 months** | | | | | | | |
| **All cases** | 0 (0) | 2 (0.92) | 31 (14.29) | 62 (28.57) | 63 (29.03) | 59 (27.19) | **217** |
| **All controls** | 1 (0.13) | 4 (0.52) | 42 (5.42) | 175 (22.58) | 267 (34.45) | 286 (36.90) | **775** |

* 43 controls which were removed if they had a history of all-cause LRTI or sepsis admission during the first six months of

**Table 4.7** Geometric mean titres and log2 titres for all cases and controls infants <6 months, *with select controls removed* *

| | | 95% confidence limit | | mean titre | 95% confidence limit | | | |
|---|---|---|---|---|---|---|---|---|
| **ED50** | **GMT** | **lower** | **upper** | **($log_2$)** | **lower** | **upper** | **p-value** | **Total** |
| **Infants <6 months** | | | | | | | | |
| All cases | 946.49 | 842.94 | 1062.76 | 9.89 | 9.72 | 10.05 | 0.0003 | **217** |
| All controls | 1191.88 | 1126.01 | 1261.61 | 10.22 | 10.14 | 10.30 | | **775** |

| | | 95% confidence limit | | mean titre | 95% confidence limit | | | |
|---|---|---|---|---|---|---|---|---|
| **ED80** | **GMT** | **lower** | **upper** | **($log_2$)** | **lower** | **upper** | **p-value** | **Total** |
| **Infants <6 months** | | | | | | | | |
| All cases | 685.94 | 610.08 | 771.22 | 9.42 | 9.25 | 9.59 | 0.0001 | **217** |
| All controls | 876.03 | 827.42 | 927.49 | 9.78 | 9.70 | 9.86 | | **775** |

* 43 controls which were removed if they had a history of all-cause LRTI or sepsis admission during the first six months of life

The risk factor analysis was repeated after the 43 selected controls were removed for both ED50 and ED80 datasets to explore for an impact (**Table 4.8 and 4.9**). As was seen with previous analyses, the cord blood titre as a unit $log_2$ was most strongly associated with a reduction in odds of RSV hospitalisation.. More specifically, for the ED50 analysis the OR for cord blood titre as unit $log_2$ was 1.39, 95% CI: 1.159–1.669, p <0.0004). The ED80 analysis were very similar.

**Table 4.8** Risk factors for RSV hospitalisation in infants <6 months, *with select controls removed* *

| Variable | ED50 <6 months OR (95% CI) | p-value |
|---|---|---|
| **Cord blood titre (log2)** | 1.391 (1.159-1.669) | 0.0004 |
| **Seasonality** | 0.496 (0.060-4.068) | 0.5135 |
| **Smoking** | 1.031 (0.357-2.980) | 0.955 |
| **Alcohol** | 0.787 (0.367-1.688) | 0.5393 |
| **Siblings <3 vs >3** | 1.503 (0.798-2.833) | 0.2073 |
| **Variable** | **ED80 <6 months OR (95% CI)** | **p-value** |
| **Cord blood titre (log2)** | 1.383 (1.158-1.652) | 0.0003 |
| **Seasonality** | 0.495 (0.060-4.081) | 0.5132 |
| **Smoking** | 1.021 (0.353-2.950) | 0.9696 |
| **Alcohol** | 0.787 (0.367-1.689) | 0.5385 |
| **Siblings <3 vs >3** | 1.499 (0.796-2.825) | 0.2100 |

* 43 controls which were removed if they had a history of all-cause LRTI or sepsis admission during the first six months of life

### 4.4 Discussion

There was a significant difference in the mean ED50 titres between RSV-LRTI hospitalized case and control infants less than six months of age. ED80 analyses in this group revealed similar directional relationships as seen with ED50 analysis with slight changes to the percentages of each group within various titre intervals. The ED80 GMT and $log_2$ values were lower overall for both cases and controls which is an expected result when the cut off is raised for defining neutralization. RSV antibody collected from cord blood and measured as a unit $log_2$ titre was the strongly associated with a reduced odds of RSV hospitalisation (OR 43%).

Similar to previous studies, a definitive threshold of RSV/A MN titre for protection against RSV-LRTI hospitalisation was not identified through this study in South Africa. Nevertheless, the inverse relationship between higher titres and lower odds of hospitalisation was observed through conditional logistic regression; similar to observed in other studies (13, 44, 95, 131). In our study, there was a reduced odds of RSV hospitalisation of 43% (95% CI 1.93–1.715, p <.0001) for every unit rise in $log_2$ titre, which was higher than point estimate range reported from previous studies ( Kenyan, 33%; Denmark, 26%; and USA Native population, 30% (47, 95, 131).

The assay used for this study was a general RSV/A microneutralization assay summarizing the totality of the mother's polyclonal antibody response to RSV/A virus acquired by the infant (i.e. in cord blood). What this assay could not describe were more specific aspects of that polyclonal response such as avidity or the targeting of a highly neutralizing epitopes found on the pre-fusion form of the F surface protein (135, 137). It is possible that a more specific assay would have revealed larger differences between case and control infants.

A sensitivity analysis which excluded controls with a history of LRTI, UTI or sepsis related hospitalisations was performed in the unlikely event that these infants may have had RSV but were not identified through the RMPRU paediatric surveillance program. However, their exclusion did not alter the results materially.

This study has several strengths and limitations. The sample size was robust and the majority (63.5%) of cases were matched to four controls on multiple variables. An important matching criterion was the infant date of birth. Cases and controls were matched on a date of birth that occurred within one week in order to control for any confounding caused by age related risk. This matching variable however could not account for differences in either the timing or the amount of infectious exposure for both mother and infant. For the mother, previous studies have shown that the amount of maternal exposure relative to the seasonal timeframe

influences the level of antibody which can be transplacentally transferred (96). For the infant, the baseline characteristics summary did reveal a higher percentage of case mothers with three completed pregnancies. This could mean that case infants had higher infectious exposure through a larger number of siblings; a known risk factor for disease (15). One limitation is that this study was unable to account for controls who may have sought care for mild to severe RSV disease outside of surveillance system at CHBAH.

There has been a large body of work dedicated to RSV sero-correlates of protection. There are important clinical patterns and evidence from passively administered monoclonal antibody which point to the promising relationship between antibody and protection against severe disease. It is unclear if further studies on natural immunity will reveal a definitive threshold of protection. The limited conclusions of the data may stem from the limitations of the human immune response itself which the virus has successfully averted as evidenced by repeated infections (138-140). Current developments in active (vaccine) and passive (monoclonal) immunization specifically aim to overcome this assumed natural limitation (43). The sero-correlate of protection may have to wait for further insights through these trials.

## Conclusion

The numbers used to describe the global paediatric burden of RSV disease are undeniable; this virus is now a leading cause of respiratory infections children under five years around the world (1, 141). Global systematic reviews and modelling indicate that the burden is unfavourably tilted towards lower and middle income countries where multiple factors may exacerbate vulnerability to more severe forms of disease and poor outcomes (1). Equally important is the fact that RSV lacks an effective treatment and the one form of prevention (a monoclonal antibody) is largely inaccessible to the vast majority who suffer from the disease. Efforts are underway to develop effective and accessible interventions including vaccination, next generation monoclonal antibodies and anti-virals. Therefore, advancing an understanding of the RSV burden at the country-level is critical so that public health authorities can be prepared to make informed decisions for their when these interventions become available.

The South African specific RSV burden has been investigated for several decades (6, 7, 9, 64, 67, 72, 74, 142, 143). Over time these studies have helped narrow the focus onto specific populations believed to be at highest risk for disease including young infants and those with HIV infection or exposure (72). This dissertation aimed to complement and expand upon the existing data by telling a story on two levels. First, by describing and comparing the hospitalized RSV burden among HIV exposed and unexposed infants through measures of hospital incidence, clinical characteristics and outcomes by month of age. And second, describing the sero-epidemiology of RSV neutralizing antibody to further inform our understanding of natural immunity in the first months of life.

In Soweto, the distribution of hospitalized disease by month of age replicated what has been described in other populations however the overall incidence among infants <12 months was lower than expected when referring to other sources of data (2, 3, 71, 74, 82). Furthermore, hospitalisation incidence rates were similar between HIV exposed and unexposed infants despite prior predictions that HIV exposed infants were at higher risk for severe RSV disease (56, 74). These differences highlight the importance of understanding key differences across populations, study design, clinical practice and statistical methods which make translating results between studies difficult. The clinical course during hospitalisation was of moderate severity with only a small percentage of cases requiring ICU admission and low mortality outcomes. Despite the lower severity and low mortality, RSV hospitalisation should be viewed as a significant medical burden for the Sowetan infant population and the hospital that

cares for them. RSV cases accounted for approximately one quarter of the total 3490 respiratory related hospitalisations captured through the two years of surveillance. During the peak of the RSV season, these cases accounted for more than half (53.2%) of all respiratory related hospitalisations with the majority (69.3%) occurring in infants <6 months of age. The majority (69%) of RSV hospitalisations received antibiotics during the course of their stay; a likely result of utilizing the WHO IMCI guidelines and a lack of testing within the standard of care. Unfortunately, it is unclear what percentage of these cases may have had a bacterial co-infection warranting antibiotics but the assumption is that most received treatment unnecessarily. Given the rising concern over antibiotic resistance, one could make the case for incorporating virologic testing to avoid inappropriate usage.

RSV clinical presentation is a respiratory syndrome which overlaps with other viral causes of respiratory infection (25). A definitive RSV diagnosis requires confirmatory testing in order to be able to differentiate it from the many possible viral aetiologies. This means that for surveillance purposes it is important to know the frequency and association of clinical signs for RSV in order to be able to efficiently screen for potential cases. The dissertation data demonstrated a clinical syndrome similar to what has been described in the past including signs of cough, wheeze and respiratory distress (25, 78, 144).

The second and third objectives of the dissertation revealed important new information about RSV disease via sero-epidemiology studies. RSV neutralizing antibody is known to traverse the placenta and is associated with protection of infants against hospitalized disease (13, 44-46). Prior studies conducted in Nepal, Bangladesh, The Gambia and the United States demonstrated efficient transfer rates ≥1 in the infant. Therefore, it was surprising to see that the overall transplacental transfer rate of RSV neutralizing antibody was less efficient among Sowetan mothers both with and without HIV infection. Covariate analysis revealed that maternal hypergammaglobulinemia contributed to lower transfer rates which makes sense when considering the mechanism of transplacental transfer is vulnerable to receptor saturation. The implications of this finding are unclear as this objective did not relate transfer rates to disease outcomes but the findings must be taken into consideration when developing RSV maternal immunization programs.

The relationship between higher RSV neutralizing antibodies and protection against hospitalized RSV was identified in the 1980's but a definitive threshold of protection remains elusive (13, 44, 95, 96, 130, 134). This dissertation attempted to identify a specific titre level which differentiated RSV hospitalized cases from non-hospitalized controls while also

examining the impact of covariates including HIV exposure, seasonality, maternal alcohol and smoking during pregnancy. There was a significant but very small difference in the titre distribution, mean GMT and mean titre ($log_2$) between HIV unexposed cases and controls but not enough to confidently draw a line in the sand between protection and no protection. The use of logistic regression was able to demonstrate a relationship between a fold rise in titre and a reduction in the odds of RSV hospitalisation. More specifically, this study identified a reduction in odds for RSV hospitalisation of 43% for every unit rise in $log_2$ titre; a finding which was in close proximity to several other studies (44, 47, 96). Although these results are disappointing, there are future avenues of exploration which could provide more promising data in the future. The recent crystallization of the RSV viral surface protein responsible for fusion between virus and host cells has informed a new era of understanding of viral host immunity. RSV vaccine programs are now able to target specific epitopes on the F protein which induce highly neutralizing antibody (137, 145). The development of assays which can measure epitope specific sites may end up being more informative about sero-correlates of protection than the general microneutralizing assay that was used in this and may prior studies. In addition, systems serology techniques promise to map out downstream antibody mediated immune functions which surely matter just as much, if not more, than the absolute titre value itself.

This thesis was researched and written over the course of six years during which time the field of RSV has continued to evolve and widen rapidly. There are currently over 40 RSV vaccine and monoclonal antibody programs in development (146). A recent phase 3 maternal immunization trial “PREPARE” sadly failed its primary endpoint but revealed several important post-hoc findings including a reduction in all-cause LRTI in the first year of life; an effect which occurred long after maternal derived RSV antibodies had waned from the infant’s circulation. This intriguing finding suggests that RSV infection and prevention may play an important role in the overall, longer term respiratory health of children. The data summarized here will hopefully be incorporated into a larger landscape of RSV knowledge and activity aimed at supporting any number of future potential interventions.

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## Appendix 1 – Ethics approvals

## Ethics approvals for the PhD study

R14/49 Dr Yasmeen Mele Agosti et al

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**

**CLEARANCE CERTIFICATE NO. M140985**

| | |
|---|---|
| **NAME: (Principal Investigator)** | Dr Yasmeen Mele Agosti et al |
| **DEPARTMENT:** | Clinical Microbiology and Infectious Diseases<br>DST/NRF Vaccine Preventable Diseases<br>Respiratory and Menigeal Pathogens Research Unit |
| **PROJECT TITLE:** | Measuring Epidemiology and Sero-Correlates of Protection against severe Respiratory Syncytical Virus (RSV) Associated Hospitilization in HIV Exposed and Unexposed South Africa Children |
| **DATE CONSIDERED:** | 03/10/2014 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | |

**APPROVED BY:** Professor P Cleaton-Jones Chairperson, HREC (Medical)

**DATE OF APPROVAL:** 21/01/2015

**This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date 21/01/2015

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

**PERMISSION TO CONDUCT RESEARCH**

Date: 12 June 2014

TITLE OF PROJECT: Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children

UNIVERSITY: Witwatersrand

Principal Investigator: Y Agosti

Department: Microbiology

Supervisor (If relevant): S Madhi

Permission Head Department (where research conducted): Yes

Date of start of proposed study: June 2014
Date of completion of data collection: December 2017

he Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 12 June 2014

.....
(Approved/Not Approved
Hospital Management
Date: 23/06/14

## Ethics approval for the paediatric surveillance program

R14/49 Prof Shabir Madhi et al

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**

**CLEARANCE CERTIFICATE NO. M140904**

| | |
|---|---|
| **NAME: (Principal Investigator)** | Prof Shabir Madhi et al |
| **DEPARTMENT:** | RMPRU<br>CHBAH |
| **PROJECT TITLE:** | Surveillance on Severe Childhood Illness in Soweto, South Africa |
| **DATE CONSIDERED:** | 03/10/2014 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | |
| **APPROVED BY:** | Professor PE Cleaton-Jones Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 05/12/2014 |

**This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**

**University of the Witwatersrand, Johannesburg**

**Human Research Ethics Committee: (Medical)**
**United States FWA Registered No IRB 00001223**
**South African National Health Research Ethics Council Registration: REC-250208-004**

**SECRETARIAT:** Suite 189, Private Bag x2600, Houghton 2041, South Africa Tel: +27-11-274 9200 Fax: +27-11-274 9281

Ms S Trenor

**FAXED & COURIERED**

Research Associate
DST/NRF - RMPRU
11th Floor, West Wing, Nurses Residence
Chris Hani Baragwanath Hospital
2013

23 February 2016

Fax: 086 262 0626

Dear Ms Trenor,

**PROTOCOL: PNEUMO & GASTRO PATHOGENS - SURVEILLANCE ON PATHOGEN-SPECIFIC CAUSES OF PNEUMONIA AND DIARRHOEA HOSPITALIZATION IN CHILDREN**

**ETHICS REFERENCE NO: 131109**

**RE : APPROVAL FOR PROTOCOL AMENDMENT VERSION 3.0 DATED 2 FEBRUARY 2016**

We acknowledge receipt of your letter dated 02 February 2016 with the following documentation pertaining to the above-captioned trial.

| | | | |
|---|---|---|---|
| Amendment Date: | 02-Feb-2016 | Amendment Version: | Protocol Version 3.0 |
| Amendment Number: | | Received Date: | 02-Feb-2016 |

The following has been approved by the Wits Human Research Ethics Committee: (Medical):

* PCV&gastro_pathogens, Protocol Version 3.0 dated 2 February 2016 (clean and tracked)
* Information Leaflet and Informed Consent Form, Version 3.0 dated 2 February 2016 (Tracke & Clean)
* Information Leaflet and Informed Consent Form for Transport, Storage and Future use of Samples, Version 2.0 dated 2 February 2016 (Tracked & Clean)

Requested amendments:
- To include the collection of bloods in all infants younger than one year of age during RSV season. Since during RSV season (approximately from end of February to August) more than 70% of all respiratory hospitalizations are due to RSV infections RMPRU believe is more practical to sample every infant.
- Bloods will be used for cytokines profiling and evaluate any association with disease severity.
- Blood will also be stored for potential future testing for host single nucleotide polymorphisms (SNP) that may be used to measure the association between selected host genetic polymorphism and altered pulmonary function including asthma among children who experienced a RSV LRTI episode during the first year of life.
- Inclusion of two new secondary objectives related to the cytokine testing.

**Ethics Approval Date: 23 February 2016**

The University of the Witwatersrand, Human Research Ethics Committee Approval Granted for the above mentioned study is valid for five years. Where required by Sponsor to have approval on a more frequent basis it remains the responsibility of the Sponsor and Investigator to apply for continuing review and approval, or for the duration of the Trial.

1. THIS APPROVAL IS SUBJECT TO THE FOLLOWING PROVISOS:

* A copy of the MCC Approval and/or MCC Notification letter must be submitted to the Ethics Regulatory Office Secretariat before the study commences / or where an Amendment may be implemented (IF MCC APPROVAL / NOTIFICATION IS APPLICABLE). It remains the responsibility of the Principal Investigator and/or Sponsor to ensure that the relevant approvals are in place.

The study is conducted according to the protocol submitted to the University of the Witwatersrand, Human Research Ethics Committee. Any amendments to the protocol must first be submitted to the Human Research Ethics Committee for approval.

* During the study, the University of the Witwatersrand, Human Research Ethics Committee is informed immediately of :
  - Any Unexpected Serious Adverse Events or Unexpected Adverse Drug Reactions, which, in the Investigator and/or the Sponsor's opinion are suspected to be related to the study drug. (Refer to POL-IEC-001 and SOP-IEC-005, Item 3.4).
  - Any data received during the trial which, may cast doubt on the validity of the continuation of the study .

* The University of the Witwatersrand, Human Research Ethics Committee is notified of any decision to discontinue the study and the reason stated.

* The Investigators authorised by this approval participate in this study. Additional Investigators shall be submitted to the University of the Witwatersrand, Human Research Ethics Committee for approval prior to their participation in the study.

* In the event of an authorised Investigator ceasing to participate in the study, the University of the Witwatersrand, Human Research Ethics Committee must be informed and the reason for such cessation given.

2. PRINCIPLES OF INFORMED CONSENT:

* The University of the Witwatersrand, Human Research Ethics Committee requires that in all studies, the Principles of Informed Consent are adhered to. This applies to volunteers as well as patients.

3. PROGRESS REPORTS:

* The University of the Witwatersrand, Human Research Ethics Committee requests that the MCC Progress Reports be submitted twice a year either in March and September or six monthly from start of study to the HREC Secretariat Office - 011 274 9281 and a report of the final results, at the conclusion of the study. (IF APPLICABLE)

4. REIMBURSEMENT TO PATIENTS FOR TRANSPORT:

* The Human Research Ethics Committee: (Medical) is in agreement that reimbursement per visit is according to the Medicines Control Council of SA and that reimbursement should be appropriate according to the situation.

5. TRANSPORT AND STORAGE OF BLOOD AND TISSUE SAMPLES IN SOUTH AFRICA:

* If blood specimens are to be stored for future analysis and is planned that such analysis will be done outside Wits, then the blood must be stored at a facility in South Africa agreed with the relevant IRB, with release of sub-samples only once projects have been approved by the local Research Ethics Committee applicable to where the analysis will be done as well as by the Wits Human Research Ethics Committee: (Medical).

6. GENETIC TESTING

* The Human Research Ethics Committee: Medical; will not approve open-ended genetic testing as this does not fit the Human Research Ethics Committee criteria.

7. GOOD CLINICAL PRACTICE

The South African Department of Health, Medicines Control Council requires Good Clinical Practice (GCP) Training for all Investigators in Clinical Trials, and that GCP training be renewed every three (3) years.

As yet, there are no National Guidelines for the content of GCP courses. Until these are available the Wits Human Research Ethics Committee (Medical) will note courses completed by Investigators without approval of the content of the individual courses.

8. THE SUPPORTING APPROVAL DOCUMENTS ARE ATTACHED:

8.1 Ethics Approval Form signed by the Chairperson of the HREC - Kindly return the copy of the Approval Form signed by the Principal Investigator /(s) per fax: 011 274 9281 for our records (this is only applicable with the initial Approval).

The above has been noted for the Ethics Committee information and records.

***KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / STUDY CO-ORDINATORS***

Regards,

**PROF PETER CLEATON-JONES**

For and on behalf of the Human Research Ethics Committee: (Medical)

## Ethics forms for 28OB GBS study

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee: (Medical)
FWA Registered No IRB 00001223

SECRETARIAT: Suite 189, Private Bag x2600, Houghton 2041, South Africa Tel: +27-11-274 9200 Fax: +27-11-274 9281

Dr CL Cutland,

Medical Officer
Respiratory & Meningeal Pathogens Resear
11th Floor, West Wing, Nurses Residence
Chris Hani Baragwanath Hospital
P O Box 90753, Bertsham
2013

Fax: 086 674 0564

**FAXED & COURIE**

02 July 2014

Dear Dr Cutland,

**PROTOCOL: V98_28OBTP - ESTABLISHING A SERO-CORRELATE OF PROTECTION AGAINST INVASIVE GROUP B STREPTOCOCCUS DISEASE IN NEWBORNS AND YOUNG INFANTS AGED ≤90 DAYS**

**ETHICS REFERENCE NO: 140203**

**RE : APPROVAL FOR PROTOCOL AMENDMENT**

We acknowledge receipt of your letter dated 01 July 2014 with the following documentation pertaining to the above-captioned trial.

| | | | |
|---|---|---|---|
| Amendment Date: | 13-Jun-14 | Amendment Version: | Version 1.1 |
| Amendment Number: | | Received Date: | 01-Jul-14 |

The following has been approved by the Wits Human Research Ethics Committee: (Medical):

* Observational Study Protocol V98_28OBTP, Version 1.1 dated 13 June 2014
* Information Leaflet and Informed Consent Form (Adult), Version 1.2 dated 20 June 2014
* Information Leaflet and Informed Consent Form (GBS case), Version 1.2 dated 2014
* Informed Consent Form Transport, Storage and Future use of Samples, Version 1.2 dated 20 June 2014

Noted:
This amendment is required due to a change in the study sponsor from Novartis Pharma Services A.G. (Novartis) to Novartis funding RMPRU as an independent investigator-driven study.

Novartis will continue to provide financial support and will retain responsibility for ELISA testing to meet primary and secondary study objectives due to the availability of a validated ELISA within Novartis.

RMPRU will take responsibility for all other key study activities including authorship of all key study documents, study operations, data analysis and reporting and monitoring.

Amendments include:

- Change in the delivery hospital centers, now restricted to CHBAH. There will, however, be ongoing surveillance for GBS cases at these hospitals.
- Study enrolment period has been extended from 12 to 24 months.
- Data collected during antenatal clinic visits will be restricted to maternal demographics and gestational age based on findings from a feasibility study (V98_22OB).
- A concurrent serotype identified between the GBS case and the vaginal swab from the corresponding mother is no longer required.
- All sample (blood and vaginal swab) processing will be completed at the RMPRU laboratory.
- RMPRU laboratory will retain right to complete testing for markers of immune function, though primary study results will be based on GBS anticapsular titres tested using a Novartis ELISA.

Clarification in the ethics section that if consent is not obtained post-cord blood collection, the relevant samples will be tracked and destroyed and will not be tested for study purposes.

The University of the Witwatersrand, Human Research Ethics Committee Approval Granted for the above mentioned study is valid for five years. Where required by Sponsor to have approval on a more frequent basis it remains the responsibility of the Sponsor and Investigator to apply for continuing review and approval, or for the duration of the Trial.

1. THIS APPROVAL IS SUBJECT TO THE FOLLOWING PROVISOS:

* A copy of the MCC Approval and/or MCC Notification letter must be submitted to the Ethics Regulatory Office Secretariat before the study commences / or where an Amendment may be implemented (IF MCC APPROVAL / NOTIFICATION IS APPLICABLE). It remains the responsibility of the Principal Investigator and/or Sponsor to ensure that the relevant approvals are in place.

* The study is conducted according to the protocol submitted to the University of the Witwatersrand, Human Research Ethics Committee. Any amendments to the protocol must first be submitted to the Human Research Ethics Committee for approval.

* During the study, the University of the Witwatersrand, Human Research Ethics Committee is informed immediately of :
  - Any Unexpected Serious Adverse Events or Unexpected Adverse Drug Reactions, which, in the Investigator and/or the Sponsor's opinion are suspected to be related to the study drug. (Refer to POL-IEC–001 and SOP-IEC–005, Item 3.4).
  - Any data received during the trial which, may cast doubt on the validity of the continuation of the study .

* The University of the Witwatersrand, Human Research Ethics Committee is notified of any decision to discontinue the study and the reason stated.

* The Investigators authorised by this approval participate in this study. Additional Investigators shall be submitted to the University of the Witwatersrand, Human Research Ethics Committee for approval prior to their participation in the study.

* In the event of an authorised Investigator ceasing to participate in the study, the University of the Witwatersrand, Human Research Ethics Committee must be informed and the reason for such cessation given.

2. PRINCIPLES OF INFORMED CONSENT:

* The University of the Witwatersrand, Human Research Ethics Committee requires that in all studies, the Principles of Informed Consent are adhered to. This applies to volunteers as well as patients.

3. PROGRESS REPORTS:

* The University of the Witwatersrand, Human Research Ethics Committee requests that the MCC Progress Reports be submitted twice a year either in March and September or six monthly from start of study to the HREC Secretariat Office - 011 274 9281 and a report of the final results, at the conclusion of the study. (IF APPLICABLE)

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* The Human Research Ethics Committee: (Medical) is in agreement that reimbursement per visit is according to the Medicines Control Council of SA and that reimbursement should be appropriate according to the situation.

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* If blood specimens are to be stored for future analysis and is planned that such analysis will be done outside Wits, then the blood must be stored at a facility in South Africa agreed with the relevant IRB, with release of sub-samples only once projects have been approved by the local Research Ethics Committee applicable to where the analysis will be done as well as by the Wits Human Research Ethics Committee: (Medical).

6. GENETIC TESTING

* The Human Research Ethics Committee: Medical; will not approve open-ended genetic testing as this does not fit the Human Research Ethics Committee criteria.

7. GOOD CLINICAL PRACTICE

The South African Department of Health, Medicines Control Council requires Good Clinical Practice (GCP) Training for all Investigators in Clinical Trials, and that GCP training be renewed every three (3) years.

As yet, there are no National Guidelines for the content of GCP courses. Until these are available the Wits Human Research Ethics Committee (Medical) will note courses completed by Investigators without approval of the content of the individual courses.

8. THE SUPPORTING APPROVAL DOCUMENTS ARE ATTACHED:

8.1 Ethics Approval Form signed by the Chairperson of the HREC - Kindly return the copy of the Approval Form signed by the Principal Investigator /(s) per fax: 011 274 9281 for our records (this is only applicable with the initial Approval).

The above has been noted for the Ethics Committee information and records.

***KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / STUDY CO-ORDINATORS***

Regards,

**PROF PETER CLEATON-JONES**
For and on behalf of the Human Research Ethics Committee: (Medical)

# INDEPENDENT ETHICS COMMITTEE APPROVAL FORM

| | | | |
|---|---|---|---|
| Ethics Reference No. | 140203 | Date of Meeting | 28 February 2014 |
| | | Recertification Due | 27 February 2015 (If applicable) |
| Principal Investigators: | Dr CL Cutland | Investigators: | Dr DPM Bhana |
| | Prof SA Madhi | | Dr CN Briner |
| | | | Dr Z Dangor |
| | | | Dr MJ Groome |
| | | | Dr R Hassan-Moosa |
| | | | Dr Andrea Hugo |
| | | | Dr SA Jones |
| | | | Dr L Jose |
| | | | Dr AL Koen |

| | |
|---|---|
| Protocol Title: | Establishing a sero-correlate of protection against invasive Group B Streptococcus disease in newborns and young infants aged ≤90 days |

| DOCUMENTS REVIEWED | | | | Tick As Appropriate | Yes | No |
|---|---|---|---|---|---|---|
| Protocol Number | V98_28OB | Date: | 11 November 2013 | | | |
| Protocol | Observational Study Protocol V98_28OB, Version 1.0 | Date: | 11 November 2013 | | ☑ | ☐ |
| Investigator's Brochure | N/A - Version: N/A - Dated: | | | | ☑ | ☐ |
| Subject Information/Consent Form | Letter to GBS colonised Women - Version: 1.0 - Dated: 24/Mar/2014 | | | | ☑ | ☐ |
| | Informed Consent Form Transport, Storage and Future use of Samples (To be signed by all participants who are older than 18 years) - Version: Final 1.1 - Dated: 24/Mar/2014 | | | | ☑ | ☐ |
| | Information Leaflet and Informed Consent Form (GBS case) (To be signed by parent of infant participants) - Version: Final 1.1 - Dated: 24/Mar/2014 | | | | ☑ | ☐ |
| | Information Leaflet and Informed Consent Form (Adult) (To be signed by all participants who are 18 years and older) - Version: Final 1.1 - Dated: 24/Mar/2014 | | | | ☑ | ☐ |
| Advertisements | Advert - Congratulations on your Pregnancy! - Version: 1.0 - Dated: 06/Feb/2014 | | | | ☑ | ☐ |
| Questionnaires | | | | | | |
| Insurance/Compensation | HDI Gerling - Certificate of Insurance - Policy No: 01475018-14031 | Valid From | 18 Mar 2014 | To: 31 Dec 2015 | ☑ | ☐ |
| Synopsis of Study/Trial Summary | Invasive Group B Streptococcus Disease | | | | ☑ | ☐ |
| Other Documentation | HREC Application Form<br>HREC Application Form - Dated: 07/Feb/2014 | | | | ☑ | ☐ |
| | NHREC Trial Application ID #: 3680 - Dated: 07/Feb/2014 | | | | ☑ | ☐ |
| | PRC Application Form - Dated: 07/Feb/2014 | | | | ☑ | ☐ |
| | Protocol Summary - Version 1.0 - Dated: 11/Nov/2013 | | | | ☑ | ☐ |
| | V98_28OB Budget Summary - Dated: | | | | ☑ | ☐ |
| | Medical Advisory Committee: Chris Hani Baragwanath Academic Hospital - Dated: 12/Mar/2014 | | | | ☑ | ☐ |
| Relevant Trial Hospital/(s) | Lillian Ngoyi Community Clinic | | | | ☑ | ☐ |
| | Johannesburg Hospital - Charlotte Maxeke JHB Academic | | | | ☑ | ☐ |
| | Coronation Hospital - Rahima Moosa Mother & Child Hospital | | | | ☑ | ☐ |
| | Chris Hani Baragwanath Hospital | | | | ☑ | ☐ |
| Syndicate and/or Research Unit | Lillian Ngoyi Clinic | | | | ☑ | ☐ |
| | JHB Paediatrics Neonatology | | | | ☑ | ☐ |
| | DST/NRF Vaccine Preventable Diseases - RMPRU | | | | ☑ | ☐ |
| | Coronation Paediatrics - Rahima Moosa Mother and Child Hosp | | | | ☑ | ☐ |
| | | | | | | |

# INDEPENDENT ETHICS COMMITTEE APPROVAL FORM

## DETAILS OF COMMITTEE

| | |
|---|---|
| Name | University of the Witwatersrand Human Research Ethics Committee (Medical) |
| Address | Research Office, Senate House<br>University of the Witwatersrand, 1 Jan Smuts Avenue, BRAAMFONTEIN, Johannesburg, 2000 |

| DETAILS OF MEETING | Yes | No |
|---|---|---|
| Is the Investigator a member of the committee ? | | ✓ |
| If "Yes" did he/she vote ? | | ✓ |
| Is the Committee organised and operated according to applicable laws and regulations together with ? | ✓ | |
| Local GCP requirements ? | ✓ | |
| ICH GCP requirements ? | ✓ | |
| FDA GCP requirements ? FWA Registered No IRB00001223 | ✓ | |
| Progress reports required either in March and September or six monthly from start of study ? | ✓ | |

| DECISION ON APPROVAL : Is approval given to conduct the trial ? | Tick As Appropriate |
|---|---|
| Yes - with no conditions | ✓ |
| Yes - with conditions | |
| Specify conditions | |
| No | |
| Specify reasons | |

## SIGNATURES

I confirm that the details on this form are correct:

| Name: | Signature: | Date |
|---|---|---|
| **Prof PE Cleaton-Jones**<br>**Chair / Deputy Chair of Committee** | | **30 June 2014** |

## DECLARATION OF INVESTIGATOR/(S)

To be completed and ONE COPY returned to the Secretariat for the HREC at Wits Health Consortium, 8 Blackwood Avenue, Parktown, 2193 or Fax To: 011 274 9281

I/We fully understand the conditions under which I am/we are authorised to carry out and complete the above-mentioned research and I/we agree to ensure full compliance with these conditions. Should any amendment, alteration or departure be contemplated from the research procedure methodology or manner of execution, I/we will communicate with the Chairman of the Human Research Ethics Committee (Medical) for approval prior to acting on any of the above mentioned proposed amendments, alterations or departures. I am/we are fully aware that any unauthorised amendment, alteration or departure as above will amount to misconduct and may lead to the institution of disciplinary procedures.

Any approval given by the HREC is conditional upon consent being obtained by the Investigator/s from the Superintendent (or equivalent official) of the Hospital, Clinic or Institution in which the research is, in part or full, to take place.

The Chairman may of course at his discretion place the matter before the full Committee.

DATE 1 JULY 14 SIGNATURE _____ NAME SHABIR MAHTA

PROTOCOL NUMBER V98_28OB ETHICS REF 140203

Date Printe 30 June 2014

**Wits Clinical Research**

8 Blackwood Avenue, Parktown, 2193, South Africa
Tel. +27-11-274-9200, Fax: +27-11-274-9281
Postnet Suite 189, Private Bag x2600, Houghton, 2041

**FAXED & COURIE**

Prof SA Madhi,

Respiratory & Meningeal Pathogens Research Uni
11th Floor, West Wing, Nurses Residence
Chris Hani Baragwanath Hospital
P O Box 90753, Bertsham
2013

Fax: 086 646 4208

Dear Prof Madhi,

**PROTOCOL NO: V98_28OB**

**PROTOCOL TITLE: Establishing a sero-correlate of protection against invasive Group B Streptococcus disease in newborns and young infants aged ≤90 days**

**PRC REFERENCE NUMBER: 140203**

Please be advised that your trial application was:

**APPROVED**

**The Expert Reviewer /(s):** Prof PA Cooper

Also reviewed by: Mrs J Palmer: Acting Chairperson Protocol Review Committee
Medical Advisory Committee: Chris Hani Baragwanath Academic Hospital

Yours sincerely

Palmer

**MRS JENNIFER PALMER (BRYCE-BORTHWICK)**
Acting **Chairperson: Protocol Review Committee**
**30 June 2014**

cc.

| | | | | | |
|---|---|---|---|---|---|
| Respiratory & Meningeal Patho | Ms C Combrinck | Tel 011 983 4283 | Cell | | Fax 086 262 0626 |
| Novartis Vaccines and Diagnos | Ms C Combrinck | Tel 011 983 4283 | Cell | | Fax 086 262 0626 |
| | Prof SA Madhi | Tel 011 386 6137 | Cell | 082 870 6672 | Fax 086 262 0626 |

# HUMAN RESEARCH ETHICS COMMITTEE MEMBERS: (MEDICAL)
# UNIVERSITY OF THE WITWATERSRAND

**Attendance Register for the Ethics Meeting held on 28 February 2014 from 12:30 - 15:00**

**Venue: PPS BOARDROOM, Faculty of Health Sciences, University of Witwatersrand 7 York Road, Parktown**

## AFFILIATED TO THE UNIVERSITY OF THE WITWATERSRAND

| Surname | Initials | Title | Discipline/s | Academic Qualifications | Gender | Present |
|---|---|---|---|---|---|---|
| Adam | Y | Dr | Obstetrics & Gynaecology | MB BCh; FCOG | F | Present |
| Cleaton-Jones | PE | Prof | Biomedical Ethics | BDS; MB BCh; PhD; DA (SA); DTM&H; DSc (Dent), FCD (SA) DPH; PhD Hon Causa, MASSAfr | M | Present |
| Conradie | FM | Dr | Infectious Diseases/HIV/TB | MB BCh; DTM&H; MSc; Dip HIV Man | F | Present |
| Cooper | PA | Prof | Paediatrics | MB BCh, PhD, DCH (SA), FCPaeds (SA) | M | Present |
| Cubasch | H | Dr | Surgery | FCS (SA) | M | Absent |
| Dessein | PHMC | Prof | Rheumatology | MD, FCP (SA), FRCP, PhD | M | Present |
| Dhai | A | Prof | Biomedical Ethics | MB ChB; FCOG; LLM; PGDipIntResEthics | F | Absent |
| Donde | B | Prof | Radiation Oncology | MB BCh, MMed Rad (T) | M | Present |
| Etheredge | H | Ms | Biomedical Ethics | MSc Med, BA | F | Present |
| Feldman | C | Prof | Pulmonology | MB BCh, PhD, DSc, FCP (SA), FRCP | M | Present |
| Gerrand | P | Ms | Social Work | MA (Social Work) | F | Present |
| Langley | G | Prof | Nursing | MSc (Nursing), PhD, MPhil (Ethics) | F | Present |
| Lownie | MA | Prof | Maxillo-Facial & Oral Surgery | BDS, BA (Hons), DipMFOS, FCMFOS(SA), MEd | F | Absent |
| Mokhachane | M | Dr | Paediatrics | MB BCh, FCP (Paeds) SA, MMed, Neonatology (SA) | F | Absent |
| Naidoo | Shan | Prof | Public Health | MB BCh, DMTH, DHSM, DOH, MMED | M | Present |
| Naran | NH | Dr | Chemical Pathology | PhD | M | Present |
| Paruk | F | Prof | Anaesthesia | MB ChB, FCOG(SA), Crit Care(SA), PhD | F | Absent |
| Penn | C | Prof | Speech Pathology | BA (Sp&HTh), PhD, CCC SL-P, OMS | F | Absent |
| Penny | C | Dr | Internal Medicine | BSc Hons, PhD | M | Present |
| Ross | M | Prof | Public Health | MB ChB, FFCH(SA), MFamMed, MFPH | F | Absent |
| Sanne | IM | Prof | Infectious Diseases/HIV/TB | MB BCh, FCP (SA), DTM&H; MMed & PhD | M | Present |
| Smith, Cora | C | Prof | Psychiatry | BA, BA (Hons), M.A (Clin.Psych), PhD | F | Present |
| Stewart | A | Prof | Physiotherapy | BSc (Physio), MSc, PhD, DPE | F | Absent |
| Szabo | CP | Prof | Psychiatry | MB BCh, MMed, MScMed, PhD; FCPsych(SA) | M | Absent |
| Thom | RGM | Prof | Psychiatry | MB ChB, DCH, FCPsych, PhD | F | Absent |
| Tsotsi | NM | Dr | | BDS; MPH; MSc Med; PGDipInt ResEthics | F | Absent |
| Van Gelderen | CJ | Prof | Obstetrics & Gynaecology | MB BCh, FRCOG, FCOG(SA) | M | Present |
| Velaphi | S | Prof | Paediatrics | MB BCh, FCPaeds, MMed | M | Absent |
| Vorster | M | Prof | Psychiatry | BA, MB BCh; MMed, FCPsych(SA), PhD PGDipIntResEthics | F | Present |
| Willem | P | Dr | Human Genetics | MD, PhD | F | Present |
| Woodiwiss | AJ | Prof | Cardiovascular Pathophysiology | BSc Physiotherapy, BSc, MSc, PhD | F | Absent |

## NOT AFFILIATED TO THE UNIVERSITY OF THE WITWATERSRAND

| Surname | Initials | Title | Discipline/s | Academic Qualifications | Gender | Present |
|---|---|---|---|---|---|---|
| Egan | A | Father | Theology | BA (Hons), MA, MDiv, STL, PhD | M | Absent |
| Guidozzi | Y | Adv | Lawyer | BSc (Nurs), LLB, MBA | F | Present |
| Ikalafeng | B | Dr | Governance | BSc (Hons), MSc, PhD | F | Present |

| | | | | | | |
|---|---|---|---|---|---|---|
| Myburgh | C | Prof | Educationist | BSc (Hons), MCom, DEd, HED | M | Present |
| Poggenpoel | M | Prof | Psychiatric Nursing | RN, PhD | F | Present |

**RETIRED MEMBER OCCASIONALLY CO-OPTED FOR SURGICAL OPINION ON A PROJECT**

| | | | | | |
|---|---|---|---|---|---|
| Oettle | GJ | Prof | Surgery | BSc (Hons), MB BCh, FRCS | M |

**Note 1:** This committee has been in continuous operation since October 1996

**Note 2:** The large committee size is to ensure a good attendance at meetings - size 23 core and 14 alternate members

**Note 3:** A Quorum is 14 members according to the 60% required by SA National Guidelines (ref 2 below)

This is to certify that the Human Research Ethics Committee: (**Medical**) of the University of the Witwatersrand operates according to the following guidelines of good clinical practice:

1. ICH Harmonised Tripartite Guideline for Good Clinical Practice.
2. SA National Department of Health 2006 Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2006).
3. Declaration of Helsinki 2013.

The Committee's United States Federal Wide Assurance details are:

1. Country code SF.
2. FWA Number: **FWA00000715.**
3. University of the Witwatersrand: IORG0000852.
4. Human Research Ethics Committee: (Medical): IRB00001223.

**Appendix 2** Informed consent forms for birth cohort and hospitalisation patients

Appendix 2 Birth Informed Consent Form – Main and Storage

**INFORMATION LEAFLET AND INFORMED CONSENT FOR**

Each ***participant*** must receive, read and understand this document **before** any study-related procedure.

**STUDY TITLE**:

Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children

| | |
|---|---|
| **INVESTIGATOR:** | Dr. YM Agosti |
| **INSTITUTION:** | DST / NRF Vaccine Preventable Diseases / Respiratory and Meningeal Pathogens Research Unit |

**DAYTIME AND AFTER HOURS TELEPHONE NUMBER(S):** 082 428 0904 / 011 983 3209
After hours 072 245 6812

**To the potential Participant:** *This consent form may contain words that you do not understand. Please ask the study doctor or staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family and friends before making your decision.*

INTRODUCTION:

**Greeting:** Hello, my name is _____ (insert name of study staff member).
I am a study staff member at DST/ NRF Vaccine Preventable Diseases/RMPRU. I would like to invite you to consider participating in a research study, entitled: **Measuring the epidemiology and sero-correlates of protection against severe Respiratory Syncytial Virus (RSV) associated hospitalizations in HIV exposed and unexposed South African children.**

Before agreeing to participate, it is important that you read and understand the following explanation of the study, the study procedures, benefits, risks, discomforts and your right to withdraw from the study at any time. This information leaflet is to help you to decide if you would like to participate. You need to understand what is involved before you agree to take part in this study. Please ask as many question as you need to before you decide to participate.You should not agree to take part unless you are satisfied with all the procedures involved. If you decide to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will be given a copy to keep.

**PURPOSE OF THE STUDY:**

We at the RMPRU are studying a germ called Respiratory Syncytial Virus or RSV. This germ gives most babies and children coughs and colds in the winter time. Sometimes, the germ makes a baby sick enough that he /she needs to come to the hospital because they have trouble breathing and eating. These babies have serious chest and lung infections. We would like to learn more about the babies who need to come into the hospital because of this germ. We will not be the doctors and nurses who care for your baby if he / she should come to the hospital with a chest or lung infection. We hope that we can learn enough from their visit to help make an injection that will stop this infection from making all South African children very sick with this germ in the future.

**LENGTH OF STUDY AND NUMBER OF PARTICIPANTS:**

Over the next two years we will be asking 30,000 moms and their new babies at Chris Hani Baragwanath Hospital to join our study. Moms and babies will join the study at the time the baby is born and then we will wait to see if the baby must come to the hospital for serious chest and lung infections. We will watch and wait until the baby is 1 year old.

PROCEDURES:

If you agree to join the study, we will take a small blood sample (less than 1 teaspoon) from you and one from the baby. This test will only take a few minutes of your time while you are still in the hospital after delivery. The blood that comes from the baby

actually comes from the umbilical cord and does not cause the baby any pain. This blood will be kept in our lab. We will also ask you questions about your health and living situation to help us understand how this infection spreads from person to person. These questions will take 20 minutes to ask. This will not keep you in the hospital longer than you need to stay after having a baby. We will place a special sticker in your baby's Road to Health card to let people know that you are a part of our study.

If and when your baby gets sick and needs to come to the hospital please bring your Road to Health card with you so that doctors and nurses will know that you and your baby are a part of our study. We will come visit your baby while he or she is in the hospital so that we can take a small amount of mucous from your baby's nose with a nasal swab. The nasal swab is a thin cotton-tipped stick that looks like an ear-bud. We will use this sample to test for the germ called RSV that can be found in the nose. The test only takes one minute and it may feel uncomfortable for your baby but will not cause pain.

We will also go back and look at the blood that we took from you and your baby when your baby was born. We will check your blood sample to see if your body made antibodies to protect your baby from this infection. Antibodies are a part of the blood that act like soldiers to fight germs. We will also look at the baby's blood sample to see how much antibody or soldiers they got from you when they were born.

We will also ask you some more questions about your baby's sickness. For example, we would like to see if your baby has had a fever or a cough or if they are eating less. These questions will take 20 minutes to ask.

If your child's HIV status is unknown, research staff will assist doctors looking after your child by drawing blood for HIV testing. Your child's doctor will ensure you are given the HIV results.

**RISKS OR WILL ANY OF THESE STUDY PROCEDURES CAUSE PAIN OR INCONVENIENCE?**

There is a small chance that you will feel some pain when you have blood taken. There is a very small chance that you can have an infection from the needle used to take the blood but our nurses are trained to do this in a very clean way so that this should not happen. Your baby will not feel the blood being taken from his/her umbilical cord when he/she is just born.

Your baby may feel something uncomfortable when the nasal swab is placed in his/her nose to take some mucous for testing. This will only happen if your baby is taken to the hospital before the age of 1 year. If you or your baby are injured during the study and this is a direct result of the blood sampling or the nasal swab sample then we will pay for any reasonable and necessary medical treatment for that injury.

**BENEFITS:**
If your baby is hospitalized and our tests show that they have this infection called RSV then we will let you know.

Apart from knowing if your baby has this kind of germ during their illness, there are no other benefits to joining this study. However, we hope that what we can learn from you and your baby in order to help future babies from getting sick with this infection called RSV.

**YOUR RIGHTS AS A PARTICIPANT:**

Your decision to be a part of this study is voluntary. Please know that it is up to you to decide to join this study. If you decide to join then you will be given this information sheet to keep and asked to sign a consent form. If you decide to not join the study, both you and your baby will still receive the care that you need from your doctors and nurses. You are also free to decide to leave the study at any time after joining.

**REIMBURSEMENT:**

You will not be paid for being a part of this study. The investigators will pay for all procedures done in this study and there will be no cost to you. .

**CONFIDENTIALITY:**

All information obtained during this study, including hospital records, personal information and test results will be kept strictly confidential. Both you and your baby will be assigned a number with your initials to guarantee your privacy. Information that may be reported in scientific journals will not include any information that identifies you or your baby as a participant in this study. Personal information will only be shared if required by law; in this case, you will be informed of my intent to disclose the information to the authorized state agency. Both your and the baby's records might be inspected by the University of the Witwatersrand, Human Research Ethics Committee (HREC) if they want to check that the study is being carried out correctly. You will be informed of any important findings on your/your baby's test results.

**ETHICAL APPROVAL:**

This clinical study protocol has been submitted to the University of the Witwatersrand, **Human Research Ethics Committee (HREC)** and written approval has been granted by that committee.

This study is sponsored by the DST/ NRF Vaccine Preventable Diseases/RMPRU.

I do not have any financial or personal interests with this organization that may bias my actions.

**ADDITIONAL INFORMATION:**

If you want any information regarding your **rights as a research participant, or complaints regarding this research study**, you may contact:

Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee established to help protect the rights of research participants at (011) 717 2301.

For **research information** you can contact Dr Yasmeen Agosti at 011-983-4283.

**INFORMED CONSENT:**

**I hereby confirm that I have been informed by the study doctor / nurse about the nature, conduct, benefits and risks of the surveillance study:** Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children

**I have also received, read and understood the above written information (Participant Informed Consent) regarding the clinical study.**

- **I am aware that the results of the study, including personal details regarding myself and my child's sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.**
- **In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by DST / NRF Vaccine preventable Diseases / RMPRU or on their behalf.**
- **I may, at any stage, without prejudice, withdraw consent for my child's participation in the study**
- **I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to allow my child participate in the study.**

☐ **Y** ☐ **N** **I agree to be interviewed and allow data from my own and my child's hospital records to be collected in order to answer the questions from the study questionnaire**

☐ **Y** ☐ **N** **I agree to the tests for germs from his / her throat and nose**

☐ **Y** ☐ **N** **I agree to the test for HIV**

**PARTICIPANT'S** name: _____

_____

Printed name of parent Signature/Mark or thumbprint

Date

I, _____(insert name of study staff member), herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

**STUDY STAFF MEMBER**:

| Printed name | Signature | Date |
|---|---|---|

TRANSLATOR/OTHER PERSON EXPLAINING INFORMED CONSENT

(Designation_____)

**WITNESS (if applicable):**

| Printed name | Signature | Date |
|---|---|---|

**INFORMATION LEAFLET AND INFORMED CONSENT FOR TRANSPORT, STORAGE AND FUTURE USE OF SAMPLE**

Each ***participant*** must receive, read and understand this document **before** any study-related procedure.

**STUDY TITLE**:

Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children


**INVESTIGATOR:** Dr. YM Agosti

**INSTITUTION:** DST / NRF Vaccine Preventable Diseases / Respiratory and Meningeal Pathogens Research Unit

**DAYTIME AND AFTER HOURS TELEPHONE NUMBER(S):** 082 428 0904 / 011 983 3209
After hours 072 245 6812


**To the potential Participant:** *This consent form may contain words that you do not understand. Please ask the study doctor or staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family and friends before making your decision.*

Goodday, my name is _____ (insert name of study staff member). I am a study staff member working at DST / NRF Vaccine Preventable Diseases / RMPRU. You have already consented to your participation in a research study entitled: "Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children".

As mentioned in the main trial informed consent form, the study doctor / nurse will perform blood samples on both mother and baby at the time of baby's birth and a nasopharyngeal swab if the baby is hospitalized for a lung and chest infection to determine which germ is making him / her ill. These samples will be processed in laboratories in Gauteng for tests detailed in the main trial ICF. Once the described tests have been completed, we would like to store the samples in a freezer at the study site for up to 15 years so that they may undergo further testing.

The additional tests which may be conducted on these samples would be quality assurance of new and existing tests for the above mentioned germs and testing for other germs. The aim of any future research is to improve the knowledge about infectious diseases, and to improve laboratory tests. These additional tests would most commonly be performed in South Africa, but the samples may also be sent to laboratories internationally, including the USA.

All additional tests besides the tests of the clinical study and test method improvement that will be performed on your stored samples will first be approved by the University of the Witwatersrand, Human Research Ethics Committee (HREC). The HREC is responsible for approving the study at this site. **It is assured that genetic tests will never be performed on the stored samples.**

All stored samples will be anonymized. That means the laboratory will **never be able** to match the coded sample with your identity. You will not be paid for allowing the investigators to keep and later use any remaining part of your samples. Samples collected

will be stored securely in Gauteng until they are needed for testing. Samples will be kept for a maximum of 15 years, before being destroyed.

You decision about storing your child's left over samples for future research does not affect your participation in the study or any care that you may receive at this clinic. You will get a copy of this form to keep. If you change your mind about storage of the samples at a later stage, the study doctor will then ask the investigator at the storage facility to destroy any remaining blood samples that have your code on them so that they cannot be used.

If you want any information regarding your rights as a research participant, or complaints regarding this research study, you may contact Professor Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC). Which is an independent committee established to help protect the rights of research participants at (011) 717 2301.

If you have any questions about this trial you should first discuss them with your doctor or the ethics committee (contact details provided on this form).

If you have any questions about this study please contact one of the study doctors at 082 428 0904 .

**INFORMED CONSENT FOR TRANSPORT, STORAGE AND FUTURE USE OF SAMPLES:**

Please indicate your choice by checking one of the two boxes below:

☐ I authorize the transport of samples to laboratories in South Africa and internationally, storage and future use of my samples by the investigators under the conditions described in this information sheet

OR

☐ I DO NOT authorize the transport of samples to laboratories in South Africa and internationally, storage and future use of my samples by the investigators under the conditions described in this information sheet.

**PARTICIPANT'S** name: _____

_____

Printed name of parent Signature/Mark or thumbprint

Date

I, _____(insert name of study staff member), herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY STAFF MEMBER:

_____

Printed name Signature Date

TRANSLATOR/OTHER PERSON EXPLAINING INFORMED CONSENT

(Designation_____)

_____

WITNESS (if applicable):

_____

Printed name Signature Date

**INFORMATION LEAFLET AND INFORMED CONSENT FOR**

Each ***participant*** must receive, read and understand this document **before** any study-related procedure.

**STUDY TITLE**:

Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children

| | |
|---|---|
| **INVESTIGATOR:** | Dr. YM Agosti |
| **INSTITUTION:** | DST / NRF Vaccine Preventable Diseases / Respiratory and Meningeal Pathogens Research Unit |

| | |
|---|---|
| **DAYTIME AND AFTER HOURS TELEPHONE NUMBER(S):** | 082 428 0904 / 011 983 3209<br>After hours 072 245 6812 |

**To the potential Participant:** *This consent form may contain words that you do not understand. Please ask the study doctor or staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family and friends before making your decision.*

INTRODUCTION:

**Greeting:** Hello, my name is _____ (insert name of study staff member).
I am a study staff member at DST/ NRF Vaccine Preventable Diseases/RMPRU. I would like to invite you to consider participating in a research study, entitled: **Measuring the epidemiology and sero-correlates of protection against severe Respiratory Syncytial Virus (RSV) associated hospitalizations in HIV exposed and unexposed South African children.**

Before agreeing to participate, it is important that you read and understand the following explanation of the study, the study procedures, benefits, risks, discomforts and your right to withdraw from the study at any time. This information leaflet is to help you to decide if you would like to participate. You need to understand what is involved before you agree to take part in this study. Please ask as many question as you need to before you decide to participate.You should not agree to take part unless you are satisfied with all the procedures involved. If you decide to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will be given a copy to keep.

**PURPOSE OF THE STUDY:**

We at the RMPRU are studying a germ called Respiratory Syncytial Virus or RSV. This germ gives most babies and children coughs and colds in the winter time. Sometimes, the germ makes a baby sick enough that he /she needs to come to the hospital because they have trouble breathing and eating. These babies have serious chest and lung infections. We would like to learn more about the babies who need to come into the hospital because of this germ. We will not be the doctors and nurses who care for your baby if he / she should come to the hospital with a chest or lung infection. We hope that we can learn enough from their visit to help make an injection that will stop this infection from making all South African children very sick with this germ in the future.

**LENGTH OF STUDY AND NUMBER OF PARTICIPANTS:**

Over the next two years we will be asking 30,000 moms and their new babies at Chris Hani Baragwanath Hospital to join our study. Moms and babies will join the study at the time the baby is born and then we will wait to see if the baby must come to the hospital for serious chest and lung infections. We will watch and wait until the baby is 1 year old.

PROCEDURES:

The study doctor / nurse will take a small amount of mucous from your baby's nose with a nasal swab. The nasal swab is a thin cotton-tipped stick that looks like an ear-bud. We will use this sample to test for the germ called RSV that can be found in the nose. The

test only takes one minute and it may feel uncomfortable for your baby but will not cause pain.

We will also ask you questions about your health and living situation to help us understand how this infection spreads from person to person. These questions will take 20 minutes to ask. This will not keep you in the hospital longer than you need to stay after having a baby.

We will also go back and look at the blood that we took from you and your baby when your baby was born. We will check your blood sample to see if your body made antibodies to protect your baby from this infection. Antibodies are a part of the blood that act like soldiers to fight germs. We will also look at the baby's blood sample to see how much antibody or soldiers they got from you when they were born.

If your child's HIV status is unknown, research staff will assist doctors looking after your child by drawing blood for HIV testing. Your child's doctor will ensure you are given the HIV results.

**RISKS OR WILL ANY OF THESE STUDY PROCEDURES CAUSE PAIN OR INCONVENIENCE?**

Your baby may feel something uncomfortable when the nasal swab is placed in his/her nose to take some mucous for testing. This will only happen if your baby is taken to the hospital before the age of 1 year. If you or your baby are injured during the study and this is a direct result of the blood sampling or the nasal swab sample then we will pay for any reasonable and necessary medical treatment for that injury.

**BENEFITS:**

If your baby is hospitalized and our tests show that they have this infection called RSV then we will let you know.

Apart from knowing if your baby has this kind of germ during their illness, there are no other benefits to joining this study. However, we hope that what we can learn from you and your baby in order to help future babies from getting sick with this infection called RSV.

**YOUR RIGHTS AS A PARTICIPANT:**

Your decision to be a part of this study is voluntary. Please know that it is up to you to decide to join this study. If you decide to join then you will be given this information sheet to keep and asked to sign a consent form. If you decide to not join the study, both you and your baby will still receive the care that you need from your doctors and nurses. You are also free to decide to leave the study at any time after joining.

**REIMBURSEMENT:**

You will not be paid for being a part of this study. The investigators will pay for all procedures done in this study and there will be no cost to you. .

**CONFIDENTIALITY:**

All information obtained during this study, including hospital records, personal information and test results will be kept strictly confidential. Both you and your baby will be assigned a number with your initials to guarantee your privacy. Information that may be reported in scientific journals will not include any information that identifies you or your baby as a participant in this study. Personal information will only be shared if required by law; in this case, you will be informed of my intent to disclose the information to the authorized state agency. Both your and the baby's records might be inspected by the University of the Witwatersrand, Human Research Ethics Committee (HREC) if they want to check that the study is being carried out correctly. You will be informed of any important findings on your/your baby's test results.

**ETHICAL APPROVAL:**

This clinical study protocol has been submitted to the University of the Witwatersrand, **Human Research Ethics Committee (HREC)** and written approval has been granted by that committee.

This study is sponsored by the DST/ NRF Vaccine Preventable Diseases/RMPRU.

I do not have any financial or personal interests with this organization that may bias my actions.

**ADDITIONAL INFORMATION:**

If you want any information regarding your **rights as a research participant, or complaints regarding this research study**, you may contact:

Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee established to help protect the rights of research participants at (011) 717 2301.

For **research information** you can contact Dr Yasmeen Agosti at 011-983-4283.

**INFORMED CONSENT:**

**I hereby confirm that I have been informed by the study doctor / nurse about the nature, conduct, benefits and risks of the surveillance study:** Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children

**I have also received, read and understood the above written information (Participant Informed Consent) regarding the clinical study.**

- **I am aware that the results of the study, including personal details regarding myself and my child's sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.**
- **In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by DST / NRF Vaccine preventable Diseases / RMPRU or on their behalf.**
- **I may, at any stage, without prejudice, withdraw consent for my child's participation in the study**
- **I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to allow my child participate in the study.**

☐ **Y** ☐ **N** **I agree to be interviewed and allow data from my own and my child's hospital records to be collected in order to answer the questions from the study questionnaire**

☐ **Y** ☐ **N** **I agree to the tests for germs from his / her throat and nose**

☐ **Y** ☐ **N** **I agree to the test for HIV**

**PARTICIPANT'S** name: _____

| Printed name of parent | Signature/Mark or thumbprint | Date |
|---|---|---|

I, _____(insert name of study staff member), herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

**STUDY STAFF MEMBER**:

| Printed name | Signature | Date |
|---|---|---|

TRANSLATOR/OTHER PERSON EXPLAINING INFORMED CONSENT

(Designation_____)

**WITNESS (if applicable):**

| Printed name | Signature | Date |
|---|---|---|

**INFORMATION LEAFLET AND INFORMED CONSENT FOR TRANSPORT, STORAGE AND FUTURE USE OF SAMPLE**

Each ***participant*** must receive, read and understand this document **before** any study-related procedure.

**STUDY TITLE**:

Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children


**INVESTIGATOR:** Dr. YM Agosti

**INSTITUTION:** DST / NRF Vaccine Preventable Diseases / Respiratory and Meningeal Pathogens Research Unit

**DAYTIME AND AFTER HOURS TELEPHONE NUMBER(S):** 082 428 0904 / 011 983 3209
After hours 072 245 6812


**To the potential Participant:** *This consent form may contain words that you do not understand. Please ask the study doctor or staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family and friends before making your decision.*

Goodday, my name is _____ (insert name of study staff member). I am a study staff member working at DST / NRF Vaccine Preventable Diseases / RMPRU. You have already consented to your participation in a research study entitled: "Epidemiology and sero-correlates of protection of severe RSV associated hospitalization in HIV exposed and unexposed South African children".

The study doctor / nurse will perform a nasopharyngeal swab to determine which germ is making him / her ill. These samples will be processed in laboratories in Gauteng for tests. Once the described tests have been completed, we would like to store the samples in a freezer at the study site for up to 15 years so that they may undergo further testing.

The additional tests which may be conducted on these samples would be quality assurance of new and existing tests for the above mentioned germs and testing for other germs. The aim of any future research is to improve the knowledge about infectious diseases, and to improve laboratory tests. These additional tests would most commonly be performed in South Africa, but the samples may also be sent to laboratories internationally, including the USA.

All additional tests besides the tests of the clinical study and test method improvement that will be performed on your stored samples will first be approved by the University of the Witwatersrand, Human Research Ethics Committee (HREC). The HREC is responsible for approving the study at this site. **It is assured that genetic tests will never be performed on the stored samples.**

All stored samples will be anonymized. That means the laboratory will **never be able** to match the coded sample with your identity. You will not be paid for allowing the investigators to keep and later use any remaining part of your samples. Samples collected will be stored securely in Gauteng until they are needed for testing. Samples will be kept for a maximum of 15 years, before being destroyed.

You decision about storing your child's left over samples for future research does not affect your participation in the study or any care that you may receive at this clinic. You will get a copy of this form to keep. If you change your mind about storage of the samples at a later stage, the study doctor will then ask the investigator at the storage facility to destroy any remaining blood samples that have your code on them so that they cannot be used.

If you want any information regarding your rights as a research participant, or complaints regarding this research study, you may contact Professor Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC). Which is an independent committee established to help protect the rights of research participants at (011) 717 2301.

If you have any questions about this trial you should first discuss them with your doctor or the ethics committee (contact details provided on this form).

If you have any questions about this study please contact one of the study doctors at

_____

**INFORMED CONSENT FOR TRANSPORT, STORAGE AND FUTURE USE OF SAMPLES:**

Please indicate your choice by checking one of the two boxes below:

☐ I authorize the transport of samples to laboratories in South Africa and internationally, storage and future use of my samples by the investigators under the conditions described in this information sheet

OR

☐ I DO NOT authorize the transport of samples to laboratories in South Africa and internationally, storage and future use of my samples by the investigators under the conditions described in this information sheet.

**PARTICIPANT'S** name: _____

Printed name of parent Signature/Mark or thumbprint

Date

I, _____(insert name of study staff member), herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY STAFF MEMBER:

Printed name Signature Date

TRANSLATOR/OTHER PERSON EXPLAINING INFORMED CONSENT

(Designation_____)

WITNESS (if applicable):

Printed name Signature Date

**Appendix 3** Supplementary tables for Chapter 2 "RSV hospitalisation incidence, prevalence, seasonality and subtype distributions in HIV-exposed and HIV-unexposed infants"

**Table 2.4** Seasonal distribution of RSV cases by reporting week, 2015 and 2016

**Seasonal distribution of RSV cases by reporting week**

**Season year 2015**

| Week ending | MMWR week | Negative RSV | Positive RSV | Total tested | RSV % |
|---|---|---|---|---|---|
| 1/10/2015 | 1 | 1 | 0 | 1 | 0 |
| 1/17/2015 | 2 | 30 | 1 | 31 | 0.032258 |
| 1/24/2015 | 3 | 21 | 2 | 23 | 0.086957 |
| 1/31/2015 | 4 | 35 | 0 | 35 | 0 |
| 2/7/2015 | 5 | 25 | 0 | 25 | 0 |
| 2/14/2015 | 6 | 20 | 3 | 23 | 0.130435 |
| 2/21/2015 | 7 | 34 | 1 | 35 | 0.028571 |
| 2/28/2015 | 8 | 31 | 3 | 34 | 0.088235 |
| **3/7/2015** | 9 | 17 | 10 | 27 | **0.37037** |
| **3/14/2015** | 10 | 22 | 9 | 31 | **0.290323** |
| 3/21/2015 | 11 | 15 | 10 | 25 | 0.4 |
| 3/28/2015 | 12 | 15 | 13 | 28 | 0.464286 |
| 4/4/2015 | 13 | 12 | 15 | 27 | 0.555556 |
| 4/11/2015 | 14 | 20 | 18 | 38 | 0.473684 |
| 4/18/2015 | 15 | 15 | 36 | 51 | 0.705882 |
| 4/25/2015 | 16 | 19 | 31 | 50 | 0.62 |
| 5/2/2015 | 17 | 31 | 32 | 63 | 0.507937 |
| 5/9/2015 | 18 | 20 | 26 | 46 | 0.565217 |
| 5/16/2015 | 19 | 24 | 33 | 57 | 0.578947 |
| 5/23/2015 | 20 | 42 | 41 | 83 | 0.493976 |
| 5/30/2015 | 21 | 33 | 32 | 65 | 0.492308 |
| 6/6/2015 | 22 | 29 | 24 | 53 | 0.45283 |
| 6/13/2015 | 23 | 29 | 6 | 35 | 0.171429 |
| 6/20/2015 | 24 | 32 | 13 | 45 | 0.288889 |
| 6/27/2015 | 25 | 37 | 9 | 46 | 0.195652 |
| 7/4/2015 | 26 | 34 | 9 | 43 | 0.209302 |
| 7/11/2015 | 27 | 28 | 11 | 39 | 0.282051 |

**Season year 2016**

| Week ending | MMWR week | Negative RSV | Positive RSV | Total tested | RSV % |
|---|---|---|---|---|---|
| 1/9/2016 | 1 | 10 | 0 | 10 | 0 |
| 1/16/2016 | 2 | 15 | 1 | 16 | 0.0625 |
| 1/23/2016 | 3 | 19 | 0 | 19 | 0 |
| 1/30/2016 | 4 | 16 | 0 | 16 | 0 |
| 2/6/2016 | 5 | 19 | 0 | 19 | 0 |
| 2/13/2016 | 6 | 21 | 2 | 23 | 0.086957 |
| 2/20/2016 | 7 | 14 | 0 | 14 | 0 |
| 2/27/2016 | 8 | 30 | 0 | 30 | 0 |
| 3/5/2016 | 9 | 19 | 0 | 19 | 0 |
| 3/12/2016 | 10 | 34 | 2 | 36 | 0.055556 |
| 3/19/2016 | 11 | 26 | 3 | 29 | 0.103448 |
| 3/26/2016 | 12 | 31 | 1 | 32 | 0.03125 |
| 4/2/2016 | 13 | 25 | 2 | 27 | 0.074074 |
| **4/9/2016** | 14 | 26 | 7 | 33 | **0.212121** |
| **4/16/2016** | 15 | 27 | 4 | 31 | **0.129032** |
| 4/23/2016 | 16 | 19 | 11 | 30 | 0.366667 |
| 4/30/2016 | 17 | 27 | 19 | 46 | 0.413043 |
| 5/7/2016 | 18 | 21 | 15 | 36 | 0.416667 |
| 5/14/2016 | 19 | 22 | 27 | 49 | 0.55102 |
| 5/21/2016 | 20 | 23 | 23 | 46 | 0.5 |
| 5/28/2016 | 21 | 22 | 35 | 57 | 0.614035 |
| 6/4/2016 | 22 | 26 | 37 | 63 | 0.587302 |
| 6/11/2016 | 23 | 26 | 36 | 62 | 0.580645 |
| 6/18/2016 | 24 | 26 | 40 | 66 | 0.606061 |
| 6/25/2016 | 25 | 20 | 23 | 43 | 0.534884 |
| 7/2/2016 | 26 | 26 | 27 | 53 | 0.509434 |
| 7/9/2016 | 27 | 14 | 19 | 33 | 0.575758 |

**Season year 2015**

| Week ending | MMWR week | Negative RSV | Positive RSV | Total tested | RSV % |
|---|---|---|---|---|---|
| 7/18/2015 | 28 | 26 | 7 | 33 | 0.212121 |
| 7/25/2015 | 29 | 34 | 12 | 46 | 0.26087 |
| 8/1/2015 | 30 | 35 | 6 | 41 | 0.146341 |
| **8/8/2015** | 31 | 27 | 1 | 28 | **0.035714** |
| **8/15/2015** | 32 | 36 | 1 | 37 | **0.027027** |
| 8/22/2015 | 33 | 28 | 3 | 31 | 0.096774 |
| 8/29/2015 | 34 | 30 | 3 | 33 | 0.090909 |
| 9/5/2015 | 35 | 28 | 4 | 32 | 0.125 |
| 9/12/2015 | 36 | 31 | 2 | 33 | 0.060606 |
| 9/19/2015 | 37 | 29 | 4 | 33 | 0.121212 |
| 9/26/2015 | 38 | 42 | 2 | 44 | 0.045455 |
| 10/3/2015 | 39 | 21 | 2 | 23 | 0.086957 |
| 10/10/2015 | 40 | 40 | 4 | 44 | 0.090909 |
| 10/17/2015 | 41 | 40 | 1 | 41 | 0.02439 |
| 10/24/2015 | 42 | 26 | 3 | 29 | 0.103448 |
| 10/31/2015 | 43 | 23 | 0 | 23 | 0 |
| 11/7/2015 | 44 | 21 | 1 | 22 | 0.045455 |
| 11/14/2015 | 45 | 23 | 0 | 23 | 0 |
| 11/21/2015 | 46 | 29 | 1 | 30 | 0.033333 |
| 11/28/2015 | 47 | 18 | 2 | 20 | 0.1 |
| 12/5/2015 | 48 | 25 | 0 | 25 | 0 |
| 12/12/2015 | 49 | 30 | 1 | 31 | 0.032258 |
| 12/19/2015 | 50 | 27 | 0 | 27 | 0 |
| 12/26/2015 | 51 | 15 | 0 | 15 | 0 |
| 1/2/2016 | 52 | 18 | 0 | 18 | 0 |

**Season year 2016**

| Week ending | MMWR week | Negative RSV | Positive RSV | Total tested | RSV % |
|---|---|---|---|---|---|
| 7/16/2016 | 28 | 20 | 12 | 32 | 0.375 |
| 7/23/2016 | 29 | 16 | 20 | 36 | 0.555556 |
| 7/30/2016 | 30 | 22 | 6 | 28 | 0.214286 |
| 8/6/2016 | 31 | 17 | 9 | 26 | 0.346154 |
| 8/13/2016 | 32 | 18 | 4 | 22 | 0.181818 |
| 8/20/2016 | 33 | 21 | 3 | 24 | 0.125 |
| 8/27/2016 | 34 | 38 | 1 | 39 | 0.025641 |
| 9/3/2016 | 35 | 25 | 5 | 30 | 0.166667 |
| **9/10/2016** | 36 | 36 | 1 | 37 | **0.027027** |
| **9/17/2016** | 37 | 29 | 0 | 29 | **0** |
| 9/24/2016 | 38 | 39 | 2 | 41 | 0.04878 |
| 10/1/2016 | 39 | 36 | 0 | 36 | 0 |
| 10/8/2016 | 40 | 36 | 0 | 36 | 0 |
| 10/15/2016 | 41 | 28 | 1 | 29 | 0.034483 |
| 10/22/2016 | 42 | 30 | 0 | 30 | 0 |
| 10/29/2016 | 43 | 19 | 0 | 19 | 0 |
| 11/5/2016 | 44 | 28 | 1 | 29 | 0.034483 |
| 11/12/2016 | 45 | 32 | 1 | 33 | 0.030303 |
| 11/19/2016 | 46 | 30 | 0 | 30 | 0 |
| 11/26/2016 | 47 | 35 | 0 | 35 | 0 |
| 12/3/2016 | 48 | 17 | 0 | 17 | 0 |
| 12/10/2016 | 49 | 37 | 1 | 38 | 0.026316 |
| 12/17/2016 | 50 | 7 | 0 | 7 | 0 |
| 12/24/2016 | 51 | 18 | 2 | 20 | 0.1 |
| 12/31/2016 | 52 | 16 | 0 | 16 | 0 |

## Appendix 4 Co-Authorship agreement form for Chapter 4 "The Transplacental transfer of RSV neutralising antibody in HIV exposed and unexposed infants"

**Declaration by student and co-author's agreement for write-up with published articles.**

I, **Yasmeen M. Agosti**, student number 1129467, declare that this dissertation is my own work and that I contributed adequately towards research findings published in the article stated below which are included in my dissertation.

| | | |
|---|---|---|
| Signature of Student | | Date: SEPT 15, 2019 |
| Name of Primary Supervisor | Shabir A. Madhi | |
| Signature of Primary Supervisor | | Date: 15 SEP 19 |

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of her dissertation. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for her degree purposes.

Article title: Impaired transplacental transfer of respiratory syncytial virus (RSV) neutralizing antibodies in HIV-infected compared to HIV-uninfected pregnant women
Clinical Infectious Diseases, 2019, 69(1): 151 -4

| Authors | Name | Signature | Date |
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| 2nd author | **Yasmeen M. Agosti** | | |
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| 6th author | Erie A.F. Simoes | | 19 SEPT 2019 |
| 7th author | Marta C. Nunes | | 15 SEP 2019 |
| 8th author | Melinda S. Suchard | | 16/9/2019 |
| 9th author | Shabir A. Madhi | | |

Comments by primary supervisor:

## Appendix 5 Original RSV antibody neutralization assay protocol

**METHODS FOR ASSAYING PLASMA SAMPLES FOR RSV NEUTRALISING ACTIVITY**
Developed by Dr. Eric Simoes

**CULTURE REAGENTS AND SOLUTIONS:**

Media and sample diluents: Cells are maintained in Minimum Essential Media (MEM – Gibco #12360038) with HEPES buffer, L-glutamine and anitibiotic/antimyotic solution. 10% fetal calf serum (FBS – Gibco #10082-147) is also added. MEM containing 2% FBS is used for all virus cultures and assays. Antibody samples are also diluted in MEM with 2% FBS.

Cell lines: HEP2 cells are obtained from American Type Culture Collection (ATCC CCL-81) and are maintained as a monolayer culture in MEM with 10% FBS. Cells are harvested by overlaying with 0.05% trypsin/0.05% EDTA (Gibco #25300054) in Hank's Balanced Salt Solution (HBSS) (Gibco #14170112). After 5 minutes at 37°C, cells are dislodged by gently knocking flasks. Cells are re-suspended in MEM with 10% FBS.

Virus Propagation: The Long strain of human RS virus (ATCC VR-26: American Type Culture Collection), isolates in Baltimore, MD in 1956, is used in these studies. Virus is propagated in HEP2 cells and infectious culture supernatant is harvested after 4-6 days at 37°C in 5% CO2 to provide virus stocks. Virus stocks stored at -70°C are passaged once, in vitro, to routinely yield virus titers of approximately 105 TCID50/ml. HEP2 cells (5 x 105) along with the virus are added in 8 mLs per tissue culture dish. After 18 hrs. at 37°C in 5% CO2, media is aspirated and virus (0.5 – 1.0 ml of stock) is added. Total volume is again adjusted to 4 mLs. Cultures are incubated 4-6 days until CPE is $\geq$ 90%. Cells are then scraped from the well using a cell scrapper, removed and centrifuged for 1 minute at 1900 rpm at 4°C to remove cell debris. The supernatant is used as the virus inoculum in the fusion-inhibition assay.

Cell Fixative/Stain: 90% H2O with 10% Glutaric Dialdehyde and Crystal Violet Powder. [for 1L – 0.67g crystal violet powder, 900 mLs H2O, & 100 mLs Glutaric Dialdehyde]

**HARDWARE:**

Plates – 96 Well, flat bottom w/lid
Sterile boats
Fine tip permanent marker
Incubator 37°C @ 5% CO2
50-300 ul multipipetter
Sterile 5mL pipettes
0-10 pipetter
Sterile 25mL pipettes
10-100 pipetter
Mechanical Pipetter
Sterile pipette tips
Hemocytometer/capillary tubes
Sterile glass pipettes
Vacuum suction pump

**REAGENTS/SOLUTIONS:**

- PHOSPHATE BUFFERED SALINE (SIGMA #1000-3; PBS: W/O Ca2+Mg2+);PH 7.4
- FETAL BOVINE SERUM (FBS – GIBCO#10082-147)
- MINIMUM ESSENTIAL MEDIA W/ HEPES BUFFER (MEM – GIBCO #12360038) SUPPLEMENTED WITH L-GLUTAMINE (GIBCO #25030-081) AND ANTIMYOTIC/ANTIBIOTIC SOLUTION. (FUNGIZONE AMPHOTERCIN B – GIBCO #15290-018 & PENICILLIN-STREPTOMYCIN – GIBCO #15140-122)
- 0.05% TRYPSIN-EDTA (GIBCO #2530054)
- IVIG DILUTION SAMPLES
- STANDARD SERA SAMPLES
- SERA SAMPLES
- GLUTARIC DIALDEHYDE, 50WT % IN AQUEOUS WATER (ALDRICH - #34,085-5)
- CRYSTAL VIOLET (SIGMA - #C3886)
- TISSUE CULTURE WATER (SIGMA - #W3500)
- RSV LONG STRAIN STOCK POOL

**PROCEDURE:**

Viral Inoculi:
The viral inoculum is made by first preparing a 4X concentrated solution. The 4X viral sample is then diluted 2-fold to 2X, 1X, and 0.5X. The 1X viral dilution is used for the assay. The 4X viral sample is prepared so that the 1X sample contains 300-500 pfu/ml of virus.

The 4X viral inoculums is made using: V=V'C'/C in 2% FBS/MEM where

V' = volume of viral sample desired (variable)
V = volume of virus stock pool that needs to be added to make the 4X viral inoculum (variable)
C' = estimated final viral titer (1X = 300-500 pfu/ml, 4X = 1200-2000 pfu/ml)
C = titer of virus stock pool (4 x 107 pfu/ml – know from plaque assay)

Viral Dilutions: Viral dilutions are prepared in four different sterile boats.

1. 4X: 50ml of 2% FBS/MEM + Virus
2. 2X: 20ml of 2% FBS/MEM + 20ml 4X
3. 1X: 25ml of 2% FBS/MEM + 25ml 2X
4. 0.5X: 3ml of 2% FBS/MEM + 3ml 1X

**CONTROL PLATES:**

Control Plate #1:

*Positive/Negative Control Plate:* A 96-well plate is divided into 2 sets of 2 rows each. The first 2 rows being RSV positive control plates and the next 2 rows being RSV negative control plates. 50 µl of 2% FBS/MEM is added to the RSV positive control wells and 100 µl of 2% FBS/MEM is added to the RSV negative control wells. 50 µl of the 1X viral sample is then added to the first 6 columns of the first 2 rows of the positive control wells. After the 1X viral sample has been added to all other plates, 50 µl of the 1X viral sample is added to the first 2 rows of the second set of 6 columns, in order to determine whether there is a significant decrease in the virus titer. No virus is added to the negative control wells.

*Backtiter/TCID Control Plate:* 100 µl of 2% FBS/MEM is added to 4 rows each with 12 wells, half of a 96-well plate. Each of the viral sample dilutions described earlier will be tittered. 50 µl of the viral inoculum (4X, 2X, 1X, & 0.5X) is added to the first 3 columns of the plate (tests are done in triplicate) and diluted 3-fold out of the plate changing tips from well to well.

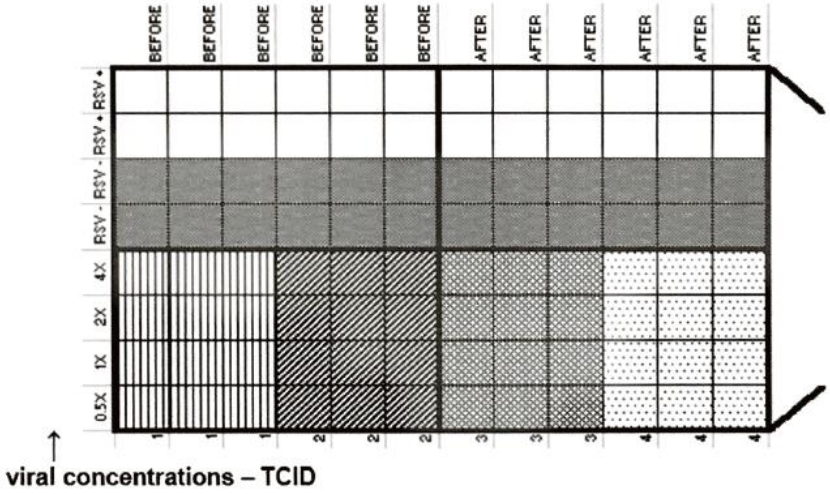
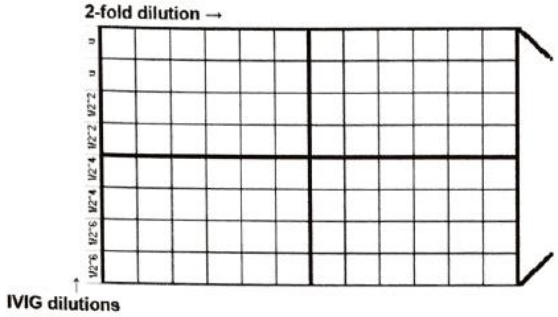


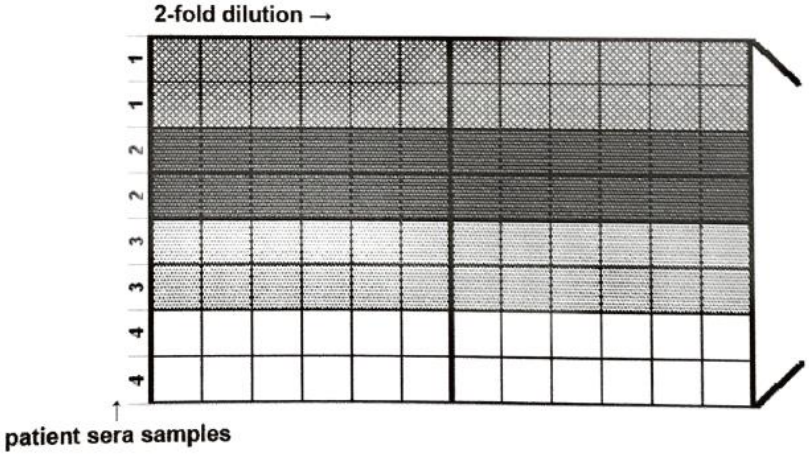

Plate 2:

*IVIG Dilutions Control Plate:* Added 50 µl 2% FBS/MEM to all the wells, except those in column 1. In column 1, 87.5 µl of 2% FBS/MEM was added instead. 12.5 µl of four different IVIG dilutions was added to column 1 ( 2 rows of each ) and then diluted 2-fold down the plate. 50 µl of the 1X viral dilution was then added to all wells.




Test Plates:

Each 96-well test plate is divided into 4 rows of 2, so that each sample is tested in duplicate. Thus 4 patient samples can be tested using one 96 well plate. 50µlof 2% FBS/MEM is added to each well, except those in column 1, for the 2-fold dilution. 87.5 µlof 2% FBS/MEM and 12.5 µl of the appropriate sera is added to each well in the column 1. For example, serum from patient #1 is added to the first 2 rows in column 1, serum from patient #2 is added to the next 2 rows in column 1 and so on. Serially dilute out 50 µl from the first wells in column 1 to wells in column 2 and so on. After each transfer the contents in each well are mixed thoroughly by pipetting the contents 3-5 times. New sterile pipettes are used between each dilution. The sera carried over from the last column is discarded. 50 µl of 1X viral solution is then added to each well.
2-fold dilution




For All Plates:

Incubation: All plates are incubated on a plate rocker for 1-1.5hrs at 37 °C and 5% CO2.

Cells: A cell suspension (250,000 cells/ml) of 2 to 5 day old Hep-2 cells in 10% FBS/MEM is prepared.

Cell Overlay: 100 µl of the 10% FBS/MEM cell suspension is added to all wells. Negative controls are done first followed by positive controls and then the other plates.

Incubation: Plates are incubated for 144 hrs (6 days) at 37 °C and 5% CO2. Plates are observed daily for virus-specific CPE.

**Data Interpretation and Analysis**

Fixing & Staining: At the end of 6 days (positive controls = 100% CPE), the medium/inoculum/serum is decanted slowly and the wells are overlayed with a crystal violet fixate as described on page 1. Plates are stained for 24 hours. The stain is then decanted and the plates are rinsed gently with water. The plates are allowed to dry upside down and then read.

Analysis of Control Plates: Each well is read as either positive (CPE present) or negative (no CPE). For control plate #1 to be valid, the negative control plate must equal 0% CPE and the positive control must equal 100% CPE. The results from the TCID plate (control plate #2) must not only show that the virus survived the entire duration (6 Days) of the experiment, but that the titer dropped consistently by 1 Log over each dilution. If any of these control plates vary from the expected outcome, the results from the test plate will be invalid and the test is re-done.

Analysis of Test Plates:

Each well is read as either positive (CPE present) or negative (no CPE). The titer is determined by the last well with at least an 80% fully protected monolayer. Since the serum is diluted in doubling dilutions, the titers are expressed in terms of Log2. Each patient sera sample is tested in duplicate and the results averaged.

All Plates are read initially and then again 2 days later to ensure accuracy. Results from both readings are then averaged.

## Appendix 6 PhD Plagiarism Declaration Form and Turn-It-In Report

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

**PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS**

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Yasmeen Mele Agosti (Student number: 1129467) am a student registered for the degree of Doctor of Philosophy in the academic year 2020.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

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