A Prospective Household observational cohort study of Influenza, Respiratory Syncytial virus and other respiratory pathogens community burden and Transmission dynamics in South Africa (The PHIRST Study)

Contents

1. Summary

Title: A Prospective Household observational cohort study of Influenza, Respiratory Syncytial virus and other respiratory pathogens community burden and Transmission dynamics in South Africa (The PHIRST Study)

Partners: National Institute for Communicable Diseases (NICD), Johannesburg, South Africa; University of Witwatersrand, School of Public Health, Johannesburg, South Africa; United States Centers for Disease Control and Prevention, Atlanta, United States of America (USA); Perinatal HIV Research Unit, Johannesburg, South Africa; MRC/Wits Rural Public Health and Health Transitions Research (Agincourt), Bushbuckridge, South Africa; Medical Research Council (Environment and Health Research Unit), Johannesburg, South Africa.

Background: Information on the community burden of influenza is essential to inform control, but is not routinely collected. For example, influenza transmission models, which are widely used to inform the efficacy and cost-effectiveness of vaccines, antivirals and non-pharmaceutical countermeasures, depend on valid epidemiological estimates of the community occurrence and transmission of disease. While community level studies such as the Flu Watch cohort study in the United Kingdom have been implemented in recent years in high-income countries, the community burden of influenza remains largely unknown in lower- and middle-income countries, especially in Africa where the burden of influenza may be higher. In addition, no data exist on the household transmission dynamics of influenza viruses. Such data could provide important insight to inform targeted interventions, for instance, how to maximize indirect effects of influenza vaccination to protect more vulnerable groups for which the available influenza vaccines are currently not licensed or the efficacy is low (e.g. children <2 years of age).

Human respiratory syncytial virus (RSV) is a major cause of childhood acute lower respiratory tract infection, especially among infants <3 months of age. While a RSV vaccine is currently not available, the efficacy of promising RSV candidate vaccines is being evaluated. Alternative strategies for RSV vaccination have been proposed, including vaccination of older age groups to indirectly protect vulnerable infants by reducing circulation of virus in the population or preventing chains of transmission to the infant. Nonetheless, to evaluate these effects knowledge of the RSV burden, transmission dynamics and source of infection for infants in the community is needed.

Pneumococcus is the leading bacterial cause of pneumonia. The pneumcoccal conjugate vaccine (PCV) was introduced into the expanded programme on immunisation in 2009. Data on carriage prevalence, dynamics of carriage and interaction with other respiratory pathogens will be helpful to guide interventions to target pneumococcal disease not prevented by vaccination. Both diphtheria and pertussis have been identified as emerging pathogens in South Africa in recent years, giving rise to outbreaks in 2014 and 2015 after years of low prevalence. Meningococcus is an epidemic-prone disease causing cyclical outbreaks in South Africa but little is known about the carriage prevalence in South Africa. Group A streptococcus (GAS) causes significant morbidity and mortality globally. Gas carriage maybe a potential source for acquisition of infections for others in the community and therefore carriage strains may be of

relevance to active disease e.g. pharyngitis. However, there is limited data on GAS carriage in South Africa. Annual incidence of tuberculosis in Southern Africa is the highest globally, with Swaziland, Lesotho and South Africa have the highest rates of tuberculosis in the world. Moreover, in South Africa tuberculosis is the leading cause of death on death notification forms and, in HIV-infected individuals (both not yet receiving antiretrovirals and whilst taking antiretrovirals), is the leading serious opportunistic infection.

Data on the carriage prevalence and/or transmission dynamics of these pathogens are important to guide control measures, including targeted vaccination strategies. Prevention efforts to limit transmission in households are often recommended but data on household transmission and the impact of HIV infection are limited to date.

Objectives

Primary

- To estimate the community burden of influenza and RSV, including: (i) the incidence of influenza and RSV infection in the community; (ii) the symptomatic fraction associated with influenza and RSV infection; (iii) the severity associated with symptomatic infections; and (iv) the fraction of individuals with symptomatic infection seeking medical care.
- To assess the transmission dynamics of influenza and RSV infections in the community, including: (i) the estimation of the household secondary infection risk (SIR), serial interval and length of shedding; (ii) the estimation of transmission of infection between age groups within the household and possibly the community; and (iii) the estimation of the effective reproductive number (R_t) and its variation over time in the community.

Secondary (selected)

- To estimate the symptomatic fraction, the severity associated with symptomatic infections and the fraction of individuals with symptomatic infection seeking medical care among influenza or RSV positive cases by HIV serostatus and age.
- To estimate the SIR and length of shedding of influenza and RSV among HIV-infected and HIV-uninfected index cases and the rate of acquisition of influenza and RSV infection among HIV-infected and HIV-uninfected household members.
- To assess the role of asymptomatic infections in the household transmission of influenza and RSV.
- Determine the heterogeneity of influenza and RSV virus strains within household clusters and describe viral evolution within and between households as well as the association between virus strains and the duration of virus shedding and HIV status
- To describe and compare nasopharyngeal pneumococcal loads in healthy individuals by age and over time, and how the loads may be altered by respiratory viral infection, HIV-status and vaccination-status
- To identify the prevalence and duration of *B. pertussis* colonization within the community by age group, vaccine status and over time, the transmission dynamics within a household, and the proportion of individuals that develop symptomatic infection

- To determine the prevalence of *N. meningitidis*, *C. diphtheriae and S. pyogenes* colonization within the community by age group at a single point in time each year
- To measure the annual incidence of tuberculosis infection in individuals living in study households and assess risk factors (including incident and prevalent HIV) for acquiring tuberculosis infection.
- To ascertain the impact of housing quality, fuel use and indoor air quality and selected measures of ventilation on tuberculosis infection and on household transmission of respiratory viruses and bacteria

Methods: We will conduct a household-level community cohort study in a rural community in Mpumalanga Province where health and demographic surveillance (HDSS) is conducted (the Agincourt HDSS surveillance site) and selected urban areas in the Matlosana Muncipality in North West Province. Prospective, hospital-based surveillance for influenza- and RSV-associated severe acute respiratory illness has been conducted at these sites since 2010. The prospective cohort study will be conducted for 3 consecutive years, from 2016-2018, to include 3 consecutive RSV and influenza seasons. Approximately one hundred households (50 households per site, with an expected average number of household members of 5 to recruit a total of 500 enrolled individuals) will be randomly selected within the study population each year and consented to participate to the study. For each household member, serum will be collected quarterly (January-February, April-May, September-October and November-December) including before and after the RSV (February to May) and influenza (May to September) seasons and a final blood draw at the end of the year. The demographic characteristics of the household members will be collected on enrollment. Starting before the RSV and influenza seasons, upper respiratory tract samples will be collected twice-a-week by trained study nurses from all household members irrespective of the presence of respiratory symptoms throughout the year. During each visit the development of symptoms (using a severity score) and healthcare seeking behavior of symptomatic cases will be recorded. In addition, during winter at one household visit, an oropharyngeal swab will be collected from each individual for testing for meningococcus, diphtheria and GAS. Nasopharyngeal samples will be tested for the detection of RSV or influenza virus and pneumococcus and *B. pertussis* infection by real-time reverse-transcriptase polymerase chain reaction (rt-PCR). Blood samples will be tested for rise in RSV, influenza and pertussis antibody titers. From consenting patients, blood samples will be tested for HIV infection. For tuberculosis infection, we will do a tuberculin skin test (TST) at baseline and the end of the first year and annually thereafter in people are found to be TST negative at their previous test. HIV testing will be offered to all participants at baseline and then six monthly thereafter. All participants will have a sputum sample taken for culture at baseline and thereafter sputum will be collected based on symptoms of cough (> 1 week duration) for TB (Xpert Mtuberculosis/Rif) and pertussis

The sample size of approximately 1500 individuals over 3 consecutive seasons will allow the estimation of 20% risk of infection and a 10% risk of illness with 95% CI and 5% desired absolute precision in the community. Individuals in the households enrolled in the first and second year of the study will be have a three follow-up visit towards in the 2nd and 3rd year of the study (February, May and October). At these visits a blood specimen for testing for, influenza and RSV serology will be collected. In addition, HIV and TST testing will be done twice a year on those who tested negative on the previous test.

Impact: This study will improve understanding of the community burden of influenza, RSV, pertussis, tuberculosis and pneumococcal infection in South Africa. It will also provide data on the carriage prevalence of meningococcus, diphtheria and GAS. The data generated from this study will also provide important information on the transmission dynamics of influenza, RSV, pertussis and pneumococcus in the community allowing to better strategize interventions (including targeted vaccination strategies) and evaluate their potential impact. Moreover, there is an absence of prospective data on tuberculosis infection from high tuberculosis burden countries. The data generated will both inform modelling of transmission, sample size for prevention studies and surveillance assessing the impact of the National Tuberculosis Control Program.

2. Investigators

Name	Role	Contact	Contribution
National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa			
Cheryl Cohen, Centre for Respiratory Diseases and Meningitis	Principal Investigator	cherylc@nicd.ac.za	Study protocol design, supervision, data entry planning, field supervision, training, data analysis
Florette Treurnicht, Centre for Respiratory Diseases and Meningitis	Co-Investigator	florettet@nicd.ac.za	Supervision, lab design, testing, data analysis
Senzo Mtshali, Centre for Respiratory Diseases and Meningitis	Co-Investigator	senzom@nicd.ac.za	Laboratory design and testing
Mpho Seleka, Centre for Respiratory Diseases and Meningitis	Co-Investigator	mphos@nicd.ac.za	Laboratory design and testing
Anne von Gottberg, Centre for Respiratory Diseases and Meningitis	Co-Investigator	annev@nicd.ac.za	Laboratory design and testing
Jocelyn Moyes, Centre for Respiratory Diseases and Meningitis	Co-Investigator	Joss.moyes@gmail.com	Study protocol design, supervision, data entry planning, field supervision, training, data analysis
Thulisa Mkhencele	Co-investigator	thulisam@nicd.ac.za	Field supervision, database design

			training, data analysis
Sibongile Walaza, Centre for Respiratory Diseases and Meningitis	Co-Investigator	sibongilew@nicd.ac.za	Study protocol design, supervision, data entry planning, field supervision, training, data analysis
Orienka Hellferscee, Centre for Respiratory Diseases and Meningitis	Co-Investigator	orienkah@nicd.ac.za	Lab design and testing
Nicole Wolter, Centre for Respiratory Diseases and Meningitis	Co-Investigator	nicolew@nicd.ac.za	Lab design and testing
~~Claire von Mollendorf~~	~~Co-Investigator~~	~~clairevm@nicd.ac.za~~	~~Study protocol design, supervision, data entry planning, field supervision, training, data analysis~~
Melinda Suchard, Centre for Vaccines and Immunology	Co-Investigator	melindas@nicd.ac.za	Lab design and testing
Site Investigators			
Kathleen Kahn	Co-Investigator	Kathleen.kahn@wits.ac.za	Field data collection and site coordination
Ryan Wagner	Co-Investigator	Ryan.wagner@wits.ac.za	Field data collection and site coordination
Neil Martinson	Co-Investigator	martinson@phru.co.za	Field data collection and site coordination
Limakatso Lebina	Co-Investigator	lebinal@phru.co.za	Field data collection and site coordination
Angela Mathee	Co-Investigator	amathee@mrc.ac.za	Field data collection (indoor air quality)
Centers for Disease Control and Prevention (CDC), Atlanta, United States			

Meredith McMorrow, Influenza Division	Co- Investigator	bwe3@cdc.gov	Study protocol design, supervision, data entry planning, field supervision, training, data analysis
Stefano Tempia, Influenza division CDC South Africa office	Co-Investigator	stefanot@nicd.ac.za	Study protocol design, supervision, data entry planning, field supervision, training, data analysis

3. Main centre(s)

1. National Institute for Communicable Diseases (NICD)
   1 Modderfontein Rd, Sandringham
   Johannesburg 2193
   South Africa
2. MRC/Wits Rural Public Health and Health Transitions Research (Agincourt)
   School of Public Health
   Faculty of Health Sciences
   University of the Witwatersrand
   New School Of Public Health Building
   Education Campus
   27 St. Andrews Road
   Parktown
   2193
   South Africa
   Email address: Dawn.Dalby@wits.ac.za
   Tel: +27-11-717-2085
3. Perinatal HIV Research Unit
   WITS Health Consortium T/A Perinatal HIV Research Unit
   New Nurses Home
   Chris Hani Baragwanath Academic Hospital
   Chris Hani Road, Diepkloof, Soweto
   Johannesburg, 1864
   South Africa
   Tel: +27 11 989 9700
   Fax: +27 11 989 9762
   Email: info@phru.co.za
4. Centers for Disease Control and Prevention (CDC),
   Influenza Division
   1600 Clifton Rd NE, Mailstop A-32,
   Atlanta, GA 30329

USA

5. Contact details

Dr Cheryl Cohen
Centre Head: Epidemiology
Centre for Respiratory Diseases and Meningitis (CRDM)
National Institute for Communicable Diseases &
Associate Professor
Wits School of Public Health
University of the Witwatersrand
1 Modderfontein Rd, Sandringham
Johannesburg 2193
South Africa
Telephone: +27 11 386 6593
Fax:+27 11 882 9979
Mobile: +27 82 803 8093
E mail: cherylc@nicd.ac.za

6. List of abbreviations

AFB	Acid-fast bacilli
ARI	Acute respiratory illness
ART	Antiretroviral treatment
CDC	United States Centres for Disease Control and Prevention
cDNA	Complementary deoxyribonucleic acid
C_t	Cycle threshold
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
FTD	Fast Track Diagnostics
GPS	Global positioning system
HA	Haemaglutinin
HAI	Haemagglutination inhibition
HIV	Human immunodeficiency virus
hMPV	Human meta pneumovirus
HDSS	Health and demographic surveillance site
ILI	Influenza-like illness
MGIT	Mycobacteria Growth Indicator Tube
NAAT	Nucleic acid amplification test
NALC-NaOH	N-Acetyl L-Cysteine sodium hydroxide
NICD	National Institute for Communicable Diseases
NP	Nasopharyngeal
NYC	New York City
OP	Oropharyngeal
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PIV	Parainfluenza virus
PM	Particulate matter
RNA	Ribonucleic acid
RTPCR	Reverse Transcriptase Polymerase Chain Reaction
RSV	Respiratory syncytial virus
Rt	Effective reproductive number
SIR	Secondary infection risk
TST	Tuberculin skin test
TU	Tuberculin units
USA	United States of America
VCT	Voluntary counselling and testing

7. Background

7.1. Influenza

Internationally, influenza surveillance provides real-time information to inform prevention and control policies[1]. However, in most cases, influenza surveillance focuses on cases seeking medical attention. Information on the community burden of influenza is key to inform control [2], but is not routinely collected. For example, influenza transmission models, which are widely used to inform the efficacy and cost-effectiveness of vaccines, antivirals and non-pharmaceutical countermeasures, depend on valid epidemiological estimates of the community occurrence of disease [3]. While community level studies such as the Flu Watch cohort study in the United Kingdom have been implemented in recent years in high-income countries [3], the community burden of influenza remains largely unknown in lower- and middle-income countries, especially in Africa where the burden of influenza may be higher. In addition, limited data exist on the household transmission dynamics of influenza virus at the individual level. Such data could provide important insight to inform targeted interventions, for instance, how to maximize indirect effects of influenza vaccination to protect more vulnerable groups for which the available influenza vaccines are currently not licensed or the efficacy is low (e.g. children <2 years of age).

7.2. Respiratory syncytial virus

Human respiratory syncytial virus (RSV) is the commonest cause of childhood acute lower respiratory tract infection, especially among infants <3 months of age [4]. While a RSV vaccine is currently not available, the efficacy of promising RSV candidate vaccines is being evaluated [5]. Nonetheless, there are considerable obstacles confronting the development of vaccines targeting young infants, including immaturity of the immune system and presence of maternal RSV-specific antibodies; both of which are associated with suboptimal vaccine responses [6]. Alternative strategies for RSV vaccination have been proposed [7], including delaying delivery to an older age [8]. In addition to direct protection of the recipient, vaccination of older age groups may lead to indirect protection of the vulnerable infant by reducing circulation of virus in the population or preventing chains of transmission to the infant [7]. Future availability of more affordable generic candidates of palivizumab (the only currently available treatment for RSV) targeting the RSV fusion (F) protein will potentially lead to more widespread use of these drugs to treat RSV disease in very young children. Reports of RSV variants with reduced susceptibility to palivizumab have been reported which also highlights the need to monitor drift in circulating strains. Nonetheless, to evaluate the impact of these interventions knowledge of the RSV burden, transmission dynamic and source of infection for infants in the community is needed.

7.3. Bacterial pathogens

The development of bacterial respiratory tract and invasive infections is preceded by nasopharyngeal colonization with the infecting pathogen. Although colonization is a prerequisite for the development of disease, the majority of individuals remain asymptomatic and only a small proportion of colonized individuals develop disease. There is a complex interplay of host, pathogen and environmental factors that determine whether the colonising organisms will be cleared by the host's immune system or transmitted to cavities such as the lungs and/or cross the mucosal barrier and invade normally sterile sites resulting in invasive disease. While factors such as underlying Human Immunodeficiency Virus (HIV) infection [9, 10] are established risk factors for developing disease, other factors remain unknown.

At any one point in time, the nasopharynx of an individual is inhabited by a complex microbiome of pathogens. Use of polymerase chain reaction (PCR) to determine the etiology of respiratory tract

infections [11-13] has highlighted the prevalence of polymicrobial respiratory tract infections [14-16]. The interaction of these co-existing pathogens in the upper respiratory tract plays an important role in the bacterial loads of the organism in the nasopharynx, and development of disease. The interaction between the pneumococcus and influenza virus is one of the most well documented synergistic bacterial-viral interactions, with influenza virus infection being a risk factor for the subsequent development of pneumococcal disease [17, 18]. In a previous study we have shown that respiratory virus infection is associated with elevated pneumococcal colonization density and, in turn, invasive pneumococcal pneumonia [19].

Pertussis, commonly known as whooping cough, is an acute respiratory illness caused by *Bordetella pertussis*. *B. pertussis* can be asymptomatically carried but may also cause severe disease, particularly in infants.[20] Pertussis is vaccine-preventable, and routine use of the whole-cell vaccine for decades has significantly reduced the burden of disease in South Africa, and around the world. However, due to the adverse side effects associated with the whole-cell vaccine many countries have changed to the acellular vaccine, including South Africa in 2009. A number of countries, such as the United States of America, have recently documented increases in pertussis disease which is thought to potentially be due to waning immunity to the acellular vaccine. There are limited data on the prevalence of *B. pertussis* carriage and disease in South Africa, and this is of particular importance as the acellular vaccine has been in use for 6 years. In 2015, data from routine surveillance, have demonstrated an increasing incidence of pertussis infection in several provinces of South Africa [21].

Invasive disease (meningitis and bacteraemia) caused by the fulminant bacterial pathogen *Neisseria meningitidis* (meningococcus) remains a significant problem worldwide. The ecological niche for *N. meningitidis* is the human nasopharynx/throat and carriage rates are approximately 5-10% during endemic disease but may be significantly higher during epidemics or in closed communities [22]. Carriage is most frequent in teenagers and young adults and overcrowding increases the risk of meningococcal carriage and disease [23]. Meningococcal disease is a rare outcome of carriage and the interaction of host, pathogen and environmental factors is poorly understood. Carriage data are thus important in trying to further our understanding of disease and potentially improve patient management. Although we have a comprehensive systematic collection of invasive meningococcal isolates collected through national surveillance since 2000, there are no carriage data for meningococcus in South Africa.[24]

The most common clinical presentation of *Corynebacterium diphtheriae* infection is an upper respiratory tract infection, most commonly involving the tonsils, pharynx, larynx, or nasal mucosa [25].The incubation period is usually between 2 to 5 days, and humans are the only known reservoir. Infection is acquired by respiratory droplet spread. In the prevaccine era, circulation of *C. diphtheriae* organisms was common, and symptomatic and asymptomatic infections provided the opportunity to acquire immunity, so that in both high- and low-income countries natural immunity was rapidly acquired, and by the age of 15 years, most individuals had acquired natural immunity[25, 26]. In countries with routine infant diphtheria vaccination programmes, the level of immunity in older children and young adults declines, especially without regular booster vaccinations[26]. The latter accumulate as a susceptible pool of adults, and together with the presence of susceptible children (e.g., due to low vaccination coverage), may allow for the possibility of outbreaks[26]. In such settings, the bacterium may be commonly carried in the nasopharynx of asymptomatic individuals, especially in settings of low vaccine coverage or waning immunity. South Africa recently experienced a large outbreak of diphtheria in KwaZulu-Natal in 2014-2015. [27] Conditions, thought to be likely causes of the outbreak i.e. very low coverage (<50%) for the 6 and 9 year diphtheria booster vaccines are widespread in all provinces of South Africa. There are no data on the prevalence of diphtheria amongst asymptomatic individuals in South Africa.

7.4. *Streptococcus pyogenes* (group A streptococcus (GAS))

GAS is associated with a range of infections including skin infections, pharyngitis and more severe invasive disease (endocarditis, septic shock syndrome and necrotizing fasciitis). Repeated GAS infections may be associated with acute post infection complications such as glomerulonephritis, acute rheumatic fever and rheumatic heart disease ([28], [29]). Invasive disease rates vary by region with between 1-3 cases per 100 000 persons described [30, 31] [32]. Globally between 5% and 15% of children carry GAS in their throats. Carriage of GAS may occur for months after resolution of an infection or may be independent of a clinical infection. Carriage maybe a potential source for acquisition of infections for others in the community[33, 34] and therefore carriage strains may be of relevance to active disease e.g. pharyngitis. Molecular characterisation of the GAS strains associated with carriage may give insights to risk factors associated with developing GAS active disease as some asymptomatic carriers have been shown to maintain the same strain when progressing to active disease[35].

In 2017 NICD was involved in investigating an outbreak of necrotising fasciitis caused by GAS, (with a high case fatality ratio, 75% among invasive cases) in a long-term care facility. In this outbreak there was also a high prevalence, 14%, of asymptomatic colonization among staff members. This is the first such outbreak to be described in South Africa. Following this outbreak gaps in surveillance for GAS have been identified, including lack of recent data on GAS carriage in healthy people, although there are a few small studies among individuals with pharyngitis or rheumatic heart disease ([36], [37]). The PHIRST study provides an unique opportunity to assess carriage of GAS in the community and will provide three consecutive years of data.

In addition, rheumatic heart disease (non-infectious complication of GAS) notifications are being reassessed with the new notifiable medical condition legislation in South Africa. These data combined with data from additional surveillance programme such as GERMS will enable us to describe to describe and better understand the epidemiology of GAS in our setting. Data on GAS carriage in South Africa will also make an important contribution to the regional and global data on GAS, a pathogen that has gained more attention in recent years as some countries have reported an increase in incidence of invasive disease [38-41].

7.5. Tuberculosis

Despite the recent evidence of initial reductions in tuberculosis rates in South Africa, tuberculosis in Southern Africa remains an extremely severe public health problem. Annual incidence of tuberculosis disease in South Africa massively increased over the past three decades, mostly as a result of the HIV epidemic. And in 2012, South Africa's annual incidence of tuberculosis is ranked third globally with almost 1% of all individuals in South Africa are diagnosed with tuberculosis disease each year. HIV-infected individuals bear the brunt of the disease as over 60% of all patients with tuberculosis are co-infected with HIV. Mortality and morbidity associated with tuberculosis is severe, ~10% of all cases die and of tuberculosis cases admitted to hospital almost one quarter die during their admission. However, details on the transmission and acquisition of tuberculosis are scanty, and data on prevalence and incidence of tuberculosis infection are unknown as there are few contemporaneous studies from Southern Africa reporting rates of tuberculosis infection in different settings, and no prospective studies that report risk factors for incident tuberculosis infection. A cross-sectional report from Cape Town suggests that scholars

who recently started high school have a dramatic increase in annual risk of tuberculosis infection [42] and our cross-sectional data from Matlosana show that the annual risk of infection in children at crèche or recently started school is 2.5% per annum [43]. People infected with tuberculosis are the reservoir of tuberculosis cases in the future and partially reflect the efficiency of the South African National Tuberculosis Control Programme to curb tuberculosis transmission by identifying infectious cases and treating them early. Having prospective estimates of incidence of tuberculosis infection and risk factors for tuberculosis infection (including the presence of a case of infectious tuberculosis in the household) will provide valuable data for subsequent clinical trials of preventing tuberculosis, modelling the impact of control measures and, assessing the impact of the National Tuberculosis Control Programme.

7.6. HIV

Although the public health antiretroviral therapy programme which has initiated over 2.6 million people on antiretroviral treatment (ART) since 2004 has had immediate and dramatic beneficial impacts on survival, risk of opportunistic infection, quality of life and household wealth; HIV incidence remains high. Recent reports of HIV incidence for the country [44], and in the non-intervention arms of clinical trials of prevention interventions show that HIV incidence overall remains high. Despite this, there is little HIV incidence data in prospectively followed cohorts. A massive condom distribution program, a nationwide male circumcision program, and the step wise increasing the CD4 threshold for initiation of antiretroviral therapy from a base of 200 cells/mm^3 to the current 500 cells/mm^3 should reduce HIV incidence. However, there is a reliance on cross-sectional surveys to assess reductions in HIV incidence. Our study should add to understanding of the impact of HIV infection on transmission and acquisition of a range of respiratory pathogens and, may also provide some insight into new HIV infections.

7.7. Indoor air pollution

Despite the rollout of a massive electrification programme over the past two decades, large numbers of South African households continue to make use of polluting fuels for cooking and space heating. Evidence reviews have shown that the risk of pneumonia in young children is increased by exposure to unprocessed solid fuels by a factor of 1.8 [45, 46]. In the South African context therefore, an examination of the role of exposure to domestic air pollution in respiratory disease transmission is of high importance.

7.7 Household contact patterns and disease transmission

Respiratory infections spread mainly through large droplets and self-inoculation from contaminated surfaces [47, 48]. Measuring the quantity and duration of face-to-face interactions among people is important to understand this transmission [49-51]. Most of the contacts we make tend to be in limited social groupings such as households, school or work setting [52], and this has also been suggested to influence the spread of respiratory pathogens [53, 54]. Despite the importance of empirical rates of contact in transmission models, data from developing countries are limited even though there is a heavy burden of respiratory and other infectious diseases from these settings [4, 55-58].

In infectious disease epidemiology, contact networks consist of individuals (nodes) with connections between (edges) that represent interactions which can lead to infection transmission [59]. For respiratory

and close contact infections, social contact networks can be used to highlight potential transmission routes [60]. Questionnaire surveys have been conventionally used to collect data on contact patterns [61-66] and networks [67-71]. Despite providing "who-contacts-whom" matrices that can be incorporated into mathematical models of infection transmission and control [65, 66, 72, 73], the questionnaire method suffers several limitations key being recall bias and low participation rates [51, 63, 65]. Current models that omit important factors such as frequency, duration and location of contacts do not capture the heterogeneity of transmission that has direct bearing on intervention measures [74, 75]. Efforts to provide methods that aim to cover these gaps have resulted in the use and advancement of automated methods of data collection. These include the wireless sensors embedded in portable devices such as mobile phones and customized wearable tags that use Bluetooth and WiFi [76, 77] or low power radio frequencies [[78, 79], respectively, to determine proximity and co-location of users.

Radio frequency (RF) proximity sensors (henceforth referred to as "tags") have been used in closed settings such as schools [49, 80, 81], hospitals[79, 82-84], work [85] and conferences [86] to characterize close contact social networks relevant to the spread of respiratory infections. The sensor platform in these studies has been designed to collect proximity data only from individuals facing each other while wearing the tags, representing conversations or actual physical touch that can lead to infection transmission (http://www.sociopatterns.org). The majority of studies using this platform reported a high participation rate (≥75%) suggesting that an unobtrusive way of data collection requiring minimal participant intervention elicits better response rates compared to paper diaries [5], especially in settings with a high proportion of illiterate individuals and have been shown to be feasible in a rural Kenyan setting [87]. Despite the relatively high initial cost of design and production, tags provide a rich data source, even for partial networks, that can be used to investigate plausible characteristics of infection transmission on networks weighted by frequency and duration of contacts. In addition, statistical methods are available to impute missing data by generating synthetic networks based on the network properties supplemented by additional demographic assumptions [33].

8. Justification for the study and study impact

This study will provide better understanding of the community burden of selected respiratory viral and bacterial pathogen infections in South Africa. The data generated from this study will also provide important information on the transmission dynamics of these pathogens in the community (including its determinants) allowing to better strategize for interventions (including potentially cocooning vaccination strategies for selected pathogens like influenza, pertussis and potentially RSV, and targeting groups at highest risk of transmission for diseases such as meningococcus, diphtheria, pertussis and pneumococcus) and evaluate their potential impact. The additional data on GAS carriage will build a new dataset to describe the prevalence of GAS carriage and molecular charecterisation of GAS strains (*emm* type profile) in our setting. In addition, for influenza, diphtheria, GAS and meningococcus, information obtained on carriage prevalence and or transmission within households will be useful to inform preparedness and response to outbreaks.

9. Objectives

9.1. Primary

9.1.1. To estimate the community burden of influenza and RSV, including:

9.1.1.1. the incidence of influenza and RSV infection in the community as determined by polymerase chain reaction (PCR) and serologic assays;

9.1.1.2. the symptomatic fraction associated with influenza and RSV infection;

9.1.1.3. the severity associated with symptomatic infections; and

9.1.1.4. the fraction of individuals with symptomatic infection seeking medical care

9.1.2. To assess the transmission dynamics of influenza and RSV infections in the community, including:

9.1.2.1. the estimation of the household secondary infection risk (SIR), serial interval and length of shedding;

9.1.2.2. the estimation of transmission of infection between individuals (both symptomatic and symptomatic) within the household and possibly the community; and

9.1.2.3. the estimation of the effective reproductive number (Rt) and its variation over time in the community.

9.2. Secondary

9.2.1. Objectives related to transmission dynamics, burden and health-seeking behavior of influenza and RSV

9.2.1.1. To estimate the symptomatic fraction, the severity associated with symptomatic infections and the fraction of individuals with symptomatic infection seeking medical care among influenza- or RSV-positive cases by HIV serostatus and age

9.2.1.2. To estimate the SIR and length of shedding of influenza and RSV among HIV-infected and HIV-uninfected index cases and the rate of acquisition of influenza and RSV infection among HIV-infected and HIV-uninfected household members

9.2.1.3. To assess the role of asymptomatic infections in the household transmission of influenza an RSV

9.2.1.4. To estimate the correlation between individuals that seroconverted for influenza and RSV and tested positive at PCR for each of the two viruses

9.2.2. Objectives related to respiratory virus characterization and evolution

9.2.2.1. Determine the contribution of specific influenza A and B subtypes or lineages to community burden of influenza

9.2.2.2. Determine the contribution of RSV-A and -B strains to the community burden of RSV

9.2.2.3. Determine the antigenic relatedness of influenza virus strains circulating within the community to the vaccine strains.

9.2.2.4. Determine the heterogeneity of influenza and RSV virus strains within household clusters and describe viral evolution within and between households as well as the association between virus strains and the duration of virus shedding and HIV status

9.2.2.5. Use molecular evolutionary analysis to better understand transmission networks associated with influenza virus and RSV spread within households and communities

9.2.2.6. Perform molecular analysis of the RSV F protein gene from circulating strains for naturally occurring polymorphisms associated with reduced susceptibility to therapeutic interventions

9.2.2.7. Assess the antigenic profile of RSV strains circulating within households and in the community

9.2.2.8. To identify the prevalence and duration colonization within the community by age group, vaccine status and over time, the transmission dynamics within a household, and the proportion of individuals that develop symptomatic infection for enterovirus, human metapneumovirus (hMPV), parainfluenza virus (PIV) 1-4, rhinovirus, coronaviruses (229E, OC43, NL63, HKU1), bocavirus, polyomavirus and adenovirus

9.2.3. Objectives related to bacterial colonization and infection

9.2.3.1. To describe and compare nasopharyngeal pneumococcal loads in healthy individuals by age and over time, and how the loads may be altered by respiratory viral infection, HIV-status and vaccination-status

9.2.3.2. To determine the prevalence of pneumococcal DNA (*lytA)* in the blood of healthy individuals at a single point in time, and the correlation between detection in the blood and the presence of nasopharyngeal colonization and nasopharyngeal pneumococcal load

9.2.3.3. To identify the prevalence and duration of *B. pertussis* colonization within the community by age group, vaccine status and over time, the transmission dynamics within a household, and the proportion of individuals that develop symptomatic infection

9.2.3.4. To determine the prevalence of *N. meningitidis* colonization within the community by age group at a single point in time each year and the predominant serogroups being carried within a household

9.2.3.5. To determine the prevalence of *C. diphtheriae* colonization within the community by age group at a single point in time each year and the characteristics of diphtheria strains being carried within a household

9.2.3.6. To determine the prevalence of GAS carriage in the community by age group (retrospectively for 2016 and 2017 and at a single point in time for 2018) and the characteristic of the GAS strains within a household.

9.2.3.7. To describe the composition of the nasopharyngeal microbiota in healthy individuals, by age and over time, and how this changes with the development of symptomatic and asymptomatic infection in the post-pneumococcal conjugate vaccine (PCV) era

9.2.4. Objectives related to important co-infections such as tuberculosis or HIV

1.1.1.1. To measure the annual incidence of tuberculosis infection in individuals living in study households and assess risk factors (including incident and prevalent HIV) for acquiring tuberculosis infection.

1.1.1.2. To evaluate whether infection with respiratory viruses leads to increased incidence of tuberculosis infection or disease

1.1.1.3. To evaluate whether tuberculosis disease is associated with increased influenza transmission in households

9.2.5. Objectives related to housing quality and exposure to indoor air pollution

9.2.5.1. To document housing quality in all dwellings

9.2.5.2. To determine the main fuels used for cooking and space and water heating;

9.2.5.3. To measure levels of indoor air pollution (particulate matter and carbon monoxide) in the study dwellings;

9.2.5.4. To measure levels of dust deposition indoors;

9.2.5.5. To examine dampness and mould in dwellings as a risk factor for respiratory symptoms

9.2.5.6. To ascertain the impact of housing quality and exposure to indoor air pollution on tuberculosis infection and on household transmission of respiratory viruses and bacteria

9.2.6. Objectives related to household contact patterns

9.2.6.1. Estimate the proportion of time spent in the home and outside the home by age group, site and time of year

9.2.6.2. Describe and compare the contact patterns and location of contacts within the house between individuals within households in a rural and peri-urban site in South Africa

9.2.6.3. Measure the number of contacts between individuals from the same household inside of the home

9.2.6.4. Estimate the association between contact patterns and transmission events between individuals within households for influenza, RSV and pneumococcus

9.2.6.5. Compare the contact patterns of individuals when they develop respiratory symptoms to the contact patterns of individuals not experiencing respiratory symptoms

9.2.6.6. Relate contact patterns and transmission between individuals to environmental conditions such as temperature, humidity and indoor air pollution

10. Methods and procedures

10.1. Definitions

Household: A group of three or more people who regularly share at least two meals in the same residence at least two days per week (residential institutions excluded).

Migrant household member: A person considered as a household member who resides elsewhere but regularly spends weekend or holiday periods within the household.

Laboratory-Confirmed Influenza Infection on PCR: An individual with a positive influenza result on real-time Reverse Transcriptase Polymerase Chain Reaction (RTPCR).

Laboratory-Confirmed Influenza Infection on Serology: An individual (who did not receive influenza vaccine) with a four-fold rise in serum titres of antibodies to influenza on the haemaglutination-inhibition test between pre- and post-influenza season serum samples.

Laboratory-Confirmed RSV Infection on PCR: An individual with a positive RSV result on real-time RTPCR.

Laboratory-Confirmed RSV Infection on Serology: An individual with a four-fold rise in serum titres of antibodies to RSV between pre- and post-RSV season serum samples.

Influenza-like illness (ILI) with documented fever: An acute respiratory infection with measured fever of ≥38 C° and cough, with onset since the last household visit or within the last ten days.

ILI with feeling feverish: An acute respiratory infection with self-reported fever (but not documented) and cough, with onset since the last household visit or within the last ten days.

Tuberculosis infection: Tuberculin skin test (TST) positivity measured at 48-72 hours after placing the TST according to HIV serostatus.

Any acute respiratory illness (ARI) not meeting the ILI case definition: self-reported cough, cold, sore throat or other respiratory symptom with onset since the last household visit or within the last ten days that does not meet the ILI case definition.

Household SIR: The proportion of household contacts of an index case that subsequently become infected with influenza or RSV or other respiratory pathogen.

Serial Interval: The time interval between onset of confirmed infection in index case and onset of confirmed illness in household contacts.

10.2. Study design overview

We will conduct a household-level prospective cohort study in one rural and one peri-urban community located in Mpumalanga Province (the Agincourt demographic surveillance site) and North West Province (Klerksdorp), respectively. The characteristics of the study population are provided in Section 10.3. The study will be conducted for 3 consecutive RSV and influenza seasons (2016, 2017 and 2018) over a 12-month period each year (January through December). One hundred households; 50 per site with expected average number of household members of 5; will be randomly selected every year within the study population and consented to participate in the study. The process of household selection is described in Section 10.5. The study will be conducted over three years because influenza transmission intensity as well as circulating influenza strains are known to vary substantially from year-to-year. If the study were conducted for only one year, biased measures may be obtained if the year is a particularly high or low transmission year. Each household member will be followed up for the calendar year of selection. In addition, for individuals enrolled in the first and second year of the study, three follow-up visits will be conducted annually to collect serum for serology of influenza and RSV. At two of the visits TST testing and HIV testing will be done/offered to those testing negative on previous tests. At the time of enrollment, the demographic characteristics of each individual within the selected household will be recorded (Appendix 2 Form 2), each household and household member will be enumerated and the HIV and tuberculosis infection status of each household member will be assessed in consenting individuals.

In Agincourt, there is a high rate of migrancy and therefore we expect that many households will have one or more household members who work and reside elsewhere but spend weekends or holiday periods within the household. These individuals will be enumerated in the baseline survey consented for study participation if they are present at any household visit. Thereafter, if they are present in the household at the time of a household visit, forms will be completed and specimens will be collected from them as per other household members for that visit. When not present, they will be recorded as absent.

10.2.1. Estimation of the community burden of influenza, RSV, *C. diphtheriae* and *B. pertussis* infection

For each household member paired sera will be collected before and after the RSV (January-February and April-May) and influenza (April-May and September-October) seasons to assess sero-conversion for the specific pathogen during the time of viral activity. An additional specimen will be collected at the end of the year to assess for viral infection out of the season.

For the assessment of RSV sero-conversion a first serum sample will be collected in January-February while for the assessment of influenza virus sero-conversion a first serum sample will be collected in April-May, this will also serve as the second serum sample for the RSV season. In addition a serum sample will be collected after the estimated end of the influenza season (September-October) and a final specimen at the end of the year (November-December). The actual time of specimen collection will be decided after reviewing data on influenza and RSV circulation. The end of the RSV or influenza season (e.g. weekly detection rate for the specific pathogen <10% for 2 consecutive weeks) will be estimated from national RSV and influenza surveillance. Serum specimens will also be tested for the presence of antibodies to *B. pertussis* and *C. diphtheriae.* The baseline survey will allow estimation of the baseline prevalence of immunity and recent infection. Testing of follow-up sera will provide information on rates of acquisition of infection.

10.2.2. Estimation of the transmission dynamics of influenza, RSV, *S. pneumonia* and *B. pertussis* in the community

Following enrollment, upper respiratory tract samples (nasopharyngeal swabs) will be collected twice a week from each household member irrespective of symptoms during the study period. Samples will be tested for RSV, influenza and other respiratory viruses as well as the selected bacteria throughout the study period. During each visit a follow up questionnaire will be administered to household members to assess development and severity of symptoms and contact patterns among individuals within the household.

10.2.3. Estimation of the carriage prevalence of *N. meningitidis , C. diphtheriae and S.pyogenes* in the community

In the month of July each year, we will conduct a cross-sectional survey to measure the carriage prevalence of *N. meningitidis* and *C. diphtheriae* in the study population. For the conduct of this survey, in addition to the nasopharyngeal swab being collected, we will collect a once-off oropharyngeal swab specimen. The nasopharyngeal and oropharyngeal specimens collected at this visit will be tested for *N. meningitidis* and *C. diphtheriae* using PCR as described below. The oropharyngeal specimens will be tested for *N. meningitidis* and *C. diphtheriae* using culture as described below.

For GAS we will retrospectively test all samples collected in 2016 and 2017 initially by PCR and prospectively in 2018 by culture, PCR and whole genome sequencing, details are described below.

10.2.4. Estimation of the transmission dynamics of *M. tuberculosis* and HIV in the community

At the time of enrollment (January-February) all consenting patients will be offered HIV testing as described in Section 10.11.5 after counselling.

At the time of enrollment (January), and again at the end of the study year (December) all individuals will be tested for tuberculosis infection using the tuberculin skin test (TST). This will allow estimation of tuberculosis incidence.

Follow-up HIV tests will be offered every six months in those who were found to be HIV-negative at baseline. Follow-up TST for all patients previously TST-negative will be placed and read in December of the first study year.

Thereafter at annual follow-up visits in October, HIV testing and TST will be performed in those who were negative previously.

We will also take specimens from anyone who is HIV-infected at baseline to assess prevalence of recent HIV infection.

In addition, at baseline, all household participants will provide a sputum sample for liquid mycobacterial culture. Thereafter, participants will be screened for symptoms of tuberculosis at least monthly and if they have symptoms of active tuberculosis, a sputum specimen for analysis by Xpert Mtuberculosis/Rif will be taken. All patients testing positive for tuberculosis will be referred to the local clinic for treatment.

10.2.5. Assessment of environmental factors associated with pathogen transmission

Information on housing type, construction materials, access to basic environmental health services, dampness and the presence of mould, and fuel use for daily cooking, space and water heating will be collected through the administration of a pre-structured questionnaire. Levels of indoor air pollution (particulate matter (PM_{10} and $PM_{2.5}$), carbon monoxide and carbon dioxide) will be measured using stationary monitoring devices over a period of one week during the winter as well as in the summer. During the same period, one member of each household will keep an air quality monitoring device on their person in order to measure air quality in all locations they visit. This device will have a GPS logger attached to it in order to measure the geographic location of air samples collected. Logtag instruments will be located in all study dwellings to monitor indoor temperature and humidity over a period of one year. Floor dust samples will be collected from each dwelling using US EPA/HUD methods once during the winter and once during the summer. Details of methods for environmental assessment are provided in Appendix 3.

10.2.6. Assessment of household social contact associated with pathogen transmission

Information on proximity data between household members will be collected from consenting household members. A pilot study, aiming to assess the acceptability, feasibility and variation in data using proximity tags will be conducted in 5 households at each site for 4 weeks during September and October of 2017. In 2018, all participating households will be asked to wear the proximity tag devices for a period of 8 – 12

days, repeated every 4 – 6 weeks. All participating household members will be asked to complete a questionnaire twice during the follow-up period on their time use and social contacts (appendix 6) which will be used to validate and supplement the proximity tag data.

10.3. Study setting and population

The study will be conducted at two sites, one in the Mpumalanga province and one in the North West Province.

10.3.1. Mpumalanga Province site

The study will be conducted within the health and socio-demographic surveillance site (HDSS) at the MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt). The population is approximately 110,000 people and is set in the rural Bushbuckridge District of Mpumalanga Province.

10.3.2. North West Province Site

Klerksdorp is located in the local municipality of Matlosana in North West Province and has a population of over 385,000 people and is 115 km^2. The city of Klerksdorp is surrounded by the townships of Jouberton, Alabama, Kanana, Khuma, and Tigane. The townships are organized into extensions that include mostly single-family houses and shacks. Prevalence of HIV in Klerksdorp is approximately 12%.[5] Annual incidence of tuberculosis is extremely high approaching 1200/100,000 with an HIV coinfection rate of over 80%.

10.4. Inclusion and exclusion criteria

Eligible households will be randomly selected households that have been residing in the selected communities for at least one year prior to the commencement of the study, that consent to participate to the study and that are planning to reside in the selected community for the duration of the study. Households should have at least 3 or more individuals in order to be eligible. In addition, at least 50% of households should have at least one child aged <5 years in the household.

Non-eligible households will be households that were not randomly selected, that were selected but did not consent to participate to the study or, that have ≥1 household members that do not consent for inclusion in the study.

10.5. Sampling and sample size

The sample size of approximately 1500 individuals over 3 consecutive years will allow the estimation of 10% risk of infection and a 5% risk of illness or higher with 95% CI and 5% desired absolute precision in the community for the selected pathogens. This should be adequate to address the objectives for influenza and RSV as infection rates of at least 10% are expected.[3, 88]

Two-hundred households (estimated mean household size: 5 members) will be initially randomly selected annually within each of the two selected communities. This will allow sufficient households to ensure that we meet the target of 50 households per site meeting study criteria and considering study refusal. The selection process will be based on a two-stage cluster sampling design whereby 3-5 township extensions (in Klerksdorp) and 3-5 villages (in Agincourt) will be randomly selected first. The same number of households will be then randomly selected within each of the selected extensions or villages. The sampling frame (e.g. list of dwellings within the selected communities) will be obtained from Statistics South Africa for Klerksdorp and the Agincourt HDSS Database. Selected household will be visited prior to the

commencement of the study to verify inclusion criteria and consenting. The verification process will continue until 50 households in each of the two selected communities will be successfully enrolled, including at least 50% of households with children aged <7 years. Five additional households in each community will be enrolled to cater for loss to follow-up. The selection process will be repeated before the beginning of each study year as described above. Households that were selected in the previous study years will not be eligible for selection in consecutive years.

A household in whom >20% of individuals decide to suspend study participation will be considered suspended. If a household is suspended or decides to withdraw from the study during the RSV season (January to April), a new household will be enrolled to replace the household for the next upcoming influenza season (May to December).

10.6. Recruitment period and follow up visits

10.6.1. Recruitment

Household identification and recruitment will be implemented from November of the year preceding the study year. Upon random selection of dwellings within the selected community, households will be visited by trained staff to verify inclusion criteria and consenting. Written informed consent will be obtained from each household member as detailed in Section 10.6.1 and Appendix 4. Each enrolled household and household member will be enumerated and the demographic characteristic of the household will be recorded. The physical address and/or global positioning system (GPS) coordinate of each dwelling will also be recorded. The starting time of the study (January of each study year) will be communicated to the primary caregiver. As household recruitment proceeds, the number of households with children aged <5 years will be reviewed. When 50% of households have been recruited the proportion of households with children aged <5 years will be evaluated and thereafter households with children may be preferentially selected until the target is met (e.g. at least 50% of households with at least one children <5 years of age).

After enrollment and enumeration of the target number of households is completed, the number of enrolled household members will be evaluated to assess that the target sample size is met. If the number of enrolled household members is below 90% of the target number of household members then additional households will be recruited until the target sample size is met.

After recruitment is completed, a first visit and follow-up plan for each household will be developed. The exact start date of the study will be then communicated to the primary caregiver by telephone or household visit 2 weeks before the assigned start date of the study. This process will be repeated for each study year and if new households are enrolled mid-year. Any babies who are born into the study household during the study period will be recruited.

- Detailed baseline questionnaires on household make-up, demographics, regular sleeping and eating arrangements, occupation and underlying illnesses will be completed (Appendix 2, Questionnaire 2 and 3)

A detailed diagram of the study visits is provided in Figure 1.

Figure 1: PHIRST Study Chronogram

* 500 individuals from 100 households.

** In addition to baseline, expectorated sputum for tuberculosis testing will be collected whenever symptoms suggestive of tuberculosis are recorded.

*** Symptoms Forms (Form 7), Hospitalization Forms (Form 9) and Death Forms (Form 10) will be administered whenever a household member was symptomatic, hospitalized or died during the twice-weekly follow-up visits.

10.6.2. First study visit

The first study visit at each household will take place between the second week of January and the second week of February of each study year. At the first study visit the household demographics on record will be verified and the follow-up schedule will be communicated and agreed upon with the head of the household and household members. A copy of the follow up visits calendar will be provided to the head of the household. During the first study visit the following specimens will be collected and assessments made for each household member or the whole household as appropriate:

- A serum sample will be collected for serological testing for influenza, RSV, pertussis and diphtheria. If serum is insufficient influenza and RSV testing will be prioritized, followed by pertussis and diphtheria.
- A tuberculin skin test will be performed and read 48-72 hours thereafter
- An expectorated sputum specimen for culture will be collected
- HIV testing will be offered as described under the section HIV testing below
- Nasopharyngeal swabs will be collected for bacterial and viral detection
- A urine specimen will be taken from all individuals who are able to provide a urine sample
- A copy of the vaccination card of all children aged <18 years and the verbal vaccination history for influenza vaccine and other vaccines outside of the routine infant immunization schedule of each household member will be collected
- A structured questionnaire will be administered to assess presence, duration and severity of symptoms as well as contact patterns among individuals within the household (Appendix 2, Questionnaire 7)
- Housing typology, visible signs of the presence of indoor air pollution (fossil fuel heating device, ashtrays or other signs of indoor smoking, number of windows and doors, number of people sleeping in each room and household, etc. will be recorded (Appendix 2, Questionnaire 4 and 5)
- Logtag instruments will be located in all study dwellings to monitor indoor temperature and humidity over a period of one year
- The date and time of the next visit will be communicated and agreed upon

10.6.3. Twice-weekly follow-up visits

After the first study visit, each household will be visited twice per week according to a pre-established schedule.

During each visit the following will take place:

- Nasopharyngeal swabs will be collected for bacterial and viral detection
- Structured questionnaires will be administered to assess presence, duration and severity of symptoms, contact patterns among individuals within the household and the history of any tuberculosis testing that the household member may have undergone outside of the study procedures
- For symptomatic individuals the following will be performed at each visit until symptoms resolve:
    - A digital temperature will be measured and documented
    - A detailed symptom history will be completed (Appendix 2, Questionnaire 7)
    - Healthcare seeking behavior will be recorded
    - For any individuals who are admitted to hospital or die a detailed form (Appendix 2, Questionnaire 9 and 10) will be completed to collect information on clinical presentation, management and laboratory results.
    - Individuals who warrant admission to hospital with respiratory illness will be referred to Matikwana or Mapulaneng Hospital in Mpumalanga and Klerksdorp or Tshepong Hospital in Klerksdorp and enrolled into the NICD pneumonia surveillance programme (Wits HREC Protocol number M140824).
- At least monthly, during the household visit, symptoms suggestive of tuberculosis[89] (cough and/or fever and/or drenching night sweats and/or unexplained weight loss) will be screened for (Appendix 2, Questionnaire 8) and if anyone is identified, the following will take place then a sputum specimen for analysis using the Xpert Mtuberculosis/Rif will be taken. All patients testing positive for tuberculosis will be referred for treatment to the local clinic.

10.6.4. Annual visit for the survey of meningococcal, diphtheria and GAS carriage

Once a year in the month of July, in addition to the nasopharyngeal specimen collected on the twice-weekly visits, we will collect an oropharyngeal swab for detection of meningococcus and diphtheria colonization by PCR and culture. For GAS the samples collected in 2016 and 2017 will be tested retrospectively.

10.6.5. Assessment of levels of indoor air pollution

Levels of indoor air pollution (particulate matter (PM_{10} and $PM_{2.5}$) and carbon monoxide) will be measured using stationary monitoring devices over a period of one week during the winter as well as in the summer. During the same period one member of each household will be asked to carry a portable air quality monitoring device with them. This will be pre-arranged with the household based on instrument availability. In addition, at the same time we will assess levels of dust in the household.

10.6.6. December end-of-year visit

Towards the end of the year, at the first household visit in December a follow-up TST will be performed on all household members who tested negative previously.

At this visit a copy of the vaccination card and the vaccination history of each household member aged <18 years will also be evaluated at this visit to ascertain if vaccination status has changed during the study year.

10.6.7. Quarterly follow-up visits

Follow-up visits for the collection of additional blood specimens will be performed in April-May (before the influenza season), September-October (after the influenza and RSV seasons) and November-December (the end of the year). The exact date within these months will be determined by reviewing data on influenza and RSV transmission to ensure that the post season specimens are taken when pathogen circulation has dropped below 10%.

At these visits all specimens and information will be collected as per twice-weekly follow-up visits. In addition, a blood specimen will be collected from each household member. At the April-May and September-October and November-December visits the detailed baseline questionnaire on household make-up, demographics, regular sleeping and eating arrangements, occupation, fuel use, underlying illnesses will be repeated (Appendix 2 Questionnaire 2, 4, 5).

10.6.8. Annual follow-up visits for individuals enrolled in the first and second year of the study

For individuals enrolled in the first and second year of the study, we will perform three visits per year in each subsequent year, one in February, one in May and one in October. This means that the first cohort of households will have 2 years of these visits and the second cohort of houses will have 1 year of these follow up visits. At these visits, a serum specimen will be collected from each individual. This specimen will be tested for influenza and RSV serology. During at least one of the visit HIV testing will be offered to those consenting individuals who previously tested HIV negative (there will be an open opportunity to test for HIV at each contact with the household to encourage people to test and access care). In addition, a TST will be performed during the February and October visit on those who tested negative on TST previously. In addition, we will ask the questions in Questionnaire 2, 3 and 8 (household enrolment form and case intake form and tuberculosis form) again.

10.6.9. Data and specimen collection

Household visits will be performed by teams of an enrolled nurse who may be accompanied by a field worker. All specimen collection and medical investigations, specifically, nasopharyngeal swabs, blood specimens, sputum specimen collection and performance of tuberculin skin tests will be performed by a nurse. All staff will be fully trained in collection of specimens and data. Details of procedures for specimen and data collection are described below.

10.7. Data collection

10.7.1. Questionnaires

The demographic characteristics of the households will be collected on enrollment by structured interview conducted by trained field workers. During each visit the development of symptoms (using a severity score) and healthcare-seeking behavior of symptomatic cases will be recorded. For each symptomatic case a structured interview will be done to assess duration and severity of symptoms. The questionnaires are included in Appendix 2, updated forms are attached as additional submissions as per the covering letter. This is due to the size and format of the download from the REDcap database.

10.7.2. Household contact data collection

We will collect information on contact patterns between individuals.

We will conduct a pilot study in 5 households at each study site for 4 weeks. The households will be selected randomly and approached to participate in the pilot study. Each household member will be asked to wear a tag (described in detail below) for 1 month (see image in Appendix 6). In order for a household to be eligible to participate in the pilot study at least 80% of household members must be willing to wear the tag. For the pilot study the tags will be packaged in waterproof cloth pouches which can be carried in the chest pocket or pinned to the inside of clothes using a safety pin. During the pilot we will explore the acceptability of this packaging and obtain input on any possible improvements.

In 2018, all participating households will be approached to participate in the household contact study. All household members will be asked to wear the device for 8 – 12 days (depending on the exact day the tag can be issued to each participant and recollected), every 4 – 6 weeks to capture information for the different seasons (summer, autumn, winter and spring) and to capture information in and out of the school holidays. To identify the location of contacts between household members within the home, tags will be placed in key locations within the houses (for example kitchen, living room, and bedrooms). During deployment, participants will be asked to complete a time sheet (Appendix 6) to indicate during what periods the tag was not worn. To enable participants to record this information, each household will be supplied with one digital clock. This clock will stay with the household after the study is completed. To measure contacts of participants of the study outside houses, participants will be asked to complete a contact diary and time use data questionnaire collecting information on the number of contacts per day, the age of the individual whom there was contact with and where the contact took place. In addition, all household members will be asked to complete a questionnaire on their time use and social contacts two times per year.

Household members will be eligible to participate in the main study even if they refuse to participate in the contact study.

Data collection infrastructure and type

The tags detect dyadic close proximity interactions between individuals separated by ≤1.5 metres (or other customized distance), suggestive of a conversation or skin-to-skin touch such as a handshake. The tags are considered to be in proximity when at least one data packet (device ID, timestamp, power level) was exchanged between them during a 20 second interval. A 20-second gap indicated a contact break. The tag will be worn as in the pilot if found to be acceptable to the community. Only face-to-face proximity relations will be detected since the radiofrequency used cannot propagate through the human body. For each deployment, field workers will issue each participating household member with a tag. Fieldworkers will ensure participants are properly trained on carriage and storage of devices. During the data collection with the devices, trouble-shooting and mitigation according to laid down SOPs will be conducted. The head of the household will also be asked to ensure that correct use of the devices is maintained throughout the study. At the end of each deployment period, fieldworkers will collect all the tags from participants and ship these to NICD where data retrieval will take place. After the data is downloaded, the tags will be shipped back to the sites where fieldworkers will issue them back to participants for the next deployment.

Community engagement and consent

A community engagement plan will be developed in consultation with site partners, including relevant stakeholders. The household head will be requested to assent to engaging the rest of the household.

Other residents will provide individual informed consent or assent as appropriate. Joint feedback meetings with study participants and other stakeholders will be organized per site in consultation with the site teams. General results of the study and experiences will be presented and discussed. The study progress and findings will also be discussed at local and international scientific meetings, and published in peer-reviewed journals as appropriate.

10.8. Specimen collection

Respiratory pathogen diagnosis depends on the collection of high-quality specimens, their rapid transport to the laboratory and appropriate storage before laboratory testing. Viruses and bacteria are best detected in specimens containing infected cells and secretions. In addition, it is important for samples for tuberculosis diagnosis as well as blood specimens for serology to be transported to the laboratory as soon as possible and cold chain maintained as delays in the transportation increase the number of contaminating bacteria and this may result in higher percentage of contaminated cultures. Specific procedures for the collection of specimens are described in Appendix 1.

Sampling from the respiratory tract is hazardous due to the generation of aerosols and droplets. Standard precautions should always be followed, and barrier protections applied whenever samples are obtained from patients. Respiratory samples (oropharyngeal (OP) swabs, nasophryngeal (NP) swabs and expectorated sputum) from pneumonia cases should be collected wearing disposable gloves and N95 particulate respirator mask.

All human biological materials collected as part of this protocol will be used only for the study described in this protocol.

A table summarizing the types of specimens to be collecting and testing to be done is included (Table 1).

Table 1: Specimens to be collected, timing, collection procedures and tests performed in the PHIRST study

Specimen type	When collected	Collection container	Storage conditions	Tests performed
Blood	Four times per year (January-February; April-May; September-October and November-December)	Clotted tube	Refrigerate specimen, spin down within 8 hours and freeze at -70 degrees	Serology for influenza, RSV, pertussis, diphtheria. HIV testing for consenting patients.
Blood	Once per year September-October	EDTA tube	Refrigerate specimen	Pneumococcal *lytA* PCR
Blood	For confirmed HIV positive participants	EDTA and Heparin tube	Sample transported within 24 hours to reference laboratory	Viral load and CD4 count test
Nasopharyngeal flocked swabs	Twice weekly	Primestore	Room temperature 30 days	Influenza, RSV, enterovirus, human metapneumovirus (hMPV), parainfluenza virus (PIV) 1-4, rhinovirus, coronaviruses (229E, OC43, NL63, HKU1), bocavirus, polyomavirus, adenovirus, *S. 33pneumoniae, B.pertussis, B. parapertussis*
Nasopharyngeal flocked swabs for cross-sectional survey	Once a year in July	Primestore	Room temperature 30 days	*C. diptheriae, N. meningitidis*
Oropharyngeal flocked swabs for cross-sectional survey	Once a year in July	Amies transport medium	Room temperature	*C. diphtheriae, N. meningitides, Group A streptococcus*
Expectorated sputum	If symptomatic of tuberculosis	Universal container	Refrigerate specimen	*M. tuberculosis* – baseline specimen will be tested by culture and follow up specimens by GeneXpert
Urine	for all who can produce a sample	Universal container	Refrigerate specimen	Quantitative Urine cotinine

10.8.1. Blood samples for serologic testing

We will collect whole blood for serologic testing from each household member four times per year as described above. In addition, for individuals enrolled in the first 2 years of the study annual, we will collect blood at annual follow-up visits in October. We will collect up to 5 ml of blood from children aged <15 years and up to 10 ml of blood from individuals aged ≥15 years at each blood draw. Detailed procedures are described in Appendix 1 Section 3.

10.8.2. EDTA tube for CD4 testing and heparin tube for viral load testing,

We will collect a sample for CD4 count and viral load testing on Individuals testing positive for HIV.

10.8.3. Blood samples for pneumococcal *lytA* testing

When blood is collected in September-October, one additional Ethylenediaminetetraacetic acid (EDTA) blood tube will be collected in addition to the clotted blood described in 10.8.1. Blood will be taken by a trained nurse. We will collect 1 ml of blood from children aged <15 years and 5ml from individuals aged ≥15 years.

10.8.4. Nasopharyngeal swabs for identification of respiratory pathogens

Nasopharyngeal flocked swabs (Copan Diagnostics, Murrieta, CA) will be collected twice weekly and inserted into Primestore medium. Procedures for collection are described in Appendix 1 Section 2.

10.8.5. Oropharyngeal swabs for identification of respiratory pathogens

Oropharyngeal flocked swabs (Copan Diagnostics, Murrieta, CA) will be collected once-off in July and inserted into Primestore medium. Procedures for collection are described in Appendix 1 Section 2.

10.8.6. Sputum samples for tuberculosis testing

Sputum samples will be collected at baseline and thereafter from any individual with tuberculosis symptoms and placed into a universal container. Procedures for collection are described in Appendix 1 Section 5.

10.8.7. Urine samples for cotinine testing

Urine samples at baseline will be collected from all individuals who are able to produce a urine specimen quantitative urine cotinine levels. Procedures for collection are described in Appendix 1 Section 4.

10.9. Specimen labeling

The collection containers will be marked with:

- The unique identifier
- Visit number
- The specimen date
- The type of specimen in the tube (e.g. nasopharyngeal swab)

Note: The tube/ universal container itself and not the cap should always be marked with identifying details. An indelible and alcohol resistant marker should be used as stick on labels can easily come off, especially when the specimens are chilled.

As soon as the specimens are collected, the relevant information should be recorded on the Laboratory Specimen Submission Form (Appendix 2 Form 11).

10.10. Specimen transport and storage

10.10.1. Nasopharyngeal specimens

Specimens should be inserted into Primestore and can be stored for up to 30 days at ambient temperature (2-30˚C). Specimens will be transported to the NICD for testing on a weekly basis. Specimen aliquots will be stored in a -70 deg C freezer until testing.

10.10.2. Oropharyngeal specimens

OP swabs will be placed in Amies Transport medium and submitted to the laboratory within 72 hours of collection.

10.10.3. Blood samples

Clotted blood samples collected in vacutainer tubes should be kept refrigerated (4°C) and should be transported as soon as possible to the laboratory on ice along with the laboratory slip. Site laboratories will spin the blood at 1000g for 10 minutes, keep tubes upright after centrifugation and ship in that manner to NICD. ETDA/heparin samples will be collected for CD4 and viral load testing, these will be shipped immediately to Lancet laboratories in either Hazyview or Klerksdorp for testing.

1.1.1. Sputum and urine samples

Sputum and urine samples should be collected in universal containers and transported on ice to the laboratory for testing

1.1.2. Packaging and transport

Detailed information about packing/transporting specimens can be found in Appendix 4.

Specimens will be transported in sealed plastic bags.

Blood and sputum specimens must be kept cold during transport and a cooler box filled with ice packs can be used for this purpose.

10.11. Laboratory methods

10.11.1. PCR testing for respiratory viruses

Nucleic acids will be extracted from Primestore medium using the MagNA Pure Total Nucleic Acid small volume kit. Upper respiratory tract samples will be tested for RSV and influenza A and B viruses by real-time RTPCR using the FTD Flu/RSV detection assay (Fast Track Diagnostics, Luxembourg). Influenza A and B positive samples will be subtyped using the CDC influenza influenza A (H1/H3/H1pdm09) subtyping and the CDC B/Yamagata- B/Victoria lineage typing kits respectively (available through Influenza Reagent Resource Program; www.influenzareagentresource.org) RSV A and B subtypes will also be determined by real-time RTPCR.

Additional respiratory viruses (rhinovirus, PIV 1,2 & 3, hMPV, adenovirus, coronavirus, bocavirus and enterovirus) will be determined using real time RTPCR.

10.11.2. PCR and culture testing for respiratory bacteria

10.11.2.1. Detection and quantification of *S. pneumoniae* in nasopharyngeal swabs

S. pneumoniae will be detected using a single-target (*lytA*) quantitative real-time PCR assay [90]. Samples with a cycle threshold (C_t) value of ≥40 will be recorded as negative. A standard curve will be prepared using serially diluted DNA extracts from a known quantity (confirmed spectrophotometrically) of *S. pneumoniae* ATCC49619, and used to calculate pneumococcal loads (genomic copies/ml).

10.11.2.2. Detection of *S. pneumoniae* in EDTA blood specimens

Total nucleic acids will be extracted using the Roche MagNA Pure 96 instrument (Roche, Mannheim, Germany) according to the manufacturer's instructions, and tested for the pneumococcus using a single-target (*lytA*) quantitative real-time PCR assay [90]. Samples with a cycle threshold (C_t) value of ≥40 will be recorded as negative.

10.11.2.3. Detection of *N. meningitidis* by PCR

N. meningitis will be detected by a singleplex real-time PCR assay targeting the *sodC* gene, which will enable the detection of groupable and non-groupable meningococci [91]. Specimens testing positive for meningococcus will be serogrouped using a previously described method which enables the identification of serogroups A,B,C,W,X and Y [92].

10.11.2.4. Detection of *Bordetella* spp. by PCR

Bordetella spp. (including *B. pertussis, B. parapertussis, B. bronchiseptica* and *B. holmesii*) will be detected by a combination of a triplex and singleplex real-time PCR assay and results interpreted as previously described [93].

10.11.2.5. Detection of *C. diphtheriae* by PCR

C. diphtheriae will be detected by targeting the *rpoB* gene, and toxigenic strains identified by the detection of the *tox* gene [94].

10.11.2.6. Detection of *C. diphtheriae* and *N. meningitidis* by culture

Each swab will be removed from Amies transport medium on arrival in the laboratory and used to inoculate the following 3 agar plates: 5% horse blood agar, New York City agar (NYC) and Hoyles and the plated out for single colonies. The blood and NYC plates will be incubated in CO_2 at 37°C and the Hoyles in O_2 at 37°C. Plates will be examined the next day for the presence of suspicious colonies, and re-incubated a further 24 hours. Suspicious colonies of *N. meningitidis* and *C. diphtheriae* will be picked off and sub cultured to confirm identity using standard laboratory methods. Serogroups *of N. meningitidis* isolates will be determined phenotypically using latex slide agglutination with antisera for capsular polysaccharides A, B, C, W, X, Y and Z. A subset of carriage isolates will be further characterised by whole genome sequencing at the NICD core sequencing facility. Toxin production of *C. diphtheriae* isolates will be determined using standard laboratory methods

10.11.2.7. Detection of *Group A streptococcus* by PCR and Culture

Retrospectively all samples collected in 2016 and 2017 (from -70°C) will be thawed and a PCR assay performed on all of these to detect the spy-gene which is specific to Streptococcus pyogenes (GAS). For

all samples that are PCR positive for GAS, 100ul of the broth will be used to inoculate a blood agar plate for the isolation of GAS. Plates will be incubated in CO2 at 37°C overnight, and suspicious beta haemolytic colonies will be further tested to confirm their identification as GAS. Plates will be checked up to 72 hours before being discarded as GAS not isolated. Confirmed isolates will be submitted for whole genome sequencing and stored in skimmed milk in -70°C for any future work-ups.

For the 2018 cohort; we will firstly attempt to isolate GAS by placing 100ul of the broth the swab was immersed in, onto blood agar plates. Plates will be incubated in CO2 at 37°C overnight, and suspicious beta haemolytic colonies will be further tested to confirm their identification as GAS. Plates will be checked up to 72 hours before being discarded as 'GAS not isolated'. Confirmed isolates will then be stored in skimmed milk in -70°C for any future work-ups, such as whole genome sequencing. A PCR assay will be performed on all of these samples to detect the spy-gene, specific to Streptococcus pyogenes (GAS).

10.11.2.8. Microbiome studies

A subset of specimens will be selected for in-depth analysis of the composition of the nasopharyngeal microbiota. The 16S gene is a conserved gene with variable regions amongst bacteria, and therefore sequencing of this gene enables the detection of all bacteria in the microbiota. Bacterial DNA will be extracted from nasopharyngeal specimens, quantified, and the 16S rRNA gene amplified and sequenced using next generation sequencing technology [95, 96]. Sequences from the 16S rRNA V5-V7 variable regions will be compared to a database of known microbial sequences to identify the microbes present. There will be no direct or intentional sequencing of human DNA, any residual human reads will be informatically filtered and discarded prior to analysis.

10.11.3. Serologic testing for immunologic response to influenza and RSV

Blood samples will be tested for rise in RSV or influenza antibody titres. Hemagglutination inhibition (HAI) assays will be performed to determine serological reactivity titres for serum samples against reference influenza virus antigens based on the selected vaccine strains and strains predominantly circulating in South Africa during each year. Turkey red blood cells will be used as indicator in the HAI assay. The protocol will be based on the method described by Rowe et al. (1999) [97].

RSV antibody titres will be determined using a enzyme-linked immunosorbent assay based microneutralization assays previously described [98, 99].

10.11.4. Serologic testing for immunologic response to bacterial respiratory pathogens

10.11.4.1. Serology for pertussis

Serology for pertussis antibody will be performed using the anti-Pertussis Toxin enzyme linked immunosorbent assay (ELISA) procedure recommended by CDC. This ELISA involves in-house coating of microtitre plates with antigen prior to incubation with serum (20 µl per well) and detection with enzyme-labelled anti-human IgG. 100 µl serum is the optimal serum volume per patient to allow for repeats. Relevant standards and controls will be ordered from the National Institute for Biological Standards (NIBSC) in the USA.

10.11.4.2. Serology for diphtheria

Serology for diphtheria will be performed using one of two commercially available ELISA kits which detect either IgG against diphtheria toxin (Virotech) or against diphtheria toxoid (Euroimmune). 10ul patient serum is incubated with toxin in each well, followed by detection with enzyme labelled anti-human IgG. 100ul serum is optimal per test, to allow for repeats. Choice of ELISA kit will depend on kit validation results at the Centre for Vaccines and Immunology.

10.11.5. HIV testing

All patients will be offered HIV testing at the baseline visit and then 6 monthly for the first year for individuals testing HIV-negative at the previous visit. For individuals in the first and second year of the study, patients will be offered HIV testing at an annual visit in October of each year.

HIV status will be defined as follows:

- If the patient has a documented positive HIV result (or evidence of ART) available in the medical records they will be recorded as HIV positive.
- If the patient is not aware of their status or a previous result documents a negative result the patient will be offered voluntary counselling and testing (VCT) by Rapid HIV-antibody detection tests or by PCR assay from dried blood spots (tested at NICD) for patient under the age of 18 months.
- From consenting patients who do not want to have a rapid HIV test as described above, blood samples already collected for serologic testing will be also tested for HIV infection. Patients will be offered the option to receive their result if they would like to.
- A documented HIV negative status for the mother would be confirmation of negative status for a child under the age of 10 years.
- In order to define HIV exposure in infants, the mother's HIV status at the time of pregnancy will need to be defined; this information will be gained verbally from the mother and confirmed by the clinic/hospital records or the child's road to health card. If mother's HIV status is unknown she will be offered VCT and rapid HIV testing as described above.
- Patients not wanting to know their HIV status or unwilling to provide an anonymous sample may still be enrolled into the study.
- For HIV positive patients, a specimen for CD4 count will be collected if there is no recent documented CD4 count results (within 6 months of household enrolment).
- Patients newly diagnosed with HIV will be referred to the local clinic for assessment for initiation of antiretroviral therapy
- For all individuals who decline HIV testing, they will be encouraged to test at follow up visits and the benefits of knowing your status emphasized.

10.11.6. Testing for tuberculosis infection and disease

10.11.6.1. Performance of the TST

The test is done by putting a small amount of tuberculosis protein (antigens) under the top layer of skin on the inner forearm. If an individual has ever been exposed to the tuberculosis bacteria (*M.tuberculosis*), their skin will react to the antigens by developing a firm red bump at the site within 2 days.

Specifically, a standard dose of 5 tuberculin units (TU) is injected intradermally to raise a wheal or bleb about 6mm in diameter in the longitudinal axis on the volar surface of the forearm and read 48-72 hours later. Reading is done by measuring the indurated area – not only the reddened area – i.e. it must be raised from surrounding skin. It is measured transversely using calipers with the ruler/reading face down to avoid digit preference, then turned over once the desired distance is fixed. The ball-pen method can be sued to identify edges of the indurated area.

10.11.6.2. Tuberculosis culture

Baseline specimens collected from all patients will be cultured for detection of *M. tuberculosis*. Sputum samples will be digested and decontaminated. Following decontamination, 0.5mls of the sediment inoculated into a Mycobacteria Growth Indicator Tube (MGIT) tube for culture and isolation of Mycobacteria in the BACTEC MGIT 960 instrument as per manufacturer's instructions. The primary isolation of mycobacterium will be done using the N-Acetyl L-Cysteine sodium hydroxide (NALC-NaOH) decontamination and MGIT culture. After inoculation, the MGIT will be placed in the BACTEC 960 instrument and incubated at 37 degrees Celsius. Cultures will be reported as negative if no growth has been observed in 42 days. When a MGIT tube "flags" positive, a smear will be assessed for acid-fast bacilli (AFB). If AFB are observed microscopically, then identification will be done using the MDRTB plus Line Probe Assay for AFB positives. This assay will confirm the presence of M. tuberculosis complex and report isoniazid and rifampicin resistance. If the MDRTB plus fails to demonstrate *M. tuberculosis* complex, despite the presence of acid fast bacilli on the Ziehl Neelsen stain, further species identification may be performed using the Mycobacterium CM/AS Line Probe Assay.

10.11.6.3. Testing for tuberculosis using Gene Xpert Mtuberculosis/RIF

Specimens in symptomatic patients identified on monthly follow-up symptom questionaires will be tested using Gene Xpert Mtuberculosis/RIF. The Xpert Mtuberculosis/RIF is based on the Cepheid GeneXpert platform, a sensitive, rapid and simple-to-use nucleic acid amplification test (NAAT). The Xpert® Mtuberculosis/RIF purifies, concentrates, amplifies (by real-time PCR) and identifies targeted nucleic acid sequences in the tuberculosis genome, and provides results from unprocessed sputum samples within 100 minutes. The Mtuberculosis Xpert/RIF detects *M. tuberculosis* as well as rifampicin resistance-conferring mutations directly from sputum.

10.11.7. Urine cotinine testing

Cotinine is an alkaloid found in tobacco and is also the predominant metabolite of nicotine. Cotinine is used as a biomarker for exposure to tobacco smoke. Cotinine has an in vivo half-life of approximately 20 hours, and is typically detectable for several days (up to one week) after the use of tobacco. The level of cotinine in the blood, saliva, and urine is proportionate to the amount of exposure to tobacco smoke, so it is a valuable indicator of tobacco smoke exposure, including secondary (passive) smoke. In urine, values between 11 ng/mL and 30 ng/mL may be associated with light smoking or passive exposure, and levels in active smokers typically reach 500 ng/mL or more. Cotinine assays provide an objective quantitative measure that is more reliable than smoking histories or counting the number of cigarettes smoked per day. Cotinine also permits the measurement of exposure to second-hand smoke (passive smoking). Drug tests can detect cotinine in the blood, urine, or saliva. Urine cotinine concentrations average fourfold to sixfold higher than those in blood or saliva, making urine a more sensitive matrix to detect low-concentration exposure.

10.11.8. Organism characterisation

10.11.8.1. Respiratory virus characterization and evolution

Identified clusters of influenza viruses and RSV in households will be investigated using molecular methods to determine the diversity of virus strains circulating within a household by using heteroduplex mobility assays to identify single strand conformational polymorphisms [100]. Sequencing of influenza A and B viruses will be done after cDNA synthesis and PCR HA gene segments or complete genomes using One-step SuperScript™ III Reverse Transcriptase PCR system (Invitrogen, USA)[101, 102].The RSV F gene will be amplified and sequenced as described previously [99]. Amplicons will be sequenced using the BigDye Terminator sequencing kit (Applied Biosystems, USA) whereas genome sequencing will be performed on the Illumina MiSeq next generation sequencing platform. Multiple sequence alignment of nucleotide sequences will be done using ClustalW algorithm embedded in BioEdit v7.0.9.1. Phylogenetic analysis will be performed on codon aligned sequences with MEGA 5.2 program [103] employing a Hasegawa-Kishino-Yano (HKY) substitution and Gamma site heterogeneity models as inferred by the data. Tree topologies and robustness will be assessed by bootstrap analysis using 100-1,000 replicates depending on whether maximum likelihood or neighbour-joining tree drawing methods are used. Representative reference sequences of relevant viral strains will be retrieved from international sequence databases (http://www.ncbi.nlm.nih.gov/genomes/). To determine antigenic characteristics of RSV strains the F protein gene sequences will be analysed to identify polymorphisms in epitopic regions identified as vaccine targets and antibody-based interventions. Additionally, samples will be shipped to the J. Craig Venter Institute (JCVI) in the United States of America, for next generation sequencing of Influenza and RSV viruses. Influenza-positive samples may also be shared with the World Health Organization Collaborating Centres as part of the Global Influenza Surveillance and Response Network.

10.11.8.2. Pneumococcal serotyping

LytA-positive specimens with a Ct-value ≤35 will be serotyped using a triplex real-time PCR assay described by Pimenta et al.[104].

10.12. Patient compensation for household visits

Each participant will be compensated for the time commitment involved in this intensive study. Each participant will be compensated for each study visit with a voucher for the local supermarket to the value of R25. An additional voucher will be issued to each participant upon return a proximity tag with no visible physical damage after each deployment. During the environmental sampling, an additional R50 electricity voucher will be provided per device placed in the household to cover electricity usage of the device.

10.13. Procedures for case enrolment and consent

Cases will be identified through stratified random sampling as described in Section 10.5. The purpose of the study will be explained to all household members in English or the local languages (as per the preference of the participant). In addition, a printed study information sheet will be provided in English and appropriate local languages for participants to read. Participants aged 18 years and older will be asked to give written consent. For participants younger than 18 years of age the parent/guardian/primary caregiver will provide for written consent for the child. Assent will be obtained from children between 7 years and 17 years by a similar process. If an individual agrees to participate but is unable to sign, a thumbprint will serve in place of a signature, and an impartial household member will sign as a witness. Consent to continue in the study will be obtained verbally at all follow up visits.

Consent and assent forms can be found in Appendix 5.

10.14. Ethical approval

Ethical approval for the study will be obtained from the University of the Witwatersrand Human Research Ethics Committee (HREC). The protocol will be submitted for a reliance from the US CDC ethics committee.

10.15. Referral to health services

If any household contact with ILI/ARI symptoms are identified that should seek medical attention, they will be referred to the relevant clinic. Patients with suspected tuberculosis will be offered tuberculosis testing by GeneXpert Mtuberculosis Rif and referred to the local clinic/hospital if indicated clinically or they test tuberculosis positive. Patients requiring hospitalization for respiratory illness will be referred to pneumonia surveillance hospitals (Matikwana and Mapulaneng in Mpumalanga and Klerksdorp and Tshepong in Northwest Province) and enrolled into the NICD pneumonia surveillance programme (Wits HREC Protocol number M140824). Patients with newly-diagnosed HIV infection will be referred to the local clinic for assessment for antiretroviral treatment including CD4+ T cell count testing.

10.16. Prevention of influenza and other infections in front line staff

Front-line staff including study nurses will be trained in infection control procedures including proper hand hygiene and the correct use of surgical face masks, not only to minimize their own risk of infection when in close contact with patients during home visits and elsewhere, but also to minimize the risk of the nurses acting as a vector of infection between household members or between households. They will also be offered influenza vaccination prior to influenza season each year. Staff will be properly trained in collection of blood specimens and disposal of sharps. Staff will be encouraged to stay home from work and practice good respiratory hygiene and handwashing if they have any respiratory illness.

A nasopharyngeal swab will be collected from all field workers on a weekly basis and tested for influenza, RSV and pertussis to document possible infection transmission to and by fieldworkers.

10.17. Study instruments

- The study forms will include: Study logs for each visit
- Forms for completion at the first study visit:
  .1. Identification form
  .2. Enrolment of household form
  .3. Enrolment log
  .4. Individual visit log
- First clinical visit
  .1. Individual visit log
  .2. Lab slip
  .3. First clinical visit
  .4. HIV testing form
  .5. Underlying conditions form
- Forms for completion at twice weekly visits:
  .1. Individual visit log
  .2. Lab slip

.3. Symptom form
.4. TB symptoms form (monthly)
.5. HIV testing form
.6. Outpatient consultation form (if triggered)
.7. Post hospitalization forms (if triggered)
.8. Death form (if triggered)
.9. Check list log

- Once off forms for each cohort
  .1. Environmental assessment form
  .2. HIV staging form
  .3. Proximity sensor time sheet
  .4. Contact diary
  .5. Time use data questionnaire

10.18. Data management

All data gathered during household enrollment, first study visit, follow-up visits and quarterly visits will be collected electronically during each household visit and uploaded directly to the REDcap server at Wits on the mobile data collection system REDcap. This will include information on household demographic, sign and symptoms, contact patterns among household members, sample collection and selected laboratory results (e.g. tuberculin skin and HIV testing results). A unique identifier including household, household member and a follow-up visit identifiers will be used for each household visit. The database will be password protected to ensure that outside parties will not have access to the database. The database will be backed up daily. A data quality/data verification process will be developed and will be implemented by a study manager and a database manager at site. This will include verification of completeness and accuracy of collected data. A study log will be maintained by study staff and compared with a pre-established study calendar to assess concordance of study implementation with study procedures.

NICD will access the database on a daily basis to check on follow up progress and data quality. Data quality and completeness will be again evaluated at NICD by a dedicated data manager.

Laboratory results will be entered into the same data system (REDcap) which will be stored on the REDcap server at Wits. Each sample will be identified by the same identifier allocated to each individual household member (including the follow-up visit identifier) and, after data quality verification, linked monthly with the central database encompassing data collected at sites.

Participant names and study identifiers will be captured in a separate table at the start of the study. All patients will be allocated a unique study number which will be used for all study information and specimen labelling. Patient identifiers will be kept in a separate database which will be hidden to data entry staff. Patient identifier data may be accessed by laboratory staff for the purposes of laboratory reporting of diseases which require patient treatment or public health action for example positive results for tuberculosis.

No personal identification will be displayed in any reports or communication regarding this investigation. All study data will be archived electronically on the REDcap server at Wits, which has additional back up.

10.18.1. Data management for the household contact study

10.18.1.1. Data Storage

Data collected by the wearable devices will be stored internally and extracted using cables. Data from the devices will be downloaded into a MySQL database. Anonymised raw data, log files and analysis code will be archived in the NICD servers by the Project Data Manager after analysis.

10.18.1.2. Data analysis

Characterizing contacts: Patterns of contact will be described using several quantities that quantify the number of unique individuals contacted (degree), number of contacts between individuals (frequency), the duration of these contacts (weight) and the cumulative time spent in contact by each pair of individuals. The statistical distribution and variations will also be assessed. Heterogeneity of the contacts and their statistical distributions will be assessed across six key variables: demography, temporality, grade, role and setting. Analysis will be conducted using R and Python.

Characterizing networks and community interactions: To describe the characteristics of networks and community interactions, network data analysis and visualization will be aggregated at the household level. Temporal data will be aggregated hourly, daily and over the entire duration of study. Networks will be stratified by the covariates (demography, grade, role, setting). Nodes and edges will represent individuals within the stratum and the occurrence of a contact between the nodes, respectively. Results obtained from this descriptive analysis will be used to determine the appropriate statistical network model to be used to better understand the heterogeneities affecting the observed network structure. This will also provide household-based mixing parameters to be used in a transmission model.

Network and Transmission models: The transmission model will investigate the role of mixing between and within households and schools on the risk of an epidemic. The network model will investigate the impact on transmission dynamics of key features identified from the household and school socio-behavioural structure. This will be undertaken to establish which quantities appear to have dominant impact on transmission rates and patterns and intervention impact on common respiratory infections such as respiratory syncytial virus.

10.19. Variables

10.19.1. Main outcomes of interest for the primary study objective

The main outcomes of interest, will be as follows:

- Laboratory-confirmed influenza-infection on PCR or serology
- Laboratory-confirmed RSV-infection on PCR or serology
- Consultation with a primary care provider
- Proportion of laboratory-confirmed influenza and RSV infections that are symptomatic

10.19.2. Main exposures of interest and secondary outcomes

10.19.2.1. Individual level exposures on questionnaire

- Age – in months for <1 year and in years thereafter
- Date of birth
- Sex

- Study year – 2016, 2017, 2018
- Study site – Mpumalanga or North West Province
- Circulating influenza subtypes – B, A(H1N1), A(H3N2), novel
- HIV status – infected, uninfected, unknown
- Underlying conditions other than HIV – the presence of immunocompromising conditions other than HIV will be recorded: Such conditions include a) prematurity or ex-prem babies with a history of chronic lung disease; b) protein-energy malnutrition; c) primary immunodeficiency states involving the adaptive and innate arms of the immune system e.g. agammaglobulinaemia, common variable immunodeficiency, DiGeorge syndrome, severe combined immunodeficiency, chronic granulomatous disease, complement deficiencies, leukocyte adhesion deficiencies; d) conditions in which there is functional or anatomic asplenia e.g. sickle cell anaemia, situs inversus with polysplenia or asplenia, splenectomy following trauma or as part of the management of chronic immune thrombocytopenic purpura; e) chronic organ dysfunction with impaired production of immune mediator proteins, or enhanced excretion of such proteins, e.g. chronic liver disease and chronic renal disease (specifically nephrotic syndrome); f) chronic lung disease, e.g. poorly-controlled asthmatics on long-term systemic steroid therapy, bronchiectasis, bronchiolitis obliterans, cystic fibrosis, Kartagener syndrome; g) conditions in which respiratory secretions are poorly mobilised due to neuromuscular disease, e.g. cerebral palsy, congenital myopathies, muscular dystrophies; h) chronic cardiac disease predisposing to recurrent episodes of cardiac failure, and/or pneumonias, e.g. atrioseptal and/or ventriculoseptal defects, cardiomyopathy; i) children on immunosuppressive therapies, e.g. cancer chemotherapy, immunomodulatory therapy for connective tissue diseases, immuosuppressive therapy post organ transplantation; j) children with metabolic diseases, e.g. congenital adrenal hyperplasia, poorly-controlled diabetes mellitus, galactosaemia; k) intrinsic genetic defects predisposing to recurrent infections, e.g. Trisomy 21.
- Type of residential dwelling categories include: formal house/apartment, informal dwelling, traditional house or l/
- Overcrowding in residences: this will be assessed by determining number of people residing in the household, as well as the number of rooms in the household (excluding bathrooms and kitchen). An index of number of people per room will be used to assess impact of crowding in the household. We will also assess the number of people sleeping in the same bedroom, as well as the number of children residing in the household.
- Cigarette smoke exposure: in children exposures to passive smoking on history will be analyzed. Passive smoking will be defined as a participant who resides in a household where there is active smoking indoors and/or where they are exposed to an indoor cigarette smoking environment (other than their residence) for more than three hours every week. For adults and teenagers a detailed smoking history will be taken and smoke exposure will also be assessed on urine cotinine testing
- Exposure to smoke in the household from indoor fires – this will be assessed on history as well as using objective measures of indoor air pollution
- Education level: the highest level of education for each participant will be determined. This will be analysed according to whether the caregiver achieved a primary, secondary or tertiary level of education.
- Socioeconomic status: various tools will be used and adapted to determine the socioeconomic status of participants. Items used by the World Bank in the Demographic Health Survey Wealth Index as well as those used in the Agincourt Health and Demographic Surveillance System Asset

status module57 will be included in the questionnaire. These items will be analysed and participants will be placed into one of five social classes ranging from wealthiest to poorest.

- Locality type: urban formal, urban informal, rural formal or rural informal.
- Race group: participants will be asked to classify which category would best describe their race. Options included in the questionnaire are: Asian, Black, Coloured or White.
- Vaccine history for individuals <18 years EPI vaccines will be assessed
- Influenza and pneumococcal vaccine in adults will be assessed in the questionnaire
- Breastfeeding: for children we will assess whether the child is currently being breastfed and breastfeeding in the first 6 months of life.
- Attendance at day care: attendance at a day-care facility with more than 5 other children for at least 3 days a week for 3 hours each day for children <7 years.
- Attendance at school: will be assessed for all individuals aged <21 years
- HIV exposure status (mothers HIV status) will be assessed for children aged <10 years
- Receipt of cotrimoxazole prophylaxis (this may be prescribed for HIV-exposed but uninfected children as well as HIV-infected patients) this will be assessed in the baseline questionnaire,
- Variables for HIV infected individuals
    - HIV stage – the clinical stage of HIV infection in HIV infected individuals will be assessed in a questionnaire and CD4 count test
    - Use of antiretrovirals (ARVs)
        - Whether the patient is currently receiving ARVs (Y/N)
    - Duration of ARV therapy (current date minus date of initiation of therapy)
        - This will be categorised into groups: <3 months; 3 - 6 months; 6 - 12 months; 12 - 36 months; > 36 months
    - Current antiretroviral drugs taken
    - Attendance at dedicated HIV clinic: whether the patient reports regular attendance (more than 2 scheduled visits attended in the last year) at an HIV clinic

10.19.2.2. Household level exposures

- Temperature
- Humidity
- Indoor air pollution

10.20. Statistical analysis

10.20.1. Estimation of the community burden of influenza, RSV, *B. pertussis,* and *M. tuberculosis* infection

Incidence of RSV, influenza virus and pertussis infection within age groups and HIV infection status will be estimated using serological and PCR data. Incidence obtained from the study subjects will be standardized to the demographic characteristic of the study sites to account for potential differences between the selected households and the population. The symptomatic fraction will be expressed as the proportion of RSV, influenza or pertussis positive cases that developed symptoms. Severity of symptoms will be assessed over a pre-established scale and analyzed using ordinal regression (proportional odd model) to discriminate between severe and mild disease. In addition, the proportion of individuals that were

hospitalized or died will be reported. The healthcare seeking behavior among the symptomatic patients will be assessed.

The annual and quarterly incidence of tuberculosis infection and symptomatic disease will be estimated using tuberculin skin test and sputum results, respectively.

10.20.2. Estimation of the prevalence of meningococcal, diphtheria and GAS carriage and HIV infection

The prevalence of meningococcal, GAS and diphtheria carriage within age groups and HIV infection status groups will be estimated using data from PCR and culture testing performed annually.

The prevalence of HIV infection in the study population will be assessed at baseline and re-evaluated quarterly.

10.20.3. Estimation of the transmission dynamics of influenza virus, RSV, *S. pneumoniae* and *B. pertussis* in the community

The incidence of infection/colonization of PCR confirmed cases will be estimated for each of the selected pathogens. The SIR for each pathogen will be evaluated among all household in which at least one household member tested positive by PCR for one of the selected pathogens and the index case was identified during the study period. The latter would be particularly relevant for *S. pneumoniae* given its high colonization rates and its prolonged colonization time. The SIR will be expressed as the proportion of infected individuals divided by the individuals at risk excluding the index case. The serial interval in the transmission chain will be expressed as the time of symptoms onset of the index case to the time of symptom onset of secondary cases within the household.

A metapopulation transmission model will be developed for each pathogen to assess the characteristics of the individuals serving as the most likely source of infection/colonization for the household as well as the transmission dynamics of infection/colonization when the pathogen is introduced in the household.

For each pathogen the intensity of the transmission will be assessed through the estimation of the effective reproductive number (R_t) and it's variation over time.

A similar analysis will be implemented for the other respiratory viruses evaluated in the study.

10.20.4. Estimation of determinant of transmission within household

For each pathogen factors associated with risk of infection will be assessed using unconditional logistic regression and survival analysis. Predictors will include individual and household level demographic characteristics, co-infection (or preceding infection) with other respiratory pathogens, co-morbidities (including HIV infection) as well as environmental and behavioral factors. A sub-analysis will be implemented to asses factors associated with high pneumococcal colonization density in the community.

10.20.5. Estimation of the prevalence of immunity to and recent infection with *C. diphtheriae* and *B. pertussis*

Baseline immunity levels for each individual will be studied by quantitating IgG to diphtheria and pertussis at the start of the study period, which will give an indication of immunity due either to natural infection or prior vaccination, and compared to immunity at the end of the study period. New seroconverters who

become IgG positive and any future timepoint will be an indication of actively circulating infection during the study period. Such results will be compared with symptoms reported and molecular results obtained from swabs during the same period.

10.21. Personnel, training and supervision

Surveillance staff (registered nurses, enrolled nurses, and/or research assistants/counsellors) will be employed at each proposed study site to assist with patient enrolment, sampling and completing questionnaires. All nurses and research assistants/counsellors will be trained in obtaining consent, completing study forms and HIV pre-and post-test counseling. The nurses will be trained in taking adequate samples.

Each study site will have a study co-ordinator who will be responsible for on-site supervision of day-to-day activities of the study team. The site co-ordinator and database manager/epidemiologist will monitor the forms submitted and review a subset of forms for completeness and consistency of data on a regular basis. Staff from NICD will visit the surveillance sites regularly to evaluate surveillance officer performance and overall site performance.

10.22. Monitoring and evaluation

Performance indicators will be developed for:

- Questionnaire completion e.g. number of fields completed, inconsistencies in data, visits with missing forms
- Completeness of visits – completeness of household visits including specimen collection and forms
- Specimen submission – numbers of cases with no specimens submitted, specimen labeling
- Data entry – consistency checks, lag in data entry etc.
- Laboratory indicators – time taken to transport specimens to laboratory, turn-around-time for results

10.23. Pilot study

A pilot study will be performed in November-December 2015. This will involve identifying pilot households, taking them through the informed consent process, also piloting data collection tools and specimen collection. Based on the findings of the pilot study, study tools and procedures will be amended.

10.24. Study limitations

The limitations of this study pertain mainly to the implementation of the study at two selected sites, which may hinder the generalisability of the study results to other areas or settings. In addition, we may have limited power to accurately assess the selected endpoints for pathogens expected to be found at low prevalence in the community such as *N. meningitidis* and *C. diphtheriae*.

10.25. Dissemination and publication of results

Finding of the study will be summarized in annual reports to all investigators. Study findings will be published in the peer-reviewed literature. The first draft of the main manuscripts will be complete in June 2019 and the final version in December 2019.

10.26. Study timelines

Activity	Date	Details
Protocol development	July 2015	
Ethics review and approvals	Submit August 2015	Approval finalized October 2015
Appoint staff and staff Training	November 2015	
Selection and enrolment of households	November - December 2015	
Pilot study	November - December 2015	
First household visits	January 2016	
Proximity tag pilot study	September – October 2017	
Enrolment end date	January 2018	
End of data collection	December 2018	
Data finalization and cleaning	January-March 2019	
First draft of main manuscripts	June 2019	
Reporting date	December 2019	

10.27. Budget and funding source

The main study is funded as a project under the Cooperative Agreement between the United States Centers for Disease Control and Prevention with the South Africa National Health Laboratory Service (NHLS) – Agreement # 5U19GH000622-03 Research Cooperative Agreement. Funding for diphtheria and pertussis serology and diphtheria carriage surveys will be provided by the NICD. Funding for additional pathogen testing will be obtained through separately sourced grant funds.

10.28. References

1. **WHO global technical consultation: global standards and tools for influenza surveillance**. *World Health Organisation* 2011.
2. Fiore AE, Uyeki TM, Broder K, Finelli L, Euler GL, Singleton JA, et al. **Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010**. *MMWR Recomm Rep* 2010; 2010/08/07:1-62.
3. Hayward AC, Fragaszy EB, Bermingham A, Wang L, Copas A, Edmunds WJ, et al. **Comparative community burden and severity of seasonal and pandemic influenza: results of the Flu Watch cohort study**. (2213-2619 (Electronic)).
4. Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. **Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis**. *Lancet* 2010; 375(9725):1545-1555.
5. Beeler JA, Eichelberger MC. **Influenza and respiratory syncytial virus (RSV) vaccines for infants: safety, immunogenicity, and efficacy**. *Microb Pathog* 2013; 55:9-15.
6. Karron RA, Wright PF, Belshe RB, Thumar B, Casey R, Newman F, et al. **Identification of a recombinant live attenuated respiratory syncytial virus vaccine candidate that is highly attenuated in infants**. *J Infect Dis* 2005; 191(7):1093-1104.

7. Anderson LJ, Dormitzer PR, Nokes DJ, Rappuoli R, Roca A, Graham BS. **Strategic priorities for respiratory syncytial virus (RSV) vaccine development**. *Vaccine* 2013; 31 Suppl 2:B209-215.
8. Nokes JD, Cane PA. **New strategies for control of respiratory syncytial virus infection**. *Curr Opin Infect Dis* 2008; 21(6):639-643.
9. Jones N, Huebner R, Khoosal M, Crewe-Brown H, Klugman K. **The impact of HIV on Streptococcus pneumoniae bacteraemia in a South African population**. *AIDS* 1998; 12(16):2177-2184.
10. Madhi SA, Kuwanda L, Cutland C, Klugman KP. **The impact of a 9-valent pneumococcal conjugate vaccine on the public health burden of pneumonia in HIV-infected and -uninfected children**. *ClinInfectDis* 2005; 40(10):1511-1518.
11. Johansson N, Kalin M, Tiveljung-Lindell A, Giske CG, Hedlund J. **Etiology of community-acquired pneumonia: increased microbiological yield with new diagnostic methods**. *Clin Infect Dis* 2010; 50(2):202-209.
12. Resti M, Moriondo M, Cortimiglia M, Indolfi G, Canessa C, Becciolini L, et al. **Community-acquired bacteremic pneumococcal pneumonia in children: diagnosis and serotyping by real-time polymerase chain reaction using blood samples**. *ClinInfectDis* 2010; 51(9):1042-1049.
13. Nolte FS. **Molecular diagnostics for detection of bacterial and viral pathogens in community-acquired pneumonia**. *Clin Infect Dis* 2008; 47 Suppl 3:S123-126.
14. Hammitt LL, Kazungu S, Morpeth SC, Gibson DG, Mvera B, Brent AJ, et al. **A preliminary study of pneumonia etiology among hospitalized children in Kenya**. *ClinInfectDis* 2012; 54 Suppl 2:S190-9. doi: 10.1093/cid/cir1071.:S190-S199.
15. van den Bergh MR, Biesbroek G, Rossen JW, de Steenhuijsen Piters WA, Bosch AA, van Gils EJ, et al. **Associations between pathogens in the upper respiratory tract of young children: interplay between viruses and bacteria**. *PLoS One* 2012; 7(10):e47711.
16. Honkinen M, Lahti E, Osterback R, Ruuskanen O, Waris M. **Viruses and bacteria in sputum samples of children with community-acquired pneumonia**. *Clin Microbiol Infect* 2012; 18(3):300-307.
17. Morens DM, Taubenberger JK, Fauci AS. **Predominant Role of Bacterial Pneumonia as a Cause of Death in Pandemic Influenza: Implications for Pandemic Influenza Preparedness**. *JInfectDis* 2008; 198(7):962-970.
18. O'Brien KL, Walters MI, Sellman J, Quinlisk P, Regnery H, Schwartz B, et al. **Severe pneumococcal pneumonia in previously healthy children: the role of preceding influenza infection**. *Clin Infect Dis* 2000; 30(5):784-789.
19. Wolter N, Tempia S, Cohen C, Madhi SA, Venter M, Moyes J, et al. **High nasopharyngeal pneumococcal density, increased by viral coinfection, is associated with invasive pneumococcal pneumonia**. *The Journal of infectious diseases* 2014; 210(10):1649-1657.
20. Crowcroft NS, Pebody RG. **Recent developments in pertussis**. *Lancet* 2006; 367(9526):1926-1936.
21. National Institute for Communicable D. **Increased detection of Bordetella pertussis in the pneumonia and influenza-like illness surveillance programmes.** **http://www.nicd.ac.za/assets/files/Pertussis.pdf Accessed 6 August 2015**. *Communicable Diseases Communique* 2015; 14(6):1-2.
22. Soriano-Gabarro M, Wolter J, Hogea C, Vyse A. **Carriage of Neisseria meningitidis in Europe: a review of studies undertaken in the region**. *Expert Rev Anti Infect Ther* 2011; 9(9):761-774.
23. Christensen H, May M, Bowen L, Hickman M, Trotter CL. **Meningococcal carriage by age: a systematic review and meta-analysis**. *Lancet Infect Dis* 2010; 10(12):853-861.
24. von Gottberg A, du Plessis M, Cohen C, Prentice E, Schrag S, de Gouveia L, et al. **Emergence of endemic serogroup W135 meningococcal disease associated with a high mortality rate in South Africa**. *ClinInfectDis* 2008; 46(3):377-386.

25. Organis WH, ation. **Module 2: Diphtheria, Update 2009 - http://www.who.int/immunization/documents/ISBN9789241597869/en/**. *Programmes; Immunizations, Vaccines and Biologicals: The Immunological Basis for Immunization Series* 2009; Accessed 3 August; 2015.
26. Galazka A. **The Changing Epidemiology of Diphtheria in the Vaccine Era**. *J Infect Dis* 2000; 181(Suppl 1):S2-9.
27. Diseases NIfC. **Diphtheria: update on outbreak in KwaZulu-Natal Province**. In: *Communicable Diseases Communique*: National Institute for Communicable Diseases; 2015.
28. Carapetis JR, Steer AC, Mulholland EK, Weber M. **The global burden of group A streptococcal diseases**. *The Lancet Infectious diseases* 2005; 5(11):685-694.
29. Cunningham MW. **Pathogenesis of group A streptococcal infections**. *Clinical microbiology reviews* 2000; 13(3):470-511.
30. Shaikh N, Leonard E, Martin JM. **Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis**. *Pediatrics* 2010; 126(3):e557-564.
31. O'Loughlin RE, Roberson A, Cieslak PR, Lynfield R, Gershman K, Craig A, et al. **The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000-2004**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007; 45(7):853-862.
32. Tyrrell GJ, Lovgren M, Kress B, Grimsrud K. **Invasive group A streptococcal disease in Alberta, Canada (2000 to 2002)**. *Journal of clinical microbiology* 2005; 43(4):1678-1683.
33. Cockerill FR, 3rd, MacDonald KL, Thompson RL, Roberson F, Kohner PC, Besser-Wiek J, et al. **An outbreak of invasive group A streptococcal disease associated with high carriage rates of the invasive clone among school-aged children**. *Jama* 1997; 277(1):38-43.
34. Weiss K, Laverdiere M, Lovgren M, Delorme J, Poirier L, Beliveau C. **Group A Streptococcus carriage among close contacts of patients with invasive infections**. *American journal of epidemiology* 1999; 149(9):863-868.
35. Martin JM, Green M, Barbadora KA, Wald ER. **Group A streptococci among school-aged children: clinical characteristics and the carrier state**. *Pediatrics* 2004; 114(5):1212-1219.
36. van Zyl ML, van Staden DA, Potgieter MD. **[Beta-hemolytic streptococci as a cause of sore throat in the Pretoria area]**. *S Afr Med J* 1981; 59(22):783-784.
37. Engel ME, Haileamlak A, Zuhlke L, Lemmer CE, Nkepu S, van de Wall M, et al. **Prevalence of rheumatic heart disease in 4720 asymptomatic scholars from South Africa and Ethiopia**. *Heart* 2015; 101(17):1389-1394.
38. Safar A, Lennon D, Stewart J, Trenholme A, Drinkovic D, Peat B, et al. **Invasive group A streptococcal infection and vaccine implications, Auckland, New Zealand**. *Emerging infectious diseases* 2011; 17(6):983-989.
39. Lamagni TL, Efstratiou A, Dennis J, Nair P, Kearney J, George R. **Increase in invasive group A streptococcal infections in England, Wales and Northern Ireland, 2008-9**. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2009; 14(5).
40. Lamagni TL, Efstratiou A, Vuopio-Varkila J, Jasir A, Schalen C. **The epidemiology of severe Streptococcus pyogenes associated disease in Europe**. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2005; 10(9):179-184.
41. Stockmann C, Ampofo K, Hersh AL, Blaschke AJ, Kendall BA, Korgenski K, et al. **Evolving epidemiologic characteristics of invasive group a streptococcal disease in Utah, 2002-2010**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2012; 55(4):479-487.

42. Middelkoop K, Bekker LG, Liang H, Aquino LD, Sebastian E, Myer L, et al. **Force of tuberculosis infection among adolescents in a high HIV and TB prevalence community: a cross-sectional observation study**. *BMC Infect Dis* 2011; 11:156.
43. Lebina L, Abraham PM, Milanovic M, Motlhaoleng K, Chaisson RE, Rakgokong M, et al. **Latent Tuberculosis in School Children and Contact Tracing in Matlosana North West, South Africa**. *Int J Tuberc Lung Dis* 2015; (In press).
44. Shisana O, Rehle T SLCZKJSP-v-WVMNVZJPWZNPPS, the SIIIIT. **South African National HIV Prevalence, Incidence, Behaviour and Communication Survey, 2008: A turning tide among teenagers?** . In. Cape Town: HSRC Press; 2009.
45. Dherani M, Pope D, Mascarenhas M, Smith KR, Weber M, Bruce N. **Indoor air pollution from unprocessed solid fuel use and pneumonia risk in children aged under five years: a systematic review and meta-analysis**. *Bull World Health Organ* 2008; 86(5):390-398C.
46. Bruce N, Pope D, Rehfuess E, Balakrishnan K, Adair-Rohani H, Dora C. **WHO indoor air quality guidelines on household fuel combustion: Strategy implications of new evidence on interventions and exposure–risk functions**. *Atmos Environ* 2015; 106:451-457.
47. Weber MW, Milligan P, Hilton S, Lahai G, Whittle H, Mulholland EK, et al. **Risk factors for severe respiratory syncytial virus infection leading to hospital admission in children in the Western Region of The Gambia**. *Int J Epidemiol* 1999; 28(1):157-162.
48. Pappas DE, Hendley JO, Schwartz RH. **Respiratory viral RNA on toys in pediatric office waiting rooms**. *Pediatr Infect Dis J* 2010; 29(2):102-104.
49. Salathe M, Kazandjieva M, Lee JW, Levis P, Feldman MW, Jones JH. **A high-resolution human contact network for infectious disease transmission**. *Proc Natl Acad Sci U S A* 2010; 107(51):22020-22025.
50. Wallinga J, Teunis P, Kretzschmar M. **Using data on social contacts to estimate age-specific transmission parameters for respiratory-spread infectious agents**. *Am J Epidemiol* 2006; 164(10):936-944.
51. Edmunds WJ, O'Callaghan CJ, Nokes DJ. **Who mixes with whom? A method to determine the contact patterns of adults that may lead to the spread of airborne infections**. *Proc Biol Sci* 1997; 264(1384):949-957.
52. Wallinga J, Edmunds WJ, Kretzschmar M. **Perspective: human contact patterns and the spread of airborne infectious diseases**. *Trends Microbiol* 1999; 7(9):372-377.
53. Elgethun K, Fenske RA, Yost MG, Palcisko GJ. **Time-location analysis for exposure assessment studies of children using a novel global positioning system instrument**. *Environ Health Perspect* 2003; 111(1):115-122.
54. Paz-Soldan VA, Stoddard ST, Vazquez-Prokopec G, Morrison AC, Elder JP, Kitron U, et al. **Assessing and maximizing the acceptability of global positioning system device use for studying the role of human movement in dengue virus transmission in Iquitos, Peru**. *Am J Trop Med Hyg* 2010; 82(4):723-730.
55. Esposito S, Gasparini R, Bosis S, Marchisio P, Tagliabue C, Tosi S, et al. **Clinical and socio-economic impact of influenza and respiratory syncytial virus infection on healthy children and their households**. *Clin Microbiol Infect* 2005; 11(11):933-936.
56. Nair H, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, et al. **Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis**. *Lancet* 2011; 378(9807):1917-1930.
57. Nair H, Simões EAF, Rudan I, Gessner BD, Azziz-Baumgartner E, Zhang JSF, et al. **Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: A systematic analysis**. *The Lancet* 2013; 381(9875):1380-1390.

58. Weber MW, Mulholland EK, Greenwood BM. **Respiratory syncytial virus infection in tropical and developing countries**. *Trop Med Int Health* 1998; 3(4):268-280.
59. Keeling MJ, Eames KT. **Networks and epidemic models**. *J R Soc Interface* 2005; 2(4):295-307.
60. Danon L, House TA, Read JM, Keeling MJ. **Social encounter networks: collective properties and disease transmission**. *J R Soc Interface* 2012; 9(76):2826-2833.
61. Fu YC, Wang DW, Chuang JH. **Representative contact diaries for modeling the spread of infectious diseases in Taiwan**. *PLoS One* 2012; 7(10):e45113.
62. Read JM, Lessler J, Riley S, Wang S, Tan LJ, Kwok KO, et al. **Social mixing patterns in rural and urban areas of southern China**. *Proc Biol Sci* 2014; 281(1785):20140268.
63. Kiti MC, Kinyanjui TM, Koech DC, Munywoki PK, Medley GF, Nokes DJ. **Quantifying age-related rates of social contact using diaries in a rural coastal population of Kenya**. *PLoS One* 2014; 9(8):e104786.
64. Hens N, Goeyvaerts N, Aerts M, Shkedy Z, Van Damme P, Beutels P. **Mining social mixing patterns for infectious disease models based on a two-day population survey in Belgium**. *BMC Infect Dis* 2009; 9:5.
65. Mossong J, Hens N, Jit M, Beutels P, Auranen K, Mikolajczyk R, et al. **Social contacts and mixing patterns relevant to the spread of infectious diseases**. *PLoS Med* 2008; 5(3):e74.
66. Horby P, Pham QT, Hens N, Nguyen TT, Le QM, Dang DT, et al. **Social contact patterns in Vietnam and implications for the control of infectious diseases**. *PLoS One* 2011; 6(2):e16965.
67. Stein ML, van Steenbergen JE, Buskens V, van der Heijden PG, Chanyasanha C, Tipayamongkholgul M, et al. **Comparison of contact patterns relevant for transmission of respiratory pathogens in Thailand and The Netherlands using respondent-driven sampling**. *PLoS One* 2014; 9(11):e113711.
68. Danon L, Read JM, House TA, Vernon MC, Keeling MJ. **Social encounter networks: characterizing Great Britain**. *Proc Biol Sci* 2013; 280(1765):20131037.
69. Conlan AJ, Eames KT, Gage JA, von Kirchbach JC, Ross JV, Saenz RA, et al. **Measuring social networks in British primary schools through scientific engagement**. *Proc Biol Sci* 2011; 278(1711):1467-1475.
70. Glass LM, Glass RJ. **Social contact networks for the spread of pandemic influenza in children and teenagers**. *BMC public health* 2008; 8:61.
71. Cauchemez S, Bhattarai A, Marchbanks TL, Fagan RP, Ostroff S, Ferguson NM, et al. **Role of social networks in shaping disease transmission during a community outbreak of 2009 H1N1 pandemic influenza**. *Proc Natl Acad Sci U S A* 2011; 108(7):2825-2830.
72. Eames KT, Tilston NL, Brooks-Pollock E, Edmunds WJ. **Measured dynamic social contact patterns explain the spread of H1N1v influenza**. *PLoS Comput Biol* 2012; 8(3):e1002425.
73. You SH, Chen SC, Wang CH, Liao CM. **Linking contact behavior and droplet patterns to dynamically model indoor respiratory infections among schoolchildren**. *J Epidemiol* 2013; 23(4):251-261.
74. Smieszek T. **A mechanistic model of infection: why duration and intensity of contacts should be included in models of disease spread**. *Theor Biol Med Model* 2009; 6:25.
75. Toth DJ, Leecaster M, Pettey WB, Gundlapalli AV, Gao H, Rainey JJ, et al. **The role of heterogeneity in contact timing and duration in network models of influenza spread in schools**. *J R Soc Interface* 2015; 12(108):20150279.
76. Eagle N, Pentland AS, Lazer D. **Inferring friendship network structure by using mobile phone data**. *Proc Natl Acad Sci U S A* 2009; 106(36):15274-15278.
77. Stopczynski A, Sekara V, Sapiezynski P, Cuttone A, Madsen MM, Larsen JE, et al. **Measuring large-scale social networks with high resolution**. *PLoS One* 2014; 9(4):e95978.
78. Cattuto C, Van den Broeck W, Barrat A, Colizza V, Pinton JF, Vespignani A. **Dynamics of person-to-person interactions from distributed RFID sensor networks**. *PLoS One* 2010; 5(7):e11596.

79. Hornbeck T, Naylor D, Segre AM, Thomas G, Herman T, Polgreen PM. **Using sensor networks to study the effect of peripatetic healthcare workers on the spread of hospital-associated infections**. *The Journal of infectious diseases* 2012; 206(10):1549-1557.
80. Fournet J, Barrat A. **Contact patterns among high school students**. *PLoS One* 2014; 9(9):e107878.
81. Stehle J, Voirin N, Barrat A, Cattuto C, Isella L, Pinton JF, et al. **High-resolution measurements of face-to-face contact patterns in a primary school**. *PLoS One* 2011; 6(8):e23176.
82. Vanhems P, Barrat A, Cattuto C, Pinton JF, Khanafer N, Regis C, et al. **Estimating potential infection transmission routes in hospital wards using wearable proximity sensors**. *PLoS One* 2013; 8(9):e73970.
83. Voirin N, Payet C, Barrat A, Cattuto C, Khanafer N, Regis C, et al. **Combining high-resolution contact data with virological data to investigate influenza transmission in a tertiary care hospital**. *Infect Control Hosp Epidemiol* 2015; 36(3):254-260.
84. Isella L, Romano M, Barrat A, Cattuto C, Colizza V, Van den Broeck W, et al. **Close encounters in a pediatric ward: measuring face-to-face proximity and mixing patterns with wearable sensors**. *PLoS One* 2011; 6(2):e17144.
85. Genois M, Vestergaard CL, Cattuto C, Barrat A. **Compensating for population sampling in simulations of epidemic spread on temporal contact networks**. *Nat Commun* 2015; 6:8860.
86. Stehle J, Voirin N, Barrat A, Cattuto C, Colizza V, Isella L, et al. **Simulation of an SEIR infectious disease model on the dynamic contact network of conference attendees**. *BMC Med* 2011; 9:87.
87. Kiti MC, Tizzoni M, Kinyanjui TM, Koech DC, Munywoki PK, Meriac M, et al. **Quantifying social contacts in a household setting of rural Kenya using wearable proximity sensors**. *EPJ Data Sci* 2016; 5:21.
88. Munywoki PK, Koech DC, Agoti CN, Lewa C, Cane PA, Medley GF, et al. **The source of respiratory syncytial virus infection in infants: a household cohort study in rural Kenya**. *J Infect Dis* 2014; 209(11):1685-1692.
89. Health Do. **National tuberculosis management guidelines. 2014. available at :http://www.sahivsoc.org/upload/documents/NTCP_Adult_tuberculosis%20Guidelines%2027.5.2014.pdf**. *National Department of Health* 2014.
90. Carvalho MG, Tondella ML, McCaustland K, Weidlich L, McGee L, Mayer LW, et al. **Evaluation and improvement of real-time PCR assays targeting lytA, ply, and psaA genes for detection of pneumococcal DNA**. *JClinMicrobiol* 2007; 45(8):2460-2466.
91. Dolan Thomas J, Hatcher CP, Satterfield DA, Theodore MJ, Bach MC, Linscott KB, et al. **sodC-based real-time PCR for detection of Neisseria meningitidis**. *PLoS One* 2011; 6(5):e19361.
92. Wang X, Theodore MJ, Mair R, Trujillo-Lopez E, du Plessis M, Wolter N, et al. **Clinical validation of multiplex real-time PCR assays for detection of bacterial meningitis pathogens**. *J Clin Microbiol* 2012; 50(3):702-708.
93. Tatti KM, Sparks KN, Boney KO, Tondella ML. **Novel multitarget real-time PCR assay for rapid detection of Bordetella species in clinical specimens**. *Journal of clinical microbiology* 2011; 49(12):4059-4066.
94. Mancini F, Monaco M, Pataracchia M, von Hunolstein C, Pantosti A, Ciervo A. **Identification and molecular discrimination of toxigenic and nontoxigenic diphtheria Corynebacterium strains by combined real-time polymerase chain reaction assays**. *Diagn Microbiol Infect Dis* 2012; 73(2):111-120.
95. Biesbroek G, Sanders EA, Roeselers G, Wang X, Caspers MP, Trzcinski K, et al. **Deep sequencing analyses of low density microbial communities: working at the boundary of accurate microbiota detection**. *PLoS One* 2012; 7(3):e32942.
96. Biesbroek G, Wang X, Keijser BJ, Eijkemans RM, Trzcinski K, Rots NY, et al. **Seven-valent pneumococcal conjugate vaccine and nasopharyngeal microbiota in healthy children**. *Emerg Infect Dis* 2014; 20(2):201-210.

97. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, et al. **Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays**. *JClinMicrobiol* 1999; 37(4):937-943.
98. Huang K, Incognito L, Cheng X, Ulbrandt ND, Wu H. **Respiratory syncytial virus-neutralizing monoclonal antibodies motavizumab and palivizumab inhibit fusion**. *Journal of virology* 2010; 84(16):8132-8140.
99. Papenburg J, Carbonneau J, Hamelin ME, Isabel S, Bouhy X, Ohoumanne N, et al. **Molecular evolution of respiratory syncytial virus fusion gene, Canada, 2006-2010**. *Emerging infectious diseases* 2012; 18(1):120-124.
100. Zou S, Stansfield C, Bridge J. **Identification of new influenza B virus variants by multiplex reverse transcription-PCR and the heteroduplex mobility assay**. *J Clin Microbiol* 1998; 36(6):1544-1548.
101. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. **Universal primer set for the full-length amplification of all influenza A viruses**. *Archives of virology* 2001; 146(12):2275-2289.
102. Zhou B, Lin X, Wang W, Halpin RA, Bera J, Stockwell TB, et al. **Universal influenza B virus genomic amplification facilitates sequencing, diagnostics, and reverse genetics**. *Journal of clinical microbiology* 2014; 52(5):1330-1337.
103. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. **MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods**. *Molecular biology and evolution* 2011; 28(10):2731-2739.
104. Pimenta FC, Roundtree A, Soysal A, Bakir M, du PM, Wolter N, et al. **Sequential triplex real-time PCR assay for detecting 21 pneumococcal capsular serotypes that account for a high global disease burden**. *JClinMicrobiol* 2013; 51(2):647-652.

Appendix 1 Specimen collection

1. Oropharyngeal (OP) swabs (throat swabs)

- Hold the tongue down with a tongue depressor
- Have the patient say “aahh” to elevate the uvula
- Use a sweeping motion to swab the posterior pharyngeal wall and tonsilar pillars.
- Avoid swabbing the soft plate and do not touch the tongue with the swab tip.(This procedure can induce the gag reflex)
- Put the swab into a collection container with viral transport medium (VTM)

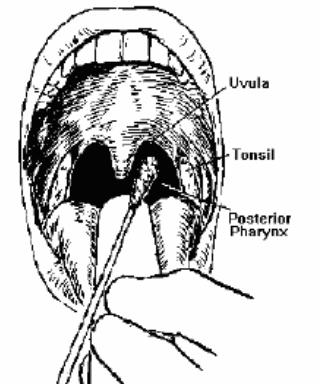


Figure 1: Oropharyngeal (OP) swab

2. Nasopharyngeal (NP) swab

- A flexible, fine-shafted flocked swab is inserted into the nostril and back to the nasopharynx until a slight resistance is met the swab is rotated two to three times and held in place for 5 seconds to ensure maximum absorbency.
- It is then slowly withdrawn with a rotating motion and placed in VTM. The tip of the swab is put into a vial containing 2–3 ml of virus transport medium and the plastic shaft is broken at the break point line so that it can fit in the VTM, taking care not to touch the tip.
- The NP swab can be put in the same container as the OP swab.
- Place the specimens on ice in laboratory cooler box.
- Complete the lab requisition forms/lab slip
- Note: Nasopharyngeal swabbing is an invasive process that can cause considerable distress to the patient.

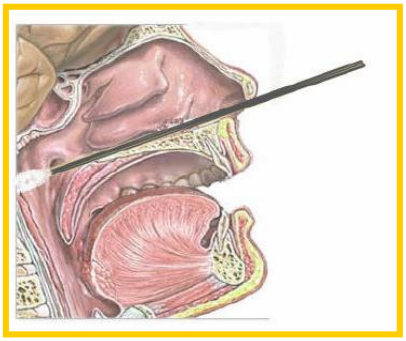


Figure 2: Nasopharyngeal (NP) swab

3. Blood specimens

- Specimens will be collected using the vacutainer collection system, as follows (see Figure 3):
- By twisting, remove the bottom white cap (D) of the needle.
- Now, firmly screw the needle in the needle holder/barrel (B).
- Remove the top cap (C) to ready the needle (A) for use just before the sample is taken.

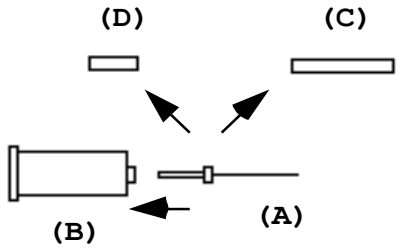


Figure 3: The Vacutainer Needle & Barrel System

- Apply the tourniquet to the patient's arm (a) and disinfect the site of venepuncture (see Figure 4).
- Hold the needle holder between thumb and index finger of the right hand and insert into the vein (b).
- Now change the position of the hand i.e. fixes the needle holder on the patient's arm with the thumb and index finger of the left hand. The left hand now serves to keep the needle Immobilized in the vein during the different actions.
- Position the first tube in the holder and press it onto the needle to allow the tube to fill (c).

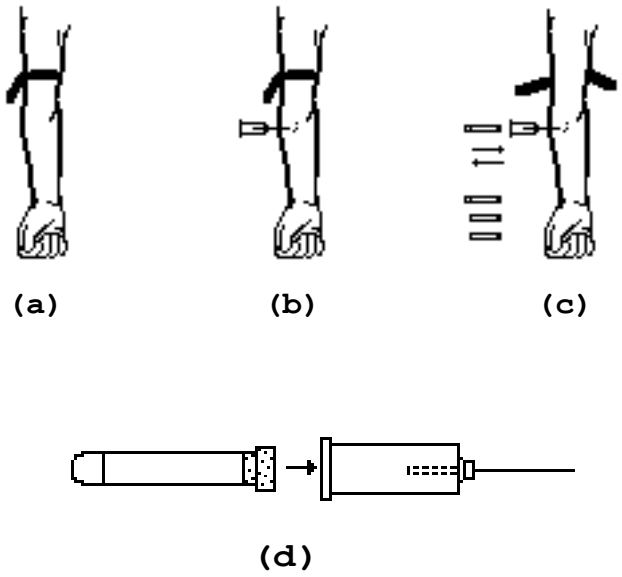


Figure 4: Collecting the Sample

- Fill the tube and remove once full.
- Release the tourniquet then remove the needle from the patients arm and immediately apply pressure to the venipuncture site. Firm pressure at this time will minimize bruising and therefore discomfort and distress to the patient.
- Ask the patient to take over applying pressure to the site.

If collecting blood in EDTA:

- Invert the ETDA, purple topped, tube gently 6 to 8 times (see Figure 5)

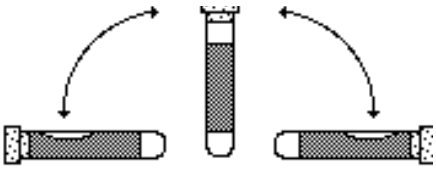


Figure 5: Inverting the ETDA

- Check that all the barcodes match and that you have marked the tube with the patient's unique bar coded study number.

Blood collection for infants and young children

- Use a winged 23 gauge needle, with a butterfly. Avoid gauge ≥25 gauge because these may cause haemolysis.
- Use a butterfly with either a syringe or an evacuated tube with an adaptor that has a barrel volume of 1-5 mls depending on collection needs.

4. Urine Samples

- This sample must be collected for all patients who are able to provide a specimen.
- For these you will need the universal container (clear plastic bottle with a green lid).
- Ask the patient to pass a fresh urine specimen into the universal container.
- Label the universal container with a unique identifier/bar code ID (NB label the container and not the lid).
- Quantitative testing for coltinine will be done at the Lancet laboratory in Cape Town

5. Expectorated sputum

- The optimum time for sputum collection is first thing in the morning but it can be collected anytime of the day.
- This sample must be collected for patients $\geq$ 5 years if able to expectorate.
- Ask the patient to gargle with water to rinse out the mouth. Brushing of teeth or rinsing the mouth thoroughly will reduce contamination.
- Instruct the patient to take a deep breath through the mouth and try to cough up mucous from deep in the chest. Patient may have to take several deep breaths and cough a lot in order to get adequate sputum. Specimen should be a deep cough specimen and not saliva.
- Patient must open the universal container and hold it close to the mouth, spit mucous into the container, without getting any of the mucous on the outside of the container.
- Patient must screw the lid of the container tightly, so that it does not leak.

Appendix 2 Forms and logs

1. Study logs : updated forms and logs are available as separate forms (version c documented in the covering letter

PHIRST Study: Study Logs

Centre for Respiratory Diseases and Meningitis (CRDM), NICD
TEL: 011 386 6410 or 011 386 6434 **FAX:** 086 723 3569

Site: □ Klerksdorp □ Agincort

Household code: _____

Household visit: □ Household enrollment □ First study visit □ Follow up visit □ Last study visit

Thick all that apply

□ Household enrollment form (for household enrollment visit only)

Form/Event	Household member														
	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____	ID:____
Household member enrollment form*	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Follow up questionnaire	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Household member symptomatic	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Symptoms Form	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Household member hospitalized	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Hospitalization Form	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Household member died	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Death Form	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Laboratory Slip	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Oropharyngeal swab	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Nasopharyngeal swabs	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Blood (EDTA)	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Blood (clotted)	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Expectorated sputum	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Urine	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
HIV rapid test	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Tuberculin skin test	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Copy of vaccination card	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□

* For household enrollment visit only

2. Household enrollment form

PHIRST Study: Enrolment of household Form

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

Household Characteristics

To be administered at the household enrolment visit after verification that enrollment criteria are met and consenting of household members.

ID Code

Date __/__/____ Time __:__

Interviewer_____

Household Characteristics *(For the head of the household)*

1. Total number of household members: ____
   1a. Total number of household members enrolled: ____
   1b. Number of temporary/transient household members:____

2. Total number of rooms in the house:____

3. Number of rooms for sleeping in the house:____

4. Does the household have windows that open? □ Yes □ No

5. If allowed or allowed with exceptions, how often does anyone smoke inside your home?
   - □ Daily
   - □ Less than every day, but at least once a week
   - □ People smoke in home, but don't know how much
   - □ Less than every week, but at least once a month
   - □ Less than once a month
   - □ No one smokes in my home

6. What type of water is *usually* available at your house? *(Choose one)*
   - □ Water taps inside home
   - □ Water tap outside Home
   - □ Water from river/canal
   - □ Rain water
   - □ Other (specify): _____

7. Do you have place to wash your hands? □ Yes □ No

If YES:

7a. Can you show me where you typically wash your hands? *(Observed)*

□ At a sink with running water

□ From a water bottle reserved for that purpose

□ At a faucet outside

□ Other (specify):_____

7b. Is water available at the hand washing area? *(Observed)* □ Yes □ No

8. What type of product is most available for family members to clean their hands in your household?

□ Soap

□ Hand gel

□ Hand wipes

□ Other (specify): _____

□ Nothing

9. What type of hand dryer is most available in your household?

□ Tissue/paper

□ Cloth towel

□ Other (specify)_____

□ None

10. Do you cook inside or outside of the home?

□ Inside

□ Outside

□ Don't know

3. Case intake form

PHIRST Study: Case Intake Form

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

Individual Member Information to be collected form each household member

Demographics

11. ID Code

12. Date Enrolled: ___/___/____

13. Relationship to head of household
- □ Self
- □ Caregiver/Parent/Guardian
- □ Sibling
- □ Child
- □ Spouse
- □ Relative

14. Date of birth: ___/___/____

15. Sex
- □ Male
- □ Female

Study/Work Environment

For students:

16. Are you a student/scholar? □ YES □ NO

(IF YES Q7-8)

(IF NO skip to Q9)

17. What type of school do you go to?
- □ Nursery/Kindergarten
- □ Primary school
- □ Secondary school
- □ Post-secondary education/University
- □ Other (specify) _____

18. What is the average class size (persons/class)?_____

(Skip to Q14)

For non-student

19. What is the highest level of education you completed?

□ No schooling / kindergarten
□ Primary
□ Secondary
□ Matriculation
□ Post-secondary

20. What is your occupation?

□ Not working (Skip to 25)
□ Agriculture
□ Mining
□ Construction
□ Sales/retail
□ Health, medical, healing
□ Domestic helper
□ Other (Specify): _____

21. Do you work indoors or outdoors?

□ Indoors
□ Outdoors

22. What type of indoor environment do you work in?

□ Office buildings
□ Hotels & boarding houses
□ Shopping centre / plaza
□ Healthcare facility
□ Mine
□ School
□ Other (Specify):_____

23. How many work colleagues do you routinely come into contact with daily? _____

Social Habits *(If ≤15 years old skip to Q16*)

24. Do you drink alcohol? □ YES □ NO

If **YES**

14a. How many units per week? _____ □ liquor
(a unit is a glass of wine, a bottle of beer, or a shot of liquor) □ wine
□ beer

25. Do you currently smoke? □ YES □ NO

If **YES**
15a., how many cigarettes do you smoke per day? _____

Hand washing / social behaviors

26. What is the average number of times in a day that you wash your hands with soap and water?

Ask: In what situations do you wash your hands with soap?

This is an open-ended question. Do not read the answer choices.

After the respondent stops listing times, ask "Are there any other situations where you wash your hands with soap?" Keep asking this question until the respondent thinks there are no other times. Mark "YES" if the respondent mentioned the critical time and "NO" if the respondent did not mention that critical time]

1. Before preparing food	□ YES □ NO
2. Before cooking food	□ YES □ NO
3. Before eating	□ YES □ NO
4. After eating	□ YES □ NO
5. Before feeding a child	□ YES □ NO
6. Before breastfeeding	□ YES □ NO
7. After cleaning a child's anus	□ YES □ NO
8. After changing a baby's nappy	□ YES □ NO
9. After disposing of children's feces	□ YES □ NO
10. After you defecate	□ YES □ NO
11. After using the latrine for any purpose	□ YES □ NO
12. After contact with any sick person in the home	□ YES □ NO
13. After helping the sick person in the home	□ YES □ NO
14. After sneezing/coughing	□ YES □ NO
15. Other (specify)	□ YES □ NO

27. When you do NOT wash your hands, why do you not wash them?

□ Inconvenient
□ Forget
□ Too busy to wash hands
□ No towel, tissue or blower to dry hands
□ No water
□ No soap
□ Unnecessary
□ Other, specify: _____

28. Do you avoid contact with the index case, the household member who first became sick?
□ Always
□ Often
□ Sometimes
□ Never

29. Do you slept in a separate bed from a person that become sick in the house?
□ Always
□ Often
□ Sometimes
□ Never

Past Medical History

30. Do you have any underlying illness or condition at the moment?

Asthma	□ YES	□ NO
Other chronic lung disease	□ YES	□ NO
CVA/Stroke	□ YES	□ NO
Cirrhosis/Liver failure	□ YES	□ NO
Chronic renal failure	□ YES	□ NO
Heart failure	□ YES	□ NO
Valvular heart disease	□ YES	□ NO
Coronary artery disease (except hypertension)	□ YES	□ NO
Pregnancy	□ YES	□ NO
Organ transplant	□ YES	□ NO
Any immunosuppressive therapy, cortisone, chemotherapy, radiation therapy	□ YES	□ NO
Sickle cell	□ YES	□ NO
Splenectomy	□ YES	□ NO
Diabetes	□ YES	□ NO
Burns	□ YES	□ NO
Immunoglobulin deficiency	□ YES	□ NO
Autoimmune disease, SLE	□ YES	□ NO
Kwashiorkor/Marasmus	□ YES	□ NO
Nephrotic syndrome	□ YES	□ NO
Spinal cord injury	□ YES	□ NO
Seizure disorder	□ YES	□ NO

Prematurity	□ YES □ NO
Obesity / BMI >=30	□ YES □ NO
COPD/Emphysema	□ YES □ NO
Malignancy/Cancer	□ YES □ NO
If yes, specify: _____	
Other (Specify): _____	

31. Are you currently being treated for tuberculosis? □ YES □ NO

Current Medications

32. Over the past 2 weeks, have you taken the following medications regularly?
□ Antivirals
□ Antibiotics
□ Neither

33. If yes, what is the name of the medication?
(Record full name of any antibiotics or antivirals used) _____
AMO Amoxicillin; AMP Ampicillin; AUG Augmentin; CEF Cefuroxime, CIP Ciprofloxacin; CLI Clindamycin; CTX Ceftriaxone; DOX Doxycycline; ERY Erythromycin; PEN Penicillin; TMX/SMX Cotrimoxazole; VAN Vancomycin; TFR Tenofovir; EFA Efavirenz; LAM Lamivudine; FDC Fixed-Dose Combined ARV; U Unknown

34. Did you receive an influenza vaccine in the past 12 months? □ YES □ NO

If YES, dose given	□ 1 □ 2
Dose 1 Date given	___/___/____
Dose 2 Date given	___/___/____

NB:ALL PATIENTS WHO DO NOT HAVE A CONFIRMED CURRENT HIV STATUS SHOULD BE OFFERED AN HIV TEST

HIV Testing: Confidential

35. Have you ever been tested for HIV? □ YES □ NO
If YES, go to Q26
If NO: and ≤10 years, skip to Q27; if >10 years, skip to Q28

36. What was the result of your most recent HIV test? □ P □ N □ Unknown
If Pos or Neg:
Date of Result: ___/___/____

What was the source of the results?
□ Road to Health Card (RTHC)
□ Laboratory report
□ Medical records
□ Verbal
□ Other (Specify):_____

If HIV positive, skip to Q29;
If Unknown or Neg with results reported verbally or documented results older than 3 months and person ≥10 years of age skip to Q28

37. What was the HIV status of the mother during pregnancy (with the child being interviewed)? □ P □ N □ Unknown
If Pos or Neg:
What was the source of the results?
□ Road to Health Card (RTHC)
□ Laboratory report
□ Medical records
□ Verbal
□ Other (Specify):_____

If Positive or Unknown or verbally reported Negative go to Q35

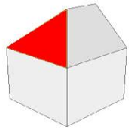
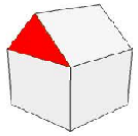
38. Would you like to be tested for HIV today? □ YES □ NO
Which test was done today?
□ Finger-prick Rapid Test
What is the test result? □ P □ N
□ Blood sample

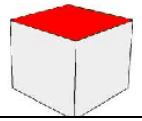
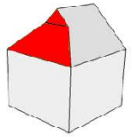
39. If HIV positive, are you on ARV treatment? □ YES □ NO

4. Housing checklist

PHIRST STUDY: HOUSING QUALITY CHECKLIST			What type of dwelling is this?	Formal House (stand alone, semi-detached, townhouse or cluster)	Flat	Single storey	Both (where both single and double storey buildings are present)
Study Site (please tick)	Agincourt	Klerksdorp		Informal dwelling	Business premises used for accommodation	Double storey	
Study code:				Backyard dwelling	Other		

BUILDINGS DESCRIPTION

ROOF			CEILING	WALLS		WINDOWS	FLOORS	TEMPERATURE CONTROL		DRINKING WATER POINTS	SHADE TREES	
Roofing material used: (please tick appropriate block)	Colour:	Shape: (please tick appropriate block)	Ceiling material used: (please tick appropriate block)	Exterior: (please tick appropriate block)	Interior: (please tick appropriate block)	Approximately, how many windows are there in the dwelling?	Flooring material used: (please tick appropriate block)	Are there any methods of temperature control used in the dwelling?		What types of drinking water points are available to the pupils? (please tick appropriate block)	Is there a tree on the western side of the house?	
Clay tiles		Hipped 	No ceiling	Stone	Stone	Do the windows have shade protection (e.g. curtains, blinds, awnings, etc.)?	Cement	Yes	No	Taps in bathrooms	Yes	No
								If yes, please indicate method used:			If yes, is it:	
Spanish tiles		Gable 	Cement	Brick	Brick	Yes / No / Some	Wood	Air conditioning		Drinking fountains in recreational areas		
						Can the windows be opened?		Ceiling fan				
Slate tiles			Wood	Plastered brick	Plastered brick		Brick	Standard fan		Taps in recreational areas		

		Flat 				Yes	No		Other (please specify)			
Metal sheeting		Gablet 	Ceiling boards	Wood	Wood	Are any of the windows broken? (please tick appropriate block)			Tiles		Container water	Deciduous	Evergreen
						Yes	No	Don't know	Linoleum	GPS coordinates:			
Asbestos		Other (please specify)	Other (please specify	Metal sheeting	Metal sheeting	Does the house have a chimney?			Earth		Other (please specify)		
Concrete				Prefabricated walling	Dry walling	Yes	No		Carpet	Address:			
Other (please specify)				Other (please specify)	Other (please specify)				Other (please specify)				

5. Environmental risk factor questionnaire

PHIRST STUDY: Environmental Risk Factor Questionnaire

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

Study Code								
Study Site	**Agincourt**					**Klerksdorp**		
Interview date	D	D	M	M	2	0	1	6
Name of Interviewer								

	INTERVIEWER: *Please record the type of dwelling used by the household. (Please choose one)*	**1 Formal house built by professional builder** **2 Formal house that was self-built** **3 Informal dwelling** **4 Backyard dwelling-formal** **5 Backyard dwelling-informal** **6 Flat** **7 Business premises used for accommodation** **8 Traditional house** **9 Other, specify** _____	
	Approximately how old is the house?	**In years**	
	How many separate rooms are there in the dwelling/house? ***Please fill in the number of each room***	**1 Kitchen (for cooking only)** **2 Bathrooms / toilets** **3 Dining rooms** **4 Lounge** **5 Bedrooms**	
	Please state whether the following are present in your dwelling.		
	1. Peeling paint (indoors)	**1=Yes 2=No 3=Don't know**	
	2. Peeling paint (outdoors)	**1=Yes 2=No 3=Don't know**	
	3. Cracks in walls	**1=Yes 2=No 3=Don't know**	
	4. Ventilation (a good supply of fresh air)	**1=Yes 2=No 3=Don't know**	
	5. Windows broken	**1=Yes 2=No 3=Don't know**	
	6. Leaks in roof	**1=Yes 2=No 3=Don't know**	

	7. Leaking water pipes in or around the dwelling	**1=Yes 2=No 3=Don't know**	
	8. Fungus or mould on walls or ceilings	**1=Yes 2=No 3=Don't know**	
	9. Odours (bad smells) in the area	**1=Yes 2=No 3=Don't know**	
	10. Overcrowding in the dwelling	1=Yes 2=No 3=Don't know	
	11 Air pollution in the neighbourhood	1=Yes 2=No 3=Don't know	
	Where do you get your drinking water from?	1 = tap inside the dwelling	
		2 = tap outside the dwelling, but on the stand (in the yard)	
		3 = tap off the property	
		4 = borehole	
		5 = river or stream or dam	
		5 = water vendor	
		6 = water truck	
		7 = water tank	
		8 = other (please specify)	
	Do you store water for drinking in a container?	1 = yes 2 = no	
	Do you have running, hot water in the house	1 = yes 2 = no	
	What type of toilet do you use?	1 = Flush toilet (waterborne)	
		2 = pit latrine	
		3 = chemical toilet	
		4 = bucket toilet	
		5 = no toilet (using open area)	
	During windy weather, does the air get very dusty/sandy in this area?	**1=Yes 0=No**	
	If yes, where do you think the dust/sand comes from?		
	Is dust inside the dwelling a major problem?	**1=Yes 0=No**	
	What do you <u>mainly</u> use to dust the house? ***Please choose one option only.***	1 A feather duster	
		2 A dry cloth	
		3 A dry cloth with furniture polish	
		4 A damp cloth	
		5 A cloth soaked in soapy water	
		6 Other (please specify) _____	
	What do you mainly use to clean the floors of the house? ***Please choose more than one if necessary.***	A dry broom to sweep **1=Yes 0=No**	
		A mop with water only **1=Yes 0=No**	
		A mop with soapy water **1=Yes 0=No**	
		Vacuum cleaner **1=Yes 0=No**	
		Other (please specify) **1=Yes 0=No** _____	

	How often do you clean the floors? ***(choose one option)***	**1 Daily** 2 More than once a week 3 Once a week 4 More than once a month 5 Seldom	
	Is there dampness in this house?	1=Yes 0=No	
	Where is the dampness in building found?	**Kitchen** 1=Yes 0=No	
		Bathrooms / toilets 1=Yes 0=No	
		Dining rooms 1=Yes 0=No	
		Lounge 1=Yes 0=No	
		Bedrooms 1=Yes 0=No	
	What do you think has caused the dampness *(e.g. plumbing leak, drying clothes, cold house, rising dampness, rotten window frames)*		
	Is there mould (fuzzy spots) in the dwelling?	1=Yes 0=No	
	In which room/s is the mould	**Kitchen** 1=Yes 0=No	
		Bathrooms / toilets 1=Yes 0=No	
		Dining rooms 1=Yes 0=No	
		Lounge 1=Yes 0=No	
		Bedrooms 1=Yes 0=No	
	What colour is the mould?		
	Have you tried to clean the mould?	1=Yes 0=No	
	How often do you clean the mould? ***Please choose one option only***	**1 Whenever you notice it** 2 Every week 3 Every month 4 Seldom	
	Has mould growth been noted on the following surfaces? *Choose all that apply*	**Brick walls** 1=Yes 0=No	
		Dry wall **1=Yes 0=No**	
		Wooden walls **1=Yes 0=No**	
		Wall-papered walls **1=Yes 0=No**	
		Carpets **1=Yes 0=No**	
		Wooden skirting **1=Yes 0=No**	
		Ceilings **1=Yes 0=No**	
		Tiles **1=Yes 0=No**	
		Shower or bath **1=Yes 0=No**	
		Curtains **1=Yes 0=No**	
		Cupboards **1=Yes 0=No**	
		Window frames **1=Yes 0=No**	
		Furniture **1=Yes 0=No**	
		Clothes **1=Yes 0=No**	
	Do you dry clothes indoors?	1=Yes 0=No	
		1 Electricity	

	What type of fuel do you mainly use for cooking? ***Please choose one***	**2 Paraffin** **3 Gas** **4 Wood** **5 Coal** **6 Other: specify**	
	Do you sometimes use a different fuel for cooking?	**1 = yes 2 = no** If yes, please specify:	
	What type of fuel do you use mainly for indoor heating? ***Please choose one.***	**1 Electricity** **2 Paraffin** **3 Gas** **4 Wood** **5 Coal** **6 Imbhawula** **7 Other: specify**	
	Do you sometimes use a different fuel for cooking?	**1 = yes 2 = no** If yes, please specify:	
	What fuel do you mainly use for heating water? ***Please choose one.***	**1 Electricity** **2 Paraffin** 3 Gas 4 Wood 5 Coal 6 Other: specify	
	Are any pets or animals kept inside the house? (If yes, please specify)	1=Yes 0=No	
	How many people in the household smoke?	**Cigarettes** 1=Yes 0=No **Pipe tobacco** 1=Yes 0=No Hookah / Hubbly bubbly **1=Yes 0=No** **Electronic cigarettes** 1=Yes 0=No **Other**_____ 1=Yes 0=No	
	Do any members of this household smoke inside the dwelling?	*Number*	

THANK YOU FOR TAKING THE TIME TO ANSWER THIS QUESTIONNAIRE.

6. Follow-up form

PHIRST Study: Follow-Up Form

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

To be administered at every follow-up visit to each household member (presence of symptoms to be verified by study staff)

Date __/__/____ Time __:__

ID Code: _____

Interviewer_____ Place of Interview □ Home □ Other (specify): _____

To be asked to the household caregiver only

1. Since our previous visit, are the same people □ YES □ NO
   still living (sleeping or eating) in this home *(to be asked to the caregiver only)*?

2. Since our previous visit was someone in the household admitted to hospital? □ YES □ NO
   If YES:
   - ID Code of person admitted to the hospital: _____
   - Complete hospital admission form

3. Since our previous visit did anyone in the household die? □ YES □ NO
   If YES:
   - ID Code of person admitted to the hospital: _____
   - Complete death form

To be asked to all household member

4. Since our previous visit, have you started to □ YES □ NO
   have any signs of illness like cough or fever?
   For each person who reports symptoms complete the follow-up visit symptom form

5. Patient symptomatic at the time of the visit □ YES
   □ NO
   (observed from study staff)?

6. Since our previous visit, have you sought care □ YES
   □ NO
   from a clinic or healer for your symptoms?

7. Symptom form

PHIRST Study: Symptom Form

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

To be administered at any follow-up visit to symptomatic household members

ID Code: _____

Date ___ /___ /____ Time ___:___

Interviewer_____ Place of Interview □ Home □ Other_____

Reason for Completing Form: □ Symptomatic Participant

1. Which of the following symptoms did you experience?
   - □ Fever — Recorded temperature: _____◦C
   - □ Sore throat — □ Mild □ Very mild □ Moderate □ Severe □ Very severe
   - □ Cough — □ Mild □ Very mild □ Moderate □ Severe □ Very severe
   - □ Runny nose — □ Mild □ Very mild □ Moderate □ Severe □ Very severe
   - □ Headache — □ Mild □ Very mild □ Moderate □ Severe □ Very severe
2. When did the symptoms start? ___ /___ /____
3. Did you seek medical or traditional medicine help with your symptoms? □ Yes □ No
4. Did you receive any medication?
   If so what medication antibiotics/symptomatic: _____
5. Were you admitted to hospital?
   If so,
   Which hospital: _____
   How long did you spend in hospital: _____ days
   What was the diagnosis? _____
6. Did you avoid contact with anyone in the house who was unwell in the past 10 days?
   □ Yes □ No □ No one sick in the household in the past 10 days
7. Since you became sick, have you kept yourself away from other members of the household?
   □ Yes □ No
8. Since you became sick, have you slept in a separate bed from other household members?
   □ Yes □ No
9. Since you became sick, have you stayed home from school, work, and social gatherings to protect others from catching your illness?
   □ Yes □ No
10. Since you became sick, do you cover your mouth and nose with a tissue when you cough or sneeze?
    □ Yes □ No

8. Tuberculosis form

PHIRST Study: TB Symptoms Form

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

To be administered at the first follow-up visit of every month.

ID Code: _____

Date ___ /___ /____ Time ___:___

Interviewer_____ Place of Interview: □ Home □ Other (specify): _____

1. Have you been coughing? □ YES □ NO
2. If yes, have you been coughing for more than 2 weeks? □ YES □ NO
3. Have you had night sweats? □ YES □ NO
4. Have you lost weight? □ YES □ NO
5. Date of symptoms onset: ___ /___ /____

If yes to any of these questions, please take sputum sample

9. Hospitalisation form

PHIRST Study: Hospitalization Form

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

To be administered to the hospitalized person when he/she returns from the hospital. If the person is not available for interview the information will be collected retrospectively form the caregiver or other household members. If the person died complete the death form.

ID Code of person admitted to hospital: _____

Relationship to hospitalized household member:
□ Self
□ Caregiver/Parent/Guardian
□ Sibling
□ Son/Daughter
□ Spouse
□ Relative

If relationship to hospitalized household member different than self, then:

ID code of respondent: _____

Date ___ /___ /____ Time ___:___

Interviewer_____ Place of Interview: □ Home □ Other (specify): _____

1. When were you/the person admitted to hospital? ___ /___ /____

2. Which hospital were you/the person admitted to?
   □ Klerksdorp
   □ Tshepong
   □ Matikwana
   □ Mapulaneng
   □ Tintswalo
   □ Other (specify): _____

3. What was the reason for hospitalization?
   □ Accident
   □ Illness
   □ Operation
   □ Childbirth
   □ Other (specify): _____
   If answer different that Illness, end.

4. If the hospitalization was for illness did you/the person have any of the following symptoms prior to the admission?
    - □ Fever
    - □ Sore throat
    - □ Cough
    - □ Runny nose
    - □ Headache
    - □ Night sweats
    - □ Difficulty breathing
    - □ Wheezing/noisy breathy

5. When did the symptoms start? ___ /___ /____

6. Did you/the person seek care before being admitted to the hospital? □ YES □ NO
   If No, skip to Q7

7. Where did you/the person seek care (tick all that apply)?

	Rank in chronological order
□ Clinic	_____
□ Private doctor	_____
□ Traditional healer	_____
□ Pharmacy	_____

8. Do you/the person have a diagnosis from the hospital? □ YES □ NO
   If No, skip to Q10.

9. What was the diagnosis?
    - □ Pneumonia
    - □ Bronchopneumonia
    - □ Bronchiolitis
    - □ Chest infection
    - □ TB
    - □ Fluid on the lungs/around the lungs
    - □ Pneumothorax
    - □ Other, (specify): _____

10. What was the outcome of the hospitalization?
    - □ Discharge
    - □ Referred to step-down facility Name of facility: _____
    - □ Transferred Name of facility: _____

11. Date of hospital outcome: ___ /___ /____

10. Death form

PHIRST Study: Death Form

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

To be administered at any follow-up visit if any household member has died

ID Code of person who died: _____

ID code of respondent: _____

Relationship of respondent to household member who died:
□ Caregiver/Parent/Guardian
□ Sibling
□ Son/Daughter
□ Spouse
□ Relative
□ Other (specify): _____

Date ___ /___ /____ Time ___:___

Interviewer_____ Place of Interview: □ Home □ Other (specify): _____

If a death certificate is available, please record the cause of death as per the death certificate: _____

_____

_____

_____

1. When did the person die? ___ /___ /____
2. What was the cause of death
   □ Accident
   □ Illness
   □ Operation
   □ Childbirth
   □ Old age
   □ Other (specify): _____
   If the cause of death was different than illness, skip to Q11

3. If the cause of death was for illness did the person have any of the following symptoms prior to dying?
    - □ Fever
    - □ Sore throat
    - □ Cough
    - □ Runny nose
    - □ Headache
    - □ Night sweats
    - □ Difficulty breathing
    - □ Wheezing/noisy breathy
4. When did the symptoms (any) start? ___ /___ /____
5. Did the person seek care before dying? □ YES □ NO
   If No, skip to Q11
6. Where did the person seek care before dying (tick all that apply)?

	Rank in chronological order
□ Hospital	_____
□ Clinic	_____
□ Private doctor	_____
□ Traditional healer	_____
□ Pharmacy	_____

7. Was the person hospitalized before dying? □ YES □ NO
   If No, skip to Q11
8. In which hospital was the person hospitalized?
    - □ Klerksdorp
    - □ Tshepong
    - □ Matikwana
    - □ Mapulaneng
    - □ Tintswalo
    - □ Other (specify): _____
9. Do you have a diagnosis from the hospital? □ YES □ NO
   If No, skip to Q11
10. What was the diagnosis?
    - □ Pneumonia
    - □ Bronchopneumonia
    - □ Bronchiolitis
    - □ Chest infection
    - □ TB
    - □ Fluid on the lungs/around the lungs
    - □ Pneumothorax
    - □ Other, (specify): _____
11. Where did the person die?
    - □ Hospital
    - □ Home
    - □ Other (specify): _____

11. Laboratory slip

PHIRST Study: Laboratory Slip

Centre for Respiratory Diseases and Meningitis (CRDM)
TEL: 011 386 6410 or 011 386 6434
FAX: 086 723 3569

ID Code: _____

Date ___ /___ /____

Interviewer_____ Time ___:___

Specimen Type	**Number of specimens (indicate 0 if not collected)**
□ Oropharyngeal swab	_____
□ Nasoparyngeal swab	_____
□ Blood (EDTA)	_____
□ Blood (clotted)	_____
□ Expectorated sputum	_____
□ Urine	_____

Appendix 3 Environmental assessment and monitoring

1. Assessment of levels of exposure to particulate matter

Levels of exposure to particulate matter (PM_{10} and $PM_{2.5}$) will be measured through the installation of portable, constant flow Gilian pumps with Dorr-Oliver cyclones as pre-separator with mixed cellulose ester filters in line. Filters will be dehydrated for 24 hours and pre-weighed using a micro-balance under controlled temperature and humidity conditions. Each filter will be assigned a unique identification number and weighed thrice. After weighing, the filter will be placed in a sealed cassette and stored in a temperature controlled environment prior to transportation to the field sites. In the field the cassette (containing the cyclone) will be inserted into the cyclone separator and attached to a Gilian pump. Pumps with cyclones will be located at the breathing height of adults. Sampling will be undertaken at a flow rate of 1.7 litres per minute. The time of switching the pump on and off (24 hours later) will be recorded. In 10% of dwellings a filter, unattached to a Gilian pump, will be installed to determine whether factors unrelated to respirable particles are influencing readings. Each Gilian pump will be serviced and calibrated beforehand. Cassettes containing filters will be sealed after sampling and carefully stored and transported to the laboratory for dehydration and weighing. A smaller version of this device will be worn by one member of each household during the environmental sampling period to monitor personal air quality. This device will have a GPS logger attached to it in order to monitor the area where the air sample was collected.

2. Assessment of exposure to carbon monoxide

ChromAir badges will be used to monitor exposure to carbon monoxide, either personally or an area within the dwelling. The carbon monoxide monitors are non-invasive and are placed on top of clothing worn by the participant. After purchase, the badges will be kept under refrigeration. In the field, before monitoring starts, all pertinent information will be entered on the I.D. label. After removing the protective strip, the badged will be attached to the participant's breathing zone (for personal monitoring) or placed in a suitable location in the dwelling. The results will be read and interpreted in line with the manufacturer's guidelines.

3. Assessment of exposure to dust

Dust within a pre-determined area of the floor will be collected using a ghost wipe. Sampling and analysis of dust samples will be conducted using US EPA/HUD methods.

4. Assessment of indoor temperature and relative humidity

Battery operated LogTag, Haxo-8 indoor temperature monitors will be placed in all dwellings to monitor indoor air temperature and relative humidity over a period of one year.

Appendix 4: Specimen packaging and transport protocol

Transport of specimens should comply with WHO Guidance on regulations for the Transport of Infectious Substances (WHO/CDS/EPR/207.2), applicable from 1 January 2007.

The receiving laboratory should be notified before shipment of specimens.

All specimens to be transported must be packed in packaging consisting of three layers. These consist of the following (Figure 6):

- Primary receptacle: A primary watertight, leak-proof receptacle containing the specimen. The receptacle is packaged with enough absorbent material to absorb all fluid in case of breakage.
- Secondary packaging: A second durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Several primary receptacles may be placed in one secondary packaging, but these must either be individually wrapped or separated so as to prevent contact between them. Sufficient additional absorbent material must be used to absorb all fluid in case of breakage.
- Outer packaging: Secondary packagings are placed in outer shipping packaging with suitable cushioning material. Outer packaging protect their contents from outside influences, such as physical damage, while in transit. The smallest overall external dimension shall be 10x10 cm.

Each completed package is required to be correctly marked and labelled with the receiver's (consignee's) name, address and telephone number.

The specimens are transported as Biological Substances, Category B. These are classified as infectious substances that are not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposed to it. This includes substances transported for diagnostic or investigational purposes.

The label UN3373 (Figure 7) is not necessary when transporting Category B substances by road.

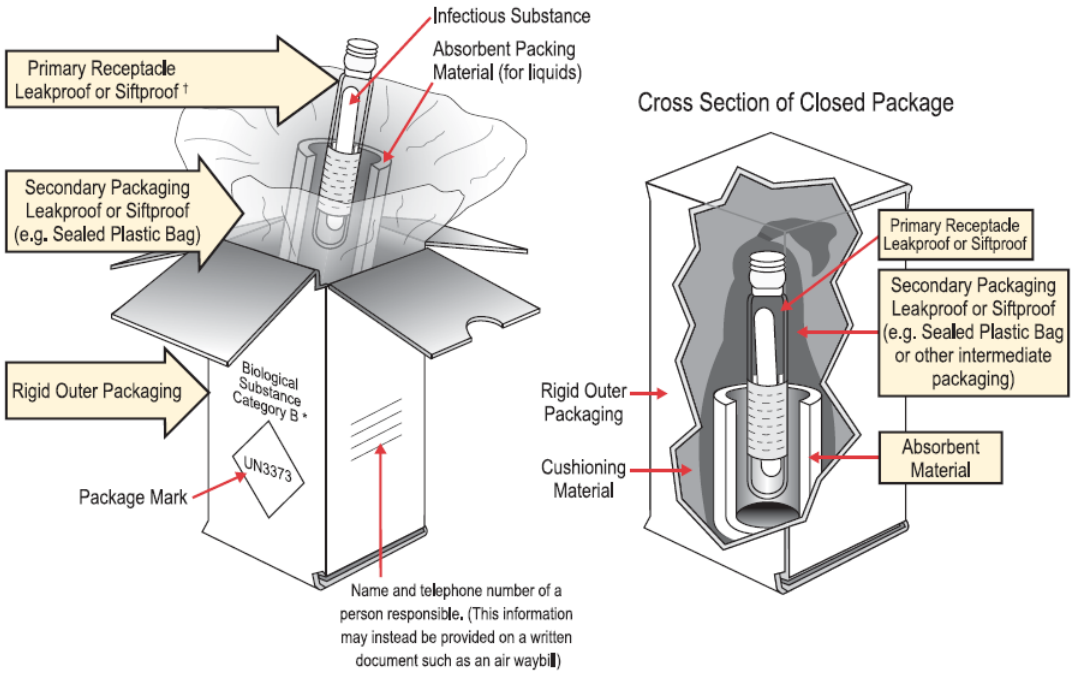


Figure 6. Example of the triple packaging system for the packing and labelling of Category B substances.

Figure 7. Label necessary for the shipment of Category B substances when transported by air.

Appendix 5 Informed consent and assent forms Updated information sheet and consent are attached as per versions on the covering letter.

1. Information leaflet for household members - Information leaflet 1 consent form for adults

STUDY TITLE: A Prospective Household observational cohort study of Influenza, Respiratory Syncytial virus and other respiratory pathogens community burden and Transmission dynamics in South Africa (The PHIRST Study)

Each participant must read this document and sign the attached informed consent before any study-related procedure is done.

Institution: National Institute for Communicable Diseases, South Africa; funded by a grant from the Centers for Disease Control and Prevention (CDC), Atlanta, United States of America.

Investigator: Prof Cheryl Cohen 011 386 6593, day time and 082 803 8093, afterhours

Hello, my name is Prof Cheryl Cohen, I am the Head of the Centre for Respiratory Disease and Meningitis at the National Institute for Communicable Diseases in Johannesburg. I would like to invite you to think about helping us with a research study called the household transmission study.

- Before you agree to take part in this study we would like you to read this information sheet about the study.
- Before you say you would like to take part please make sure you understand what you need to do.
- You should also make sure you understand the purpose of the study, the study procedures, benefits, risks, discomforts, and precautions as well as the alternative procedures that are available to you, 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.
- If you have any questions, do not hesitate to ask me or the study staff that will introduce the study to you.
- You should not agree to take part unless you are satisfied with all the procedures involved.
- Please be open with me regarding your health history, since you may otherwise harm yourself by participating in this study.
- 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.

Background/Purpose

Infectious diseases are caused by different germs (virus, bacteria or parasites). By infectious we mean that the illness can be passed from one person to the next. It is important to understand the way these infections are passed on to other people. The household (home) is an important place for transmission (passing on) of these infections because people spend time together in close contact. It is also possible that someone is infected with the virus or bacteria but does not become ill. It is important to understand the number of people who get infected and do not get ill. The information from the study will help to make guidelines for the use of vaccines and other interventions to help prevent these illnesses in people.

We are conducting a study to try and understand how infectious diseases are passed on in households. We are interested in viruses and bacteria that cause respiratory illness (for example influenza and tuberculosis (TB)). We are also interested in the bacteria that causes meningitis called meningococcus. The names of all these germs are confusing so it is easier to group them into the germs that cause respiratory illness (colds, flu and pneumonia (chest infections)) and the germs that cause meningitis (infection in the brain).

We know that some people are at risk of getting very severe forms of these illnesses, these groups include very young children, the elderly, people with other illnesses (like heart disease, diabetes and other lung conditions), and people who have weak immune systems (like people with HIV). We would like to know how these infections enter the household and then how they are passed on in the house. This is why we are doing this study in the household. There are important factors in households, including the compositions of the household (how many young children, children who go to school, people who work and people who stay in the household). There are also important factors like smoking and making of fires inside the house which also play a role in the transmission of infections.

In order to help us explain all these things we are planning a study that will enroll 100 household in the Bushbuckridge district and in Klerksdorp each year. Each household will be asked to be part of the study for one year. During this year we will do a number of things after the household members have signed consent to participate, details of each of these procedure are outlined later in this form.

1. Complete a detailed questionnaire for each person in the home including questions about your age, the kind of work you do, what illness you may have and some questions about your home (number of rooms, cooking etc).
2. We will ask about your HIV status and if necessary do testing for HIV. If you test positive for HIV we will counsel you and refer you for treatment.
3. We will take blood samples (10mls (two teaspoon) from adults and 5mls (one teaspoon) from children aged <15 years, at the beginning of the study and then three times during the year. These blood samples will be tested for bacteria and viruses and for your bodies' reaction to these viruses.
4. In September we will take one additional blood sample which will be 1ml (less than one teaspoon) from children aged <15 years and 5mls (one teaspoon) from adults. These blood samples will be tested for a particular bacterium, streptococcus, which causes pneumonia and meningitis.
5. Take a sputum sample at the beginning of the study, this sample will be tested for TB; if you test positive for TB we will refer you for treatment.

6. Each month we will ask you if you have any symptoms of TB and if you have these symptoms we will take a sputum specimen for TB testing. If you test positive for TB we will refer you for treatment.
7. At the beginning of the year and again at the end of the year we will to a skin test to check for TB infection
8. Take a urine sample at the beginning of the study. This urine sample will be tested for a chemical that tells us how much you smoke (this will not be done on children under 12 years of age).
9. At the enrolment visit and following that at the twice weekly household visit we will take a swab from each member of the household. At the follow up visits we will ask a few simple questions about any respiratory symptoms you may have experienced. These swabs will be tested for the germs that cause respiratory illness. If you have experienced/are experiencing any respiratory symptoms we can refer you for treatment.
10. Towards the middle of the year in July, we will take a throat swab; this swab will be tested for the bacteria that cause meningitis.
11. We will also ask if anyone has been in hospital or died. We will ask questions as about why they went to hospital and what they died from.
12. We will put a small machine in the house that will measure the temperature and humidity in your house. These machines are similar to a thermometer and will not interfere with your day to day activities.
13. At two points in the study (once in the summer months and once in the winter months) we will also put a machine in your house that measures the amount of carbon monoxide (smoke) in your home. These machines will not interfere with your day to day activities.
14. After the first year of the study is finished we will visit you at your house once a year for the next 2 year in October to take a blood sample of 5mls (one teaspoon) from children aged <15 years and 10mls (two teaspoon) from people aged ≥15 years, perform a tuberculosis test and perform an HIV test.

Length of study and number of participants

- The study will be performed in South Africa only.
- Approximately 1500 participants will participate in this study, 100 households each year with an average of 5 people in each household.
- Every household member, regardless of their age will be asked to participate.
- We will visit the household twice a week for the period of one year. After that we will visit the household once a year for a maximum of two years.

Study procedures

1. Blood samples: Blood will be taken from your arm; this will be done by a trained nurse.
2. HIV testing
    a. If you are HIV-positive and have some way of showing us this, either by a clinic record or your ARV treatment we will not need to test you for HIV.
    b. We can do a rapid HIV test at your house by doing a small prick on your finger and you will be able to receive the result immediately

c. We can also test the blood sample that we have taken at the beginning of the study or four times per year for HIV in the laboratory in Johannesburg. If you choose for us to do this we can still give you your results if you would like to know them

3. Naso-pharyngeal swab (swabs are like a long cotton bud), the nurse will put a swab into your nose until it touches the back of your throat.
4. Throat swabs. After asking you to open your mouth wide, the nurse will put the swab into the back of your mouth and touch the back of your throat with the swab.
5. Sputum samples, we will ask you to cough into a small bottle.
6. Urine will be collected in a small bottle, the nurse will explain how to do this and you will collect the sample yourself in the bathroom.
7. Skin test for TB, the nurse will give you a very small injection into the skin with a very small needle, this may cause a slight swelling on your arm. After 2 to 3 days the nurse will come back to measure how big the swelling is.

Your rights as a participant

It is your right to choose if you want to take part in this study. If you chose not to participate this will not affect your right to health care, other services or your right to participate in future studies.

Expected duration of participation

The first visit will take about an hour per person including taking the blood samples; the follow-up visits will be about 15 minutes per person. We will try to arrange these visits when most people in the household are present and this may involve us visiting your household in the evening or over weekends.

Risks of this study

There is minimal risk to you in this study as we are only asking questions about your normal behaviour at home. You may find some of the questions uncomfortable. There may be minimal discomfort when we take the nasopharyngeal or throat sample. Some people may gag when we take the sample, but the procedure only takes a few seconds.

The TB skin test has a slight risk of discomfort and may result in slight swelling of the arm.

Venipunctures (i.e. drawing blood) are normally done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site. There is also a slight possibility of infection. Your protection is that experienced personnel perform the procedures under sterile conditions.

Testing for HIV may be stressful, we will have a trained counsellor to explain all about the test and what the results will mean to you. We will also refer you directly into the treatment programme if you do test positive for HIV.

Benefits of this study

By taking part in this study, you will help us learn more about how certain infectious diseases affect different people and who may benefit from vaccines. You will have a chance to get the results of your HIV and TB tests and if you test positive for these infectious you will get the benefit of early treatment.

Confidentiality

Every effort will be made to protect your confidentiality: study forms and blood samples will be marked with a number and not a name. Study staff will keep a log of household members and identifying details of household members, these will be kept in secure locked offices. No reference to personal detail will be made in any study report or in the final results of the study.

Withdrawal from the study

Your participation in this study is entirely voluntary and you can decline to participate, or stop at any time, without stating any reason. Your withdrawal will not affect your access to other medical care.

- The investigators retain the right to withdraw you from the study if it is considered to be in your best interest.
- If you did not give an accurate history or did not follow the guidelines of the study and the regulations of the study facility, you may be withdrawn from the study at any time.

Reimbursement for Participation

You will not be paid to participate in this study. However we will offer you some reimbursement for the time and inconvenience of participating in this study. We will offer you a voucher to be redeemed at a supermarket for items sold at that supermarket. Each member of the household will receive a voucher to the value of R25 for each of the visits. For a household of 5 people counting the twice weekly visits this would be in the region of R1000 worth of vouchers for an average month.

The study will not pay for any care that you need for the any illness you may experience during this study. We will share the results of the HIV and TB tests with you so that you will know if you have been infected with these illnesses. In addition, we are able to refer you for medical help to your nearest clinic if this is necessary.

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.
- The study has been structured in accordance with the **Declaration of Helsinki** (last updated: October 2013), which deals with the recommendations guiding doctors in biomedical research involving human participants. A copy may be obtained from me should you wish to review it.
- I do not have any financial or personal interests with this organisation that may bias my actions.
- 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.

2. Information leaflet for household members – Information leaflet 2: assent for children ages 7 to 17 years

STUDY TITLE: A Prospective Household observational cohort study of Influenza, Respiratory Syncytial virus and other respiratory pathogens community burden and Transmission dynamics in South Africa (The PHIRST Study)

Each participant must read this document and sign the attached informed consent before any study-related procedure is done.

Parental consent is require for each person younger than 18

Institution: National Institute for Communicable Diseases, South Africa; funded by a grant from the Centers for Disease Control and Prevention (CDC), Atlanta, United States of America.

Investigator: Prof Cheryl Cohen 011 3866593, day time and 082 803 8093, afterhours

Hello, my name is Prof Cheryl Cohen, I am the Head of the Centre for Respiratory Disease and Meningitis at the National Institute for Communicable Diseases in Johannesburg. I would like to invite you to think about helping us with a research study called the household transmission study.

- Before you agree to take part in this study we would like you to read this information sheet about the study.
- Before you say you would like to take part please make sure you understand what you need to do.
- You should also make sure you understand what the study is about and what the study would like to say at the end.
- Make sure you understand the study procedures (what will be done to you in the study), the benefits, risks and discomforts.
- You have the right to ask to stop being in the study if you wish, at any time in the study.
- 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.
- If you have any questions, please ask me or the study staff that will introduce the study to you.
- You should not agree to take part unless you are satisfied with all the procedures involved.
- Please be open with me regarding your health history, since you may otherwise harm yourself by participating in this study.
- 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.

Background/Purpose

Infectious diseases are caused by different germs (virus, bacteria or parasites). By infectious we mean that the illness can be passed from one person to the next. It is important to understand the way these infections are passed on to other people. The household (home) is an important place for transmission (passing on) of these infections because people spend time together in close contact. It is also possible

that someone is infected with the virus or bacteria but does not become ill. It is important to understand the number of people who get infected and do not get ill. The information from the study will help to make guidelines for the use of vaccines and other interventions to help prevent these illnesses in people.

We are conducting a study to try and understand how infectious diseases are passed on in households. We are interested in viruses and bacteria that cause respiratory illness (for example influenza and tuberculosis (TB)). We are also interested in the bacteria that causes meningitis(infection of the brain) called meningococcus. The names of all these germs are confusing so it is easier to group them into the germs that cause respiratory illness (colds, flu and pneumonia (chest infections)) and the germs that cause meningitis (infection in the brain).

We know that some people are at risk (have a bigger chance) of getting very severe (bad) forms of these illnesses, these groups include very young children, the elderly, people with other illnesses (like heart disease, diabetes and other lung conditions), and people who have weak immune systems (like people with HIV).

We would like to know how these infections enter the household and then how they are passed on in the house. This is why we are doing this study in the household. There are important factors in households, including the compositions of the household (how many young children, children who go to school, people who work and people who stay in the household). There are also important factors like smoking and making of fires inside the house which also play a role in the transmission of infections.

In order to help us explain all these things we are planning a study that will enroll 100 household in the Bushbuckridge district and in Klerksdorp each year. Each household will be asked to be part of the study for one year. During this year we will do a number of things after the household members have signed consent to participate, details of each of these procedure are outlined later in this form.

15. Complete a detailed questionnaire (ask a number of questions) for each person in the home including questions about your age, the kind of work you do, what illness you may have and some questions about your home (number of rooms, cooking etc).
16. We will ask about your HIV status and if necessary do testing for HIV. If you test positive for HIV we will counsel you and refer you for treatment.
17. We will take blood sample 5mls (one teaspoon) from children aged <15 years and 10mls (two teaspoon) from people aged ≥15 years, at the beginning of the study and then three more times during the year. These blood samples will be tested for bacteria and viruses and for your bodies' reaction to these viruses.
18. In September we will take one additional blood sample which will be 1ml (less than one teaspoon) from children aged <15 years and 5mls (one teaspoon) from people aged ≥15 years. These blood samples will be tested for a particular bacterium, streptococcus, which causes chest and brain infections.
19. Take a sputum (spit that is coughed up from the chest) sample at the beginning of the study, this sample will be tested for TB; if you test positive for TB we will refer you for treatment.

20. Each month we will ask you if you have any symptoms of TB (like coughing a lot or losing weight) and if you have these symptoms we will take a sputum specimen for TB testing. If you test positive for TB we will refer you for treatment.
21. At the beginning of the year and again at the end of the year we will to a skin test to check for TB infection
22. Take a urine sample at the beginning of the study. This urine sample will be tested for a chemical that tells us how much you smoke (this will not be done on children under 12 years of age).
23. Visit the household twice a week to take a swab (swabs are like a long cotton bud) from each member of the household and to ask a few simple questions about any respiratory symptoms you may have experienced. These swabs will be tested for the germs that cause respiratory illness. If you have experienced/are experiencing any respiratory symptoms we can refer you for treatment.
24. Towards the middle of the year in July, we will take a throat swab; this swab will be tested for the bacteria that cause meningitis (infection around the brain).
25. We will put a small machine in the house that will measure the temperature and humidity in your house. These machines are similar to a thermometer and will not interfere with your day to day activities.
26. At two points in the study (once in the summer months and once in the winter months) we will also put a machine in your house that measures the amount of carbon monoxide (smoke) in your home. These machines will not interfere with your day to day activities.
27. After the first year of the study is finished we will visit you at your house once a year for the next 2 year in October to take a blood sample of 5mls (one teaspoon) from children aged <15 years and 10mls (two teaspoon) from people aged ≥15 years, perform a tuberculosis test and perform an HIV test.

Length of study and number of participants

- The study will be performed in South Africa only
- Approximately 1500 participants will participate in this study, 100 households each year with an average of 5 people in each household.
- Every household member, regardless of their age will be asked to participate.
- We will visit the household twice a week for the period of one year. After that we will visit the household once a year for a maximum of two years.

Study procedures

8. Blood samples: Blood will be taken from your arm; this will be done by a trained nurse.
9. HIV testing
    a. If you are HIV-positive and have some way of showing us this, either by a clinic record or your ARV treatment we will not need to test you for HIV.
    b. We can do a rapid HIV test at your house by doing a small prick on your finger and you will be able to receive the result immediately
    c. We can also test the blood sample that we have taken at the beginning of the study or four times per year for HIV in the laboratory in Johannesburg. If you choose for us to do this we can still give you your results if you would like to know them

10. Naso-pharyngeal swab (swabs are like a long cotton bud), the nurse will put a swab into your nose until it touches the back of your throat.
11. Throat swabs (swabs are like a long cotton bud). After asking you to open your mouth wide, the nurse will put the swab into the back of your mouth and touch the back of your throat with the swab.
12. Sputum samples, we will ask you to cough into a small bottle.
13. Urine will be collected in a small bottle, the nurse will explain how to do this and you will collect the sample yourself in the bathroom.
14. Skin test for TB, the nurse will give you a very small injection into the skin with a very small needle, this may cause a slight swelling on your arm. After 2 to 3 days the nurse will come back to measure how big the swelling is.

Your rights as a participant

It is your right to choose if you want to take part in this study. If you chose not to participate this will not affect your right to health care, other services or your right to participate in future studies.

Expected duration of participation

The first visit will take about an hour per person including taking the blood samples; the follow-up visits will be about 15 minutes per person. We will try to arrange these visits when most people in the household are present and this may involve us visiting your household in the evening or over weekends.

Risks of this study

There is minimal risk to you in this study as we are only asking questions about your normal behaviour at home. You may find some of the questions uncomfortable. There may be minimal discomfort when we take the nasopharyngeal or throat sample. Some people may gag when we take the sample, but the procedure only takes a few seconds.

The TB skin test has a slight risk of discomfort and may result in slight swelling of the arm.

Venipunctures (i.e. drawing blood) are normally done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site. There is also a slight possibility of infection. Your protection is that experienced personnel perform the procedures under sterile conditions.

Testing for HIV may be stressful, we will have a trained counsellor to explain all about the test and what the results will mean to you. We will also refer you directly into the treatment programme if you do test positive for HIV.

Benefits of this study

By taking part in this study, you will help us learn more about how certain infectious diseases affect different people and who may benefit from vaccines. You will have a chance to get the results of your HIV and TB tests and if you test positive for these infectious you will get the benefit of early treatment.

Confidentiality

Every effort will be made to protect your confidentiality (privacy): study forms and blood samples will be marked with a number and not a name. Study staff will keep a log of household members and identifying details of household members, these will be kept in secure locked offices. No reference to personal detail will be made in any study report or in the final results of the study.

Withdrawal from the study

Your participation in this study is entirely voluntary and you can decline to participate, or stop at any time, without stating any reason. Your withdrawal will not affect your access to other medical care.

- Your withdrawal will not affect your access to other medical care.
- The investigators retain the right to withdraw you from the study if it is considered to be in your best interest.
- If you did not give an accurate history or did not follow the guidelines of the study and the regulations of the study facility, you may be withdrawn from the study at any time.

Reimbursement for Participation

We will offer you some reimbursement for the time and inconvenience of participating in this study. We will offer you a voucher to be redeemed at a supermarket for items sold at that supermarket. Each member of the household will receive a voucher to the value of R25 for each of the visits. For a household of 5 people counting the twice weekly visits this would be in the region of R1000 worth of vouchers for an average month.

The study will not pay for any care that you need for the any illness you may experience during this study. We will share the results of the tests with you so that you will know if you have been infected with any of the illnesses we are studying. In addition we are able to refer you for medical help to your nearest clinic if this is necessary.

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.
- The study has been structured in accordance with the **Declaration of Helsinki** (last updated: October 2013), which deals with the recommendations guiding doctors in biomedical research involving human participants. A copy may be obtained from me should you wish to review it.
- I do not have any financial or personal interests with this organisation that may bias my actions.
- 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.

3. Informed consent form

STUDY TITLE: A Prospective Household observational cohort study of Influenza, Respiratory Syncytial virus and other respiratory pathogens community burden and Transmission dynamics in South Africa (The PHIRST Study)

INFORMED CONSENT:

- I hereby confirm that I have been informed by the study team member, ... *(INSERT NAME OF STUDY TEAM MEMBER)*, about the nature, conduct, benefits and risks of the Household transmission study
- I have also received, read and understood the above written information (Participant Information Leaflet and Informed Consent) regarding the clinical study.
- I am aware that the results of the study, including personal details regarding my 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 computerised system by NICD or on their behalf.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- By signing this form I agree to participate in the following study procedures

	Yes	No
My blood being taken at the beginning of the study and three more times a year		
A rapid HIV test and I wish to get my result		
An HIV test using the blood taken at the beginning of the study and three more times a year and I wish to get my result		
An HIV test using the blood taken at the beginning of the study and three more times a year and I do not wish to get my result		
To give a sputum sample at the beginning of the study and at anytime during the study should I have symptoms of TB		
To have a skin test for tuberculosis infection once at the beginning of the year and once at the end of the year		
To give a urine sample at the beginning of the study		
A throat swab taken once a year		
A swab through my nose to the back of my throat, twice a week for year		
My blood being taken, a tuberculosis test and an HIV test once a year for two years after the first year of the study is complete		

PARTICIPANT:

Printed Name Signature / Mark or Thumbprint Date and Time

I, ... *(INSERT NAME OF STUDY TEAM MEMBER)*, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY team member:

Printed Name	Signature	Date and Time

TRANSLATOR / OTHER PERSON EXPLAINING INFORMED CONSENT……**(DESIGNATION):**

Printed Name	**Signature**	**Date and Time**

WITNESS (If applicable)**:**

Printed Name	Signature	Date and Time

SEPARATE INFORMED CONSENT FOR PARENTS/LEGAL GUARDIANS:

(On behalf of minors under 18 years old)

CONFIRM THAT IF THE PARTICIPANT IS 7-17 YEARS OF AGE THAT THEY HAVE READ THE ASSENT FORM Y/N

- ..... *(INSERT NAME OF STUDY TEAM MEMBER)* has provided me with a copy of the Participant Information Leaflet and Consent regarding the Household transmission study and has fully explained to me the nature, risks, benefits and purpose of the study.
- The study doctor has given me the opportunity to ask any questions concerning the study.
- It has been explained to me that I will be free to withdraw my child from the study at any time, without any disadvantage to future care.
- I have understood everything that has been explained to me and I consent for my child to participate in this clinical study.
- By signing this form I agree to:

	Yes	No
My child having blood being taken at the beginning of the study and three more times a year		
A rapid HIV test and I wish to get my child's result		
An HIV test using the blood taken at the beginning of the study and three more times a year and I wish to get my child's result		
An HIV test using the blood taken at the beginning of the study and three more times a year and I do not wish to get my child's result		
My child to give a sputum sample at the beginning of the study and at anytime during the study should I have symptoms of TB		
My child to have a skin test for tuberculosis infection once at the beginning of the year and once at the end of the year		
My child to give a urine sample at the beginning of the study		
My child to have a throat swab taken once a year		
My child to have a swab through his/her nose to the back of his/her throat, twice a week for year		
My child's blood being taken, a tuberculosis test and an HIV test once a year for two years after the first year of the study is complete		

PARENT/LEGAL GUARDIAN:

Printed Name	Signature / Mark or Thumbprint	Date and Time

PARTICIPANT ASSENT: * (7-17 years of age)

Printed Name	**Signature / Mark or Thumbprint**	**Date and Time**

(* Minors competent to understand must participate as fully as possible in the entire procedure)

STUDY DOCTOR:

Printed Name	Signature	Date and Time

TRANSLATOR / OTHER PERSON EXPLAINING INFORMED CONSENT:……**(DESIGNATION):**

Printed Name	Signature	Date and Time

WITNESS (If applicable)**:**

Printed Name	Signature	Date and Time

VERBAL PARTICIPANT INFORMED CONSENT:

(Applicable when participants cannot read or write or are incapable of giving consent)

- I, the undersigned, ... *(INSERT NAME OF STUDY TEAM MEBER)* have read and have explained fully to the participant, named ..... and/or his/her relative/friend/legal representative, ..., the participant information leaflet.
- The account I have given has explained both the possible risks and benefits of the study as well as the alternative treatments available for his/her illness. The participant and/or his/her relative/friend/legal representative understands these.
- The participant and/or his/her relative/friend/legal representative indicated that he/she understands that the participant will be free to withdraw from the study at any time for any reason and without jeopardising his/her subsequent treatment.
- I have also informed the participant and/or his/her relative/friend/legal representative of the existence of relevant compensation arrangements in case of an injury attributable to the medicine(s) used in the clinical study, to which he/she agrees.
- By putting my finger print on this form I agree to:

	Yes	No
My blood being taken at the beginning of the study and three more times a year		
A rapid HIV test and I wish to get my result		
An HIV test using the blood taken at the beginning of the study and three more times a year and I wish to get my result		
An HIV test using the blood taken at the beginning of the study and three more times a year and I do not wish to get my result		
To give a sputum sample at the beginning of the study and at anytime during the study should I have symptoms of TB		
To have a skin test for tuberculosis infection once at the beginning of the year and once at the end of the year		
To give a urine sample at the beginning of the study		
A throat swab taken once a year		
A swab through my nose to the back of my throat, twice a week for year		
My blood being taken, a tuberculosis test and an HIV test once a year for two years after the first year of the study is complete		

I hereby certify that, the participant and/or his/her relative/friend/legal representative, acting on his/her behalf, has agreed to participate in this study.

PARTICIPANT:

Printed Name	Mark or Thumbprint (if applicable)	Date and Time

STUDY DOCTOR:

Printed Name	Signature	Date and Time

TRANSLATOR / OTHER PERSON EXPLAINING INFORMED CONSENT:……**(DESIGNATION)**

Printed Name	Signature	Date and Time

PARENT/LEGALGUARDIAN/LEGALREPRESENTATIVE/FRIEND:……**(RELATIONSHIP)**

Printed Name	Signature / Mark or Thumbprint	Date and Time

WITNESS:

Printed Name	Signature	Date and Time

Appendix 6: Proximity study collection tools

Figure 1: Proximity sensor device

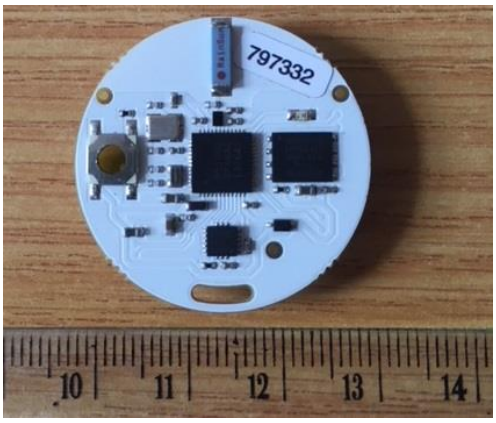


Figure 2: Packaging of proximity sensor

PHIRST Study: Proximity sensor time sheets

Individual ID: _____

Please fill in the date, time and reason for every time you remove the tag.

Monday	D D / M M	Time tag put on in the morning	___ : ___	Time tag taken off in the evening	___ : ___
Monday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Monday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Tuesday	D D / M M	Time tag put on in the morning	___ : ___	Time tag taken off in the evening	___ : ___
Tuesday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Tuesday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Wednesday	D D / M M	Time tag put on in the morning	___ : ___	Time tag taken off in the evening	___ : ___
Wednesday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Wednesday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Thursday	D D / M M	Time tag put on in the morning	___ : ___	Time tag taken off in the evening	___ : ___
Thursday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Thursday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Friday	D D / M M	Time tag put on in the morning	___ : ___	Time tag taken off in the evening	___ : ___
Friday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Friday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Saturday	D D / M M	Time tag put on in the morning	___ : ___	Time tag taken off in the evening	___ : ___
Saturday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Saturday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Sunday	D D / M M	Time tag put on in the morning	___ : ___	Time tag taken off in the evening	___ : ___
Sunday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	
Sunday	D D / M M	Other period when tag was not worn	Taken off: ___ : ___ Put on: ___ : ___	Reason: ☐ Bath ☐ Church ☐ Work ☐ Other (specify)_____	

PHIRST Study: Contact Diary **Individual ID: ______________________** **Date: _______________**

Age (or range*) in years	Gender		Did you touch his/ her skin?		Member of your household?		Total time spent with person during whole day					Where did you have contact? (tick all that apply)					How often do you have contact with this person in general?				
	M	F	Yes	No	Yes	No	Less than 5 mins	5-15 mins	15 mins – 1 hr	1-4 hrs	More than 4 hrs	Home	School	Work	Trans-port	Other	Daily or almost day	Once or twice a week	Once or twice a month	Less than one month	Never met before
10 - ( )	☐	☑	☑	☐	☑	☐	☐	☐	☐	☐	☑	☑	☐	☐	☐	☐	☑	☐	☐	☐	☐
15 - (20)	☑	☐	☐	☑	☐	☑	☐	☑	☐	☐	☐	☐	☐	☐	☑	☐	☐	☐	☐	☐	☑
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
- ( )	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

PHIRST Study: Time use data questionnaire **Individual ID: ______________________** **Date: _______________**

	00:00	01:00	02:00	03:00	04:00	05:00	06:00	07:00	08:00	09:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00	21:00	22:00	23:00
Location of activity																								
Home	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Someone else's home	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Church	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
School	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Place of work	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Shebeen/pub/bar	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Shop	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Other public area	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Personal																								
Sleep/rest	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Bathing/dressing	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Eating	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Personal services	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Household																								
Cooking	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Shopping	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Housekeeping	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Work/Education																								
Paid work	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
School	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Looking for work	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Free time																								
Reading	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Socializing	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Sport	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Other activities																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23