Cohort profile: A Prospective Household cohort study of Influenza, Respiratory syncytial virus and other respiratory pathogens community burden and Transmission dynamics in South Africa, 2016–2018

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Abstract
Purpose: The PHIRST study (Prospective Household cohort study of Influenza, Respiratory Syncytial virus, and other respiratory pathogens community burden and Transmission dynamics in South Africa, 2016–2018)
Transmission dynamics in South Africa) aimed to estimate the community burden of influenza and respiratory syncytial virus (RSV) including the incidence of infection, symptomatic fraction, and to assess household transmission.

Participants: We enrolled 1684 individuals in 327 randomly selected households in a rural and an urban site over three consecutive influenza and two RSV seasons. A new cohort of households was enrolled each year. Participants were sampled with nasopharyngeal swabs twice-weekly during the RSV and influenza seasons of the year of enrolment. Serology samples were collected at enrolment and before and after the influenza season annually.

Findings to Date: There were 122 113 potential individual follow-up visits over the 3 years, and participants were interviewed for 105 783 (87%) of these. Out of 105 683 nasopharyngeal swabs, 1258 (1%) and 1026 (1%) tested positive on polymerase chain reaction (PCR) for influenza viruses and RSV, respectively. Over one third of individuals had PCR-confirmed influenza each year. Overall, there was influenza transmission to 10% of household contacts of an index case.

Future Plans: Future planned analyses include analysis of influenza serology results and RSV burden and transmission. Households enrolled in the PHIRST study during 2016–2018 were eligible for inclusion in a study of SARS-CoV-2 transmission initiated in July 2020. This study uses similar testing frequency to assess the community burden of SARS-CoV-2 infection and the role of asymptomatic infection in virus transmission.

KEYWORDS: burden, cohort profile, influenza, respiratory syncytial virus, South Africa, transmission

1 | INTRODUCTION

In 2015, lower respiratory tract infections caused an estimated 2.7 million deaths globally. Among children aged <5 years, the highest mortality rates are in sub-Saharan Africa where the HIV-epidemic has increased morbidity of severe pneumonia. Influenza, respiratory syncytial virus (RSV), pertussis and pneumococcus are among the leading causes of pneumonia globally.

Approximately 30% of influenza and RSV transmission is estimated to occur within households. Data on community burden and transmission of respiratory pathogens are important to guide vaccination strategies such as reduced pneumococcal conjugate vaccine dose schedules, optimal timing of booster doses and vaccinating community transmitters. Illness episodes in the community may be associated with substantial community impact including absenteeism from school or work and loss of income.

The PHIRST study aimed to estimate the community burden of influenza and RSV (including the incidence of infection and symptomatic fraction) and to assess household transmission of influenza and RSV. Secondary objectives included describing the community burden and transmission of Streptococcus pneumoniae and Bordetella pertussis, estimating the impact of HIV infection and age on disease burden, estimating rates of tuberculosis infection and transmission and investigating the interaction between respiratory viruses and bacteria. We also aimed to evaluate the role of asymptomatic influenza and RSV infection in household transmission.

2 | COHORT DESCRIPTION

2.1 | Study population and household eligibility criteria

A prospective cohort study of randomly selected households in South Africa was conducted in a rural and an urban site, each with established surveillance for pneumonia and influenza-like illness. The rural site in Mpumalanga Province (Agincourt sub-district) is part of a health and socio-demographic surveillance system (HDSS), including approximately 116 000 people in 31 contiguous villages. Approximately 30% of the population are former Mozambicans who migrated there in the 1980s. The urban site, Jouberton Township in Klerksdorp, is part of the municipality of Matlosana in North West Province, with a population of approximately 180 000 people. Mining of gold and uranium, although declining, remains a primary driver of the local economy.

We aimed to enroll approximately 1500 individuals (approximately 500 individuals per year) over three consecutive influenza and RSV seasons to allow the estimation of 20% risk of infection and a
10% risk of illness with 95% confidence intervals (CIs) and 5% absolute precision. Assuming an average household size of five individuals and a loss to follow-up of 10%, based in previous studies, we aimed to enroll approximately 55 households with >2 household members per site each year with at least 50% having at least one child aged <5 years in the house.

In rural Agincourt, each year, we purposively selected two different villages within the HDSS. Within these villages, we randomly selected households with >2 members from an enumerated list obtained from the HDSS. In urban Jouberton township, we generated a list of 450 random global positioning system (GPS) coordinates located within a polygon defining the township boundaries using Google Earth. Study staff navigated to the location represented by the coordinates and selected the nearest house. If there was no dwelling within 30 m, the coordinates were discarded. Households were approached consecutively until the desired sample size was reached. If a household withdrew during January–April of each year, it was replaced by a new household, selected consecutively, for the remaining follow-up period.

At each household with >2 members, study staff requested permission from the head of household to inform members about the study purpose, risks and benefits. If the head of household was a minor or unavailable after three attempts, the household was excluded. Written informed consent was required to participate in the study from all household members aged ≥18 years; assent was required from children aged 7–17 years, and consent from a parent/guardian of children aged <18 years. We included households where ≥80% of household members consented.

### 2.2 Frequency of follow-up

Each year, a new cohort of individuals was enrolled and following enrolment, all participants and households had a period of active twice-weekly follow-up for 6–10 months. During the year of active follow-up, participants received twice-weekly scheduled follow-up visits (once during Monday–Wednesday and again during Thursday–Saturday) to the household during May–October in 2016 (due to delayed start in the first study year), and January–October in 2017 and 2018 (Figure 2) for the collection of symptom data and nasopharyngeal swabs.

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**FIGURE 1** Location of rural (Agincourt) and urban (Jouberton) study sites in South Africa

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**Strengths and limitations of this study**

- PHIRST was conducted in urban and rural African settings with high HIV prevalence, allowing assessment of the effect of HIV on community burden and transmission dynamics of respiratory pathogens.
- Households were selected randomly to provide a representative sample of the community. Twice-weekly sampling from each cohort of individuals for 6–10 months irrespective of symptoms allows estimation of community burden, household secondary infection risk, and serial interval including asymptomatic or paucisymptomatic episodes.
- Polymerase chain reaction testing of >100,000 nasopharyngeal swab samples for multiple pathogens (influenza, respiratory syncytial virus, pertussis and Streptococcus pneumonia) allows detailed examination of disease burden and transmission and pathogen interactions.
- PHIRST was not powered to assess severe outcomes (i.e. hospitalisation and death).
- We only examined four pathogens, but other microorganisms may be important. Samples have been stored which could allow us to implement broader multi-pathogen testing in the future.
Household surveys were conducted once during the follow-up period to evaluate household income, housing quality, oropharyngeal carriage of meningococcus, Corynebacterium diphtheriae and Group A streptococcus and presence of S. pneumoniae DNA in blood by polymerase chain reaction (PCR). Serum samples were also collected at enrolment, before the influenza season, and at the end of the active follow-up period. In addition, sera were also collected from the 2016 and 2017 cohorts in subsequent years (Figures 2 and S1). Environmental assessments including respirable particulate matter and temperature were undertaken twice a year (summer and winter) (Table 1).

2.3 Baseline, symptom and health contact data

Data were collected using REDCap (Research Electronic Data Capture)\textsuperscript{19}. Following enrolment, a baseline questionnaire was completed for each household including information on household members, relationships, sleeping arrangements and housing. For each individual, we collected baseline information on demographics, underlying illnesses, vaccinations and occupation. During the twice-weekly follow-up phase, at each visit, for each participant, a questionnaire assessing presence of symptoms, absenteeism and health system contacts was completed and nasopharyngeal (NP) swabs were collected regardless of the presence or absence of symptoms (Table 1). Field workers were trained in the identification of respiratory signs and symptoms at the beginning of each year. Symptoms assessed at twice-weekly visits included fever (self-reported or measured tympanic temperature $\geq 38^\circ C$), cough, difficulty breathing, sore throat, nasal congestion, chest pain, muscle aches, headache, vomiting or diarrhoea. In 2017 and 2018, procedures for collection of symptom data were improved following review of symptom data from 2016. These improvements included simplification of the symptom collection form and monthly training of fieldworkers on signs and symptoms identification and recording and frequent emphasis of the importance of symptom reporting to participants.

2.4 Laboratory measurements

NP samples were collected using flexible nasopharyngeal nylon flocked swabs (PrimeSwab\textsuperscript{™}, Longhorn Vaccines & Diagnostics, San Antonio, Texas, USA), placed in PrimeStore\textsuperscript{®} Molecular Transport Medium (MTM) (Longhorn Vaccines & Diagnostics, San Antonio, Texas, USA) and transported at 2–8°C to the National Institute for Communicable Diseases (NICD) in Johannesburg, where the samples were tested, aliquoted and stored at 2–8°C before freezing at −70°C. Nucleic acids were extracted from PrimeStore\textsuperscript{®} MTM using the Roche MagNA Pure 96 instrument (Roche, Mannheim, Germany) according to the manufacturer’s instructions. NP samples were tested for RSV and influenza A and B viruses by real-time PCR (RT-PCR) using the FTD Flu/RSV detection assay (Fast Track Diagnostics, Luxembourg). Influenza A positive samples were subtyped using the Centers for Disease Control and Prevention (CDC) influenza A (H1/H3/H1pdm09) subtyping kit and influenza B lineage was determined using the CDC B/Yamagata-B/Victoria lineage typing kit (available through International Reagent Resource Program; http://www.internationalreagentresource.org) using SuperScript\textsuperscript{™} III One-Step RT-PCR System with Platinum\textsuperscript{™} Taq DNA Polymerase.
| Type of data                      | Details                                                                 | Frequency                                      |
|----------------------------------|--------------------------------------------------------------------------|------------------------------------------------|
| Data collection tool             | Data collected                                                           |                                                 |
| **Enrolment form**               | Household characteristics (number of individuals and rooms, water source, electricity, smoking in house, cooking in house and relationships) | Enrolment                                      |
| **Case intake form**             | Age, sex, education, occupation, daily contacts, smoking, alcohol, hand washing, past medical history, documented vaccinations, HIV status and treatment | Enrolment                                      |
| **Household income form**        | Total household income category                                          | Annual survey                                  |
| **Housing quality checklist**    | Type and condition of dwelling, roofing, ceiling, walls, windows, floors, temperature control, security, type of toilet, dampness, cooking and space heating fuels, ventilation and pets | Annual survey                                  |
| **Environmental assessment**     | Respirable particulate matter (PM$_2.5$), carbon dioxide, temperature and relative humidity | Bi-annual survey (aim for 1 in winter and 1 in summer) |
| **TB form**                      | Cough, night sweats or weight loss for >2 weeks                          | Monthly TB visits                              |
| **Follow-up visit form**         | Symptoms (cough, fever, sore throat, runny nose, headache, body pains, difficulty breathing, chest pain, vomiting and diarrhoea), medication, outpatient visits, pharmacist, traditional healer, hospitalisation and death | Twice-weekly follow-up visits                  |
| **Proximity survey**             | Number and duration of contact events (≤1.5 m apart) between participants wearing sensors | Proximity surveys (4 in 2018)                  |
| **Contact diary**                | Age, gender and household member of each individual participant had contact with as well as type of contact, where contact took place and duration of contact | One day in August – October period 2018        |
| **Time use survey**              | Activity and location for each hour of the day                           | One day in August – October period 2018        |
| **Costing survey**               | Out of pocket costs related to illness among individuals reporting symptoms | October–November 2018                          |

| Specimens                        | Tests performed                                                         | Frequency                                      |
|----------------------------------|-------------------------------------------------------------------------|------------------------------------------------|
| Nasopharyngeal flocked swabs     | PCR for influenza, RSV, B. pertussis, and S. pneumoniae                 | Enrolment and twice-weekly follow-up visits    |
| Clotted blood                    | Serology for influenza, RSV, pertussis HIV testing for consenting patients | Enrolment and at blood draws (2–3 times per year) |
| EDTA blood                       | CD4+ T cell count and HIV viral load for HIV-infected individuals        | Enrolment and end of year                      |
| Blood drop                       | Rapid HIV test for consenting patients                                   | Enrolment                                      |
| Urine                            | Quantitative cotinine                                                    | Enrolment                                      |
| EDTA blood                       | Pneumococcal lytA PCR                                                    | Annual survey                                  |
| Oropharyngeal flocked swabs      | C. diptheriae, N. meningitidis and Streptococcus pyogenes                | Annual survey                                  |
| Expectorated sputum              | M. tuberculosis culture, B. pertussis PCR                                 | Monthly TB visits if symptoms of tuberculosis  |

Abbreviations: EDTA, ethylenediamine tetraacetic acid; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; TB, tuberculosis.
Participants were considered HIV infected if they had one of the following during the follow-up period: two positive rapid HIV tests, evidence of a positive HIV laboratory result or evidence of antiretroviral treatment. Participants were considered HIV uninfected if they had a documented negative HIV test result during the study. A documented HIV negative status for the mother was considered confirmation of HIV negative status for a child aged <10 years. Infants were defined as HIV exposed but uninfected if they were HIV uninfected, but the mother was HIV infected. HIV infection was confirmed by PCR in children aged <18 months. For all HIV-infected individuals, specimens were collected for CD4+ T cell and quantitative HIV viral load testing. Patients newly diagnosed with HIV were referred to the local clinic for assessment and initiation of antiretroviral treatment.

2.5 | Environmental assessment

Environmental monitoring was conducted in a convenience subset of 150 households for one week each year during summer and winter. Respirable particulate matter <4-μm diameter (PM<sub>4</sub>) concentrations were measured indoors using a stationary photometric monitor (DustTrak II Model 8530, TSI Incorporated, Shoreview, MN, USA) and gravimetric filter sampling to measure the quantity of airborne particulate matter. During the same period, one member of each household carried a personal exposure monitoring device (SidePak AM510, TSI Incorporated, Shoreview, MN, USA) throughout the day-time. Indoor carbon dioxide and ambient PM<sub>4</sub> (ES-642, MetOne Instruments, Inc, Grants Pass, OR, USA) levels were measured during 2018. Thermochron® iButton sensors (Maxim Integrated, San Jose, California, USA) were located in the indoor (all households) and ambient (subset) environments to measure temperature (Model DS1921G-F5) and relative humidity (Model RS1923F5 in 2018) concurrently with the air pollution monitoring in 2016 and continuously during 2017–2018.

2.6 | Housing quality survey

Information on housing type, construction, materials and condition, water sources, water security and water storage, fuel use and expenditure for cooking, space and water heating, waste removal services, visible dampness and smoking practices was collected annually for all households.

2.7 | Proximity and contact study

In 2018, four surveys of household contact using proximity monitors (http://www.sociopatterns.org) were conducted to capture information on intra-household contact patterns for three seasons (summer, autumn and winter). To measure contacts of participants of the study outside the home, participants were interviewed by field workers to complete a contact diary and time-use questionnaire for one day between August and October 2018.

2.8 | Costing survey

We surveyed all symptomatic household members during August–October 2018 to assess cost of medically attended and non-medically attended illness episodes.
3 | FINDINGS TO DATE

3.1 | Household and individual characteristics

From 2016 to 2018 at both sites combined, 881 households were approached: for 409 (46%) households, the head of household agreed to participate and 327 (78%) of these were included in the final analysis (Figures 3a and 2b). There were 1861 individuals residing in the 327 included households, of whom, 1684 (90%) consented and were included in the final analysis. Reasons for non-inclusion are shown in Figure 3a.b. A higher percentage of approached houses were included at the rural site (159/267, 60%) compared to the urban site (168/614, 27%). This is because rural site houses with >2 members were pre-selected from the HDSS database and a higher proportion of household members consented to participate in the study among eligible households (209/252, 83% vs. 200/326, 61%, p < .001).

At the rural site, characteristics of included households were similar to those of households within the HDSS that were not included (Table 2a). However, when compared to individuals from the HDSS who were not included, included individuals were less likely to be aged 15–44 years, male, employed or have completed secondary education. This likely reflects migrant worker patterns and the fact that within included households, males and individuals aged 15–44 years were less likely to participate in the study (Table S2). At the urban site, characteristics of households and individuals included in PHIRST were compared to those completing follow-up (Table S3).

Among 327 households included in the study, the median household size was five individuals (interquartile range 3–10) and 160 (49%) reported crowding (>2 people per sleeping room) (Table 3). Among 1684 study participants, 16% were aged <5 years, 32% aged 5–14 years and 60% were female. Compared to the urban site, households in the rural site were more likely to have a child aged <5 years in the house and to use wood as fuel for cooking, and less likely to report smoking in the house or to have a handwashing place with water. Compared to the urban site, individuals in the rural site were more likely to be aged <15 years, female, unemployed and less likely to drink alcohol or smoke.

Among 1684 included individuals, 1605 (95%) were present at end of the twice-weekly follow-up phase. Of 79 lost to follow-up, 53 (67%) left the study sites, 21 (27%) withdrew and 5 (6%) died (Table 4). Just over half of the individuals in the 2016 and 2017 cohorts completed three serology blood draws in the years after completion of the swabbing phase. Individuals lost to follow-up were more likely to be aged 15–44 years, possibly due to economic migration, compared to those completing follow-up (Table S3).

3.2 | Samples collected and symptoms

There were 122 113 potential individual follow-up visits over the 3 years, and participants were interviewed for 105 783 (87%) of these. In 2017–2018, there were 94 786 potential visits, of which participants were interviewed for 81 943 (86%) and 81 928 (>99%) had available data on symptoms. At least one symptom was reported for 8% (6692) of visits overall. At least one symptom over the follow-up period in 2017–2018 was reported by 89% (1012/1142) of individuals and was more commonly reported among children aged <5 years (97%, 180/185) compared to older individuals (5–18 years 87%, 404/466; 19–65 years 87%, 390/450; >65 years 93%, 38/41, p < .001 chi-squared test). The commonest symptoms reported were cough (76%, 863/1142) and runny nose (74%, 841/1142). The rate of clinic visits in 2017–2018 for acute complaints was 1.3 per 100 person weeks of follow-up (n = 520) (2.1 in <5 years, 0.9 in 5–18 years, 1.3 in 19–65 years and 2.0 in >65 years) and of hospitalisations was 0.05 per 100 person weeks of follow-up (n = 36) (0.09 in <5 years, 0.05 in 5–18 years, 0.10 in 19–65 years and 0.3 in >65 years).

From May 2016 to December 2018, a total of 105 683 nasopharyngeal swabs, 4217 clotted blood samples, 1442 whole blood samples, 1567 urine samples and 741 sputum samples were collected. Out of 105 683 nasopharyngeal swabs from follow-up visits collected and tested from 1684 participants, in 327 households, 1258 (1%), 273 (<1%), 38 829 (37%) tested positive on PCR for influenza viruses, RSV, pertussis and pneumococcus, respectively.

Analysis of data from 2017–2018 on influenza identified high attack rates with 79% of households had at least one individual testing influenza positive each season and 37% of household members infected at least once with PCR-confirmed influenza each year. Incidence was similar in the urban and rural site. Repeat influenza infections within the same season were identified in 17% of individuals experiencing at least one influenza infection and were more common in children. The incidence of PCR-confirmed influenza infection was highest among children aged <5 years and decreased with increasing age. Overall, 56% of infections were associated with ≥1 symptom and 35% of these had fever and cough. The proportion of symptomatic infections was higher in children aged <5 years and decreased with increasing age. Overall, 56% of infections were associated with ≥1 symptom and 35% of these had fever and cough. The proportion of symptomatic infections was higher in children aged <5 years (74% in this age group vs. 39% in those aged 19–44 years). Overall, there was influenza transmission to 10% of household contacts of an index case. Transmission was highest among children and individuals with ≥2 symptoms (17%); however, asymptomatic individuals did transmit influenza to 6% of household contacts.

3.3 | Future plans

Households enrolled in the PHIRST study during 2016–2018 were eligible for inclusion in a study of SARS-CoV-2 transmission initiated in July 2020. This study uses similar testing frequency and household selection methods to assess the community burden of SARS-CoV-2 infection and the role of asymptomatic infection in virus transmission.
4.1 Strengths

PHIRST was conducted in urban and rural African settings. Situation in a high HIV prevalence setting with high study uptake of HIV testing (97%) allows assessment of the effect of HIV on community burden and transmission dynamics of respiratory pathogens. Households were selected randomly to provide a representative sample of the community. Sampling from individuals irrespective of symptoms allows estimation of community burden, household secondary infection risk and serial interval including asymptomatic or paucisymptomatic episodes. It also allows the estimation of the proportion of transmission from asymptomatic individuals. PHIRST utilised multiple laboratory-confirmed infection endpoints including PCR and serology, which provide additional data on the community burden of these pathogens and allows evaluation of the correlation between pathogen detection and serological response in individuals of different age and HIV-infection status. Laboratory confirmation of multiple respiratory pathogens simultaneously allows study of
the effect of respiratory co-infections on disease severity and transmissibility and the interaction between different pathogens. Our twice-weekly sampling strategy was unlikely to miss many episodes of infection and allowed accurate ascertainment of first and subsequent infections from the same or different pathogens in the household. Our long period of active follow-up for each cohort allowed us to describe burden and transmission of infection within described seasons.

4.2 Limitations

This study was not powered to assess severe outcomes (i.e., hospitalisation and death). Repeated assessment of symptoms at twice-weekly visits over an extended period may lead to possible fatigue and under-reporting by participants. High rates of migration and movement in communities under study affected follow-up rates. The study was intensive, and nasopharyngeal swabs are

| Characteristic | Included n/N (%) or number (range) | Not included n/N (%) or number (range) | p |
|----------------|-----------------------------------|----------------------------------------|---|
| Household level |                                   |                                        |   |
| Mean number (range) members in household | 4 (1–20) | 4 (1–15) | .479 |
| Modern house (vs. traditional) | 136/136 (100) | 16 738/16 785 (99.7) | .587 |
| Toilet site |                                   |                                        |   |
| In house | 2/136 (1) | 268/16 782 (2) | .658 |
| In yard | 121/136 (89) | 15 249/16 782 (91) |   |
| Other | 13/136 (10) | 1265/16 782 (8) |   |
| Type of toilet |                                   |                                        |   |
| Modern | 3/136 (2) | 245/16 780 (1) | .151 |
| VIP | 7/136 (5) | 1841/16 780 (11) |   |
| Pit latrine | 116/136 (85) | 13 676/16 780 (82) |   |
| Other | 10/136 (7) | 1018/16 780 (6) |   |
| Source of energy for cooking |                                   |                                        |   |
| Electricity | 58/136 (43) | 8929/16 781 (53) | .050 |
| Wood | 77/136 (57) | 7759/16 781 (46) |   |
| Gas/Paraffin (kerosene)/Other | 1/136 (1) | 93/16 781 (1) |   |

Note: Only households and individuals with data available in the Agincourt HDSS included, for individual level analysis only permanent (non-migrant) household members included. Data are mean (range) or n/N (%).

Abbreviation: VIP, ventilated improved pit latrine.

aThe highest level an individual has attained at the time of observation.

bIndividuals aged ≥17 years.
uncomfortable for participants, which may have resulted in fewer participants consenting and reduced follow-up rates. Quality of specimen collection may have varied; however, >99% of samples tested positive for the presence of human DNA. We only examined four pathogens, but other micro-organisms may be important. Samples have been stored which could allow us to implement broader multi-pathogen testing in the future. We did not test staff members for the presence of organisms under study, because a previous similar study in which staff were sampled weekly did not identify any influenza or RSV infections among field staff.33

**TABLE 2B** Characteristics of included participants at the urban site (Jouberton) during 2016–2018 from PHIRST database and characteristics of the general population from the 2011 Census29

| Characteristic                              | Enrolled n/N (%) or number (range) | General population\(^a\) n/N (%) or number (range) |
|---------------------------------------------|-------------------------------------|-------------------------------------------------|
| **Household level**                         |                                     |                                                 |
| Mean number (range) members in household    | 5 (3–14)                            | 4 (3–5)\(^b\)                                  |
| Mean number (range) rooms in household      | 5 (2–11)                            |                                                 |
| **Monthly household income**                |                                     |                                                 |
| ≤R800 ($≤54)                                | 18/167 (11)                         | 7119/32 136 (22)                               |
| R801–R1600 ($55–$108)                      | 35/167 (21)                         | 2835/32 136 (9)                                |
| R1601–R3200 ($109–$116)                    | 49/167 (29)                         | 6507/32 136 (20)                               |
| R3201–R6400 ($117–$232)                    | 34/167 (20)                         | 7251/32 136 (23)                               |
| R6401–R12800 ($233–$464)                   | 9/167 (5)                           | 4851/32 136 (15)                               |
| >R12800 (> $464)                           | 5/167 (3)                           | 3558/32 136 (11)                               |
| Did not disclose                            | 17/167 (10)                         | 15/32 136 (0)                                  |
| **Type of dwelling**                        |                                     |                                                 |
| Formal house                                | 158/167 (95)                        | 23 379/32 130 (73)                             |
| Informal dwelling                           | 6/167 (4)                           | 6777/32 130 (21)                               |
| Formal dwelling in backyard                 | 0/167 (0)                           | 882/32 130 (3)                                 |
| Flat                                        | 0/167 (0)                           | 225/32 130 (1)                                 |
| Traditional dwelling                        | 0/167 (0)                           | 93/32 130 (0)                                  |
| Other                                       | 3/167 (2)                           | 774/32 130 (2)                                 |
| **Individual level**                        |                                     |                                                 |
| Age group (years)                           |                                     |                                                 |
| <1                                          | 27/858 (3)                          | 2664/111 936 (2)                               |
| 1–4                                         | 89/858 (10)                         | 10 590/111 936 (9)                             |
| 5–14                                       | 238/858 (28)                        | 21 051/111 936 (19)                            |
| 15–44                                      | 334/858 (39)                        | 54 297/111 936 (49)                            |
| 45–64                                      | 124/858 (14)                        | 18 684/111 936 (17)                            |
| 65+                                        | 46/858 (5)                          | 4650/111 936 (4)                               |
| Female sex                                  | 487/859 (57)                        | 57 129/111 939 (51)                            |
| Level of education\(^c\)                   |                                     |                                                 |
| No schooling                                | 26/443 (6)                          | 7872/98 442 (8)                                |
| Primary schooling                           | 116/443 (26)                        | 34 767/98 442 (35)                             |
| Some secondary                              | 207/443 (47)                        | 35 295/98 442 (36)                             |
| Secondary completed                         | 81/443 (18)                         | 18 015/98 442 (18)                             |
| Post-secondary                              | 13/443 (3)                          | 2493/98 442 (3)                                |
| Currently working\(^d\)                    | 154/444 (35)                        | 24 642/72 981 (34)                             |

\(^a\)Data on general population obtained from census 2011\(^29\) unless otherwise indicated. Data are median (IQR) or n/N (%).

\(^b\)Source: Wong et al.\(^18\)

\(^c\)>18 years PHIRST, >5 years in census.

\(^d\)>18 years PHIRST, >15 years in census.
TABLE 3 Baseline characteristics of households and participants included in the final cohort by site, a rural and an urban site in South Africa, PHIRST study, 2016–2018

| Characteristic                                      | Overall n (%) or median (IQR) | Rural n (%) or median (IQR) | Urban n (%) or median (IQR) | OR (95% CI) | p     |
|-----------------------------------------------------|-------------------------------|-----------------------------|----------------------------|-------------|-------|
| **Household level characteristics**                 |                               |                             |                            |             |       |
| N                                                   | 327                           | 159                         | 168                        |             |       |
| Year                                                |                               |                             |                            |             |       |
| 2016                                                | 100 (31)                      | 50 (31)                     | 50 (30)                    | Reference   | .932  |
| 2017                                                | 109 (33)                      | 53 (33)                     | 56 (33)                    | 1.1 (0.6–1.8)|       |
| 2018                                                | 118 (36)                      | 56 (35)                     | 62 (37)                    | 1.1 (0.6–1.9)|       |
| **Number of household members**                     |                               |                             |                            |             |       |
| 3–5                                                 | 196 (60)                      | 89 (56)                     | 107 (64)                   | Reference   | .341  |
| 6–10                                                | 119 (36)                      | 63 (40)                     | 56 (33)                    | 0.7 (0.5–1.2)|       |
| >10                                                 | 12 (4)                        | 7 (4)                       | 5 (3)                      | 0.6 (0.2–1.9)|       |
| **Number of household members**                     | 5 (3–10)                      | 5 (3–11)                    | 5 (3–10)                   | Not estimated| .442  |
| **Number of rooms**                                 | 5 (2–9)                       | 5 (1–10)                    | 5 (2–8)                    | Not estimated| .453  |
| **Number of rooms for sleeping**                    | 2 (1–4)                       | 3 (1–4)                     | 2 (1–4)                    | Not estimated| .256  |
| **Crowding (>2 people per sleeping room)**          | 160 (49)                      | 83 (52)                     | 77 (46)                    | 0.8 (0.5–1.2)| .250  |
| **Child aged <5 years in house**                    | 225 (69)                      | 141 (89)                    | 84 (50)                    | 0.1 (0.1–0.2)| <.001 |
| **HIV-infected household member**                   | 172 (53)                      | 80 (50)                     | 92 (55)                    | 1.2 (0.8–1.8)| .421  |
| **Cigarette smoke in house**                        | 71 (22)                       | 18 (11)                     | 53 (32)                    | 3.6 (2.0–6.5)| <.001 |
| **Main water source tap inside (vs. tap outside)**  | 154 (47)                      | 71 (45)                     | 83 (49)                    | 0.8 (0.5–1.3)| .390  |
| **Handwashing place with water in house**           | 264 (81)                      | 100 (63)                    | 164 (98)                   | 24.2 (8.6–68.3) | <.001 |
| **Main fuel for cooking**                           |                               |                             |                            |             |       |
| **Electricity**                                     | 244 (75)                      | 89 (56)                     | 155 (93)                   | 121.9 (16.6–892.8) | <.001 |
| **Wood**                                            | 71 (22)                       | 70 (44)                     | 1 (1)                      | Reference   |       |
| **Paraffin (kerosene)/gas/other**                   | 10 (3)                        | 0 (0)                       | 10 (6)                     | Not estimated|       |
| **Monthly household income**                        |                               |                             |                            |             |       |
| ≤R800 (<$54)                                       | 39 (12)                       | 21 (14)                     | 18 (11)                    | Reference   | .807  |
| R801–R1600 ($55–$108)                               | 75 (24)                       | 36 (23)                     | 39 (24)                    | 1.3 (0.6–2.7)|       |
| R1601–R3200 ($109–$116)                             | 109 (34)                      | 56 (36)                     | 53 (33)                    | 1.1 (0.5–2.3)|       |
| R3201–R6400 ($117–$232)                             | 70 (22)                       | 32 (21)                     | 38 (23)                    | 1.4 (0.6–3.0)|       |
| R6401–R12800 ($233–$464)                            | 18 (6)                        | 8 (5)                       | 10 (6)                     | 1.5 (0.5–4.5)|       |
| >R12800 (> $464)                                    | 7 (2)                         | 2 (1)                       | 5 (3)                      | 0.9 (0.5–16.8)|       |
| **Individual level characteristics**                |                               |                             |                            |             |       |
| N                                                   | 1684                          | 849                         | 835                        |             |       |
| Age group (years)                                   |                               |                             |                            |             |       |
| <1                                                  | 36 (2)                        | 15 (2)                       | 21 (3)                     | 2.5 (1.2–5.1)| <.001 |
| 1–4                                                 | 243 (14)                      | 156 (18)                    | 87 (10)                    | Reference   |       |
| 5–14                                                | 547 (32)                      | 309 (36)                    | 238 (29)                   | 1.4 (1.1–1.9)|       |
| 15–44                                               | 590 (35)                      | 265 (31)                    | 325 (39)                   | 2.1 (1.6–3.0)|       |
| 45–64                                               | 195 (12)                      | 74 (9)                      | 121 (14)                   | 2.9 (2.0–4.3)|       |
| 65+                                                 | 73 (4)                        | 30 (4)                      | 43 (5)                     | 2.6 (1.5–4.4)|       |
| **Female sex**                                      | 1009 (60)                     | 533 (63)                    | 476 (57)                   | 0.8 (0.6–0.9)| .016  |
| **Year**                                            |                               |                             |                            |             |       |
| 2016                                                | 542 (32)                      | 280 (33)                    | 262 (31)                   | Reference   | .768  |
| 2017                                                | 577 (34)                      | 289 (34)                    | 288 (34)                   | 1.1 (0.8–1.3)|       |
| 2018                                                | 565 (34)                      | 280 (33)                    | 285 (34)                   | 1.1 (0.9–1.4)|       |

(Continues)
| Characteristic | Overall n (%) or median (IQR) | Rural n (%) or median (IQR) | Urban n (%) or median (IQR) | OR (95% CI) | p  |
|---------------|-----------------------------|-----------------------------|-----------------------------|-------------|----|
| **Level of education**<sup>b</sup> | | | | | |
| No schooling  | 90 (12) | 65 (22) | 25 (6) | Reference | <.001 |
| Primary schooling | 158 (22) | 60 (20) | 98 (23) | 4.2 (2.4–7.5) | |
| Some secondary | 285 (39) | 85 (28) | 200 (47) | 6.1 (3.6–10.3) | |
| Secondary completed | 174 (24) | 87 (29) | 87 (21) | 2.6 (1.5–4.5) | |
| Post-secondary | 19 (3) | 5 (2) | 14 (3) | 7.3 (2.4–22.3) | |
| **Employment**<sup>b</sup> | | | | | |
| Unemployed | 400 (55) | 182 (60) | 218 (51) | Reference | .014 |
| Employed | 267 (37) | 88 (29) | 179 (42) | 1.7 (1.2–2.3) | |
| Student | 59 (8) | 32 (11) | 27 (6) | 0.7 (0.9–1.5) | |
| Reported alcohol use<sup>c</sup> | 337 (39) | 57 (15) | 280 (57) | 7.3 (5.2–10.2) | <.001 |
| Reported current cigarette smoking<sup>c</sup> | 139 (16) | 22 (6) | 117 (24) | 4.9 (3.0–7.9) | <.001 |
| Reported current snuff smoking<sup>c</sup> | 97 (11) | 5 (1) | 92 (19) | 17.0 (6.8–42.1) | <.001 |
| Reported current any smoking<sup>c</sup> | 242 (28) | 28 (8) | 214 (44) | 9.5 (6.2–14.4) | <.001 |
| Cigarette smoke inside house<sup>e</sup> | 99 (41) | 7 (25) | 92 (43) | 2.3 (0.9–5.5) | .048 |
| **Urine cotinine (all ages)**<sup>b</sup> | | | | | |
| Negative | 649 (42) | 530 (68) | 119 (16) | Reference | <.001 |
| Passive exposure | 660 (43) | 222 (28) | 438 (57) | 8.8 (6.8–11.4) | |
| Active smoking | 241 (16) | 31 (4) | 210 (27) | 30.2 (19.7–46.2) | |
| Unknown | 134 | 66 | 68 | Not included | |
| **HIV status**<sup>e</sup> | | | | | |
| Uninfected | 1379 (85) | 715 (86) | 664 (83) | Reference | .158 |
| Infected | 249 (15) | 117 (14) | 132 (17) | 1.2 (0.9–1.6) | |
| Unknown | 56 | 17 | 39 | Not included | |
| Previous tuberculosis | 88 (5) | 15 (2) | 73 (9) | 5.3 (3.0–9.4) | <.001 |
| Current tuberculosis | 24 (1) | 3 (<1) | 21 (3) | 7.3 (2.2–24.5) | <.001 |
| Other underlying illness<sup>f</sup> | 50 (3) | 5 (1) | 45 (5) | 9.6 (3.8–24.3) | <.001 |
| Influenza vaccination current year | 2 (<1) | 1 (<1) | 1 (<1) | 1.0 (0.1–16.2) | .990 |
| Pneumococcal vaccine up to date for age<sup>g</sup> | | | | | |
| Yes | 221 (81) | 137 (81) | 84 (80) | 0.2 (0.1–1.3) | .182 |
| No | 7 (3) | 2 (1) | 5 (5) | Reference | |
| No data | 46 (17) | 30 (18) | 16 (15) | 0.2 (0.1–1.2) | |
| DTaP-IPV/Hib vaccine up to date for age<sup>g</sup> | | | | | |
| Yes | 222 (81) | 135 (80) | 87 (83) | 0.9 (0.2–3.9) | .735 |
| No | 7 (3) | 4 (2) | 3 (3) | Reference | |
| No data | 45 (16) | 30 (18) | 15 (14) | 0.7 (0.1–3.4) | |

<sup>a</sup>Data available for 318 households, 155 rural and 163 urban.

<sup>b</sup>Individuals aged ≥18 years N = 726, 302 at rural and 424 at urban site.

<sup>c</sup>Self-reported, individuals aged ≥15 years N = 858, 369 at rural and 489 at urban site.

<sup>d</sup>Amongst those reporting any current smoking.

<sup>e</sup>% and p value among individuals with known status.

<sup>f</sup>Self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes.

<sup>g</sup>Individuals aged <5 years N = 274, 169 at rural site and 105 at urban site, 229 with available vaccination data, 139 at the rural site and 90 at the urban site.
**TABLE 4** Follow-up rates by site and year among 1684 individuals included in the analysis of the PHIRST study, South Africa, 2016–2018

| Characteristic                                         | Overall N (%) | Rural N (%) | Urban N (%) | 2016 N (%) | 2017 N (%) | 2018 N (%) |
|--------------------------------------------------------|---------------|-------------|-------------|------------|------------|------------|
| **Twice-weekly follow-up phase**                       |               |             |             |            |            |            |
| Individuals                                             |               |             |             |            |            |            |
| Total included in analysis                              | 1684          | 849         | 835         | 542        | 577        | 565        |
| Initially enrolled                                     | 1555 (92)     | 790 (93)    | 765 (92)    | 505 (93)   | 551 (95)   | 499 (88)   |
| Late enrolments for replacement or in-migration to      | 129 (8)       | 59 (7)      | 70 (8)      | 37 (7)     | 26 (5)     | 66 (12)    |
| household                                              |               |             |             |            |            |            |
| Lost to follow-up                                      | 79 (5)        | 32 (4)      | 47 (6)      | 21 (4)     | 31 (5)     | 27 (5)     |
| Withdrawal n (%)                                       | 21 (27)       | 13 (41)     | 8 (17)      | 9 (43)     | 6 (19)     | 6 (22)     |
| Left study site n (%)                                   | 53 (67)       | 17 (53)     | 36 (77)     | 12 (57)    | 21 (68)    | 20 (74)    |
| Death n (%)                                            | 5 (6)         | 2 (6)       | 3 (6)       | 0 (0)      | 4 (13)     | 1 (4)      |
| Completed follow-up                                     | 1605 (95)     | 817 (96)    | 788 (94)    | 521 (96)   | 546 (95)   | 538 (95)   |
| Households                                              |               |             |             |            |            |            |
| Total included                                         | 327           | 159         | 168         | 100        | 109        | 118        |
| Initially enrolled                                     | 308 (94)      | 148 (93)    | 160 (95)    | 96 (96)    | 105 (96)   | 107 (91)   |
| Late enrolments for replacement or in-migration to      | 19 (6)        | 11 (7)      | 8 (5)       | 4 (4)      | 4 (4)      | 11 (9)     |
| household                                              |               |             |             |            |            |            |
| Lost to follow-up                                      | 6 (2)         | 1 (1)       | 5 (3)       | 1 (1)      | 2 (2)      | 3 (3)      |
| Withdrawal n (%)                                       | 3 (1)         | 1 (1)       | 2 (1)       | 1 (1)      | 2 (2)      | 0 (0)      |
| Left study site n (%)                                   | 3 (1)         | 0 (0)       | 3 (2)       | 0 (0)      | 0 (0)      | 3 (3)      |
| Completed follow-up                                     | 321 (98)      | 158 (99)    | 163 (97)    | 99 (99)    | 107 (98)   | 115 (97)   |
| Serology phase individuals^b                           | n/N (%)       | n/N (%)     | n/N (%)     | NA         | NA         | NA         |
| 2016 cohort: three blood draws in serology phase 2017   | 297/542 (55)  | 116/280 (41) | 181/262 (69)| NA         | NA         | NA         |
| 2016 cohort: three blood draws in serology phase 2018   | 295/542 (54)  | 117/280 (42) | 178/262 (68)| NA         | NA         | NA         |
| 2017 cohort: three blood draws in serology phase 2018   | 303/577 (53)  | 118/289 (41) | 185/288 (64)| NA         | NA         | NA         |

Abbreviation: NA, not applicable.

^aBefore 31 March for 2016, before 31 January for 2017 and 2018.

^bTiming of blood draws indicated graphically in Figure 3. Participants in the 2016 cohort had two blood draws during 2016 and three each in 2017 and 2018, participants in the 2017 cohort had three blood draws in 2017 and three in 2018, participants in the 2018 cohort had three blood draws in 2018.

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**ETHICAL REVIEW**

The protocol was registered on http://clinicaltrials.gov on 6 August 2015 (Reference NCT02519803) and was approved by the University of the Witwatersrand Human Research Ethics Committee (Reference 150808) and the US CDC’s Institutional Review Board relied on the local review (#6840). Participants provided individual written consent or assent prior to enrolment and received a grocery store voucher of ZAR25-30 (USD 2–2.5) per visit for their time.

**PATIENT AND PUBLIC INVOLVEMENT**

Both study sites have community advisory boards (CAB) consisting of representatives from community-based and faith-based organisations who were involved in the planning of the PHIRST study. The CABs meet regularly and give advice on protocols, consents and recruitment plans and also provide feedback to communities on results of studies.

In addition, feedback sessions on study findings were held for participating families.

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CONFLICT OF INTEREST
Cheryl Cohen has received research grants awarded to her institution from Sanofi Pasteur, US Centers for Disease Control and Prevention. Cheryl Cohen has had costs for travel to a meeting supported by Parexel. Maimuna Carrim was awarded the Robert Austrian Research Award in Pneumococcal Vaccinology sponsored by Pfizer. Neil Martinson has a research grant awarded to his institution by Pfizer South Africa. Anne von Gottberg has received research grants awarded to her institution from Sanofi Pasteur, Pfizer and US Centers for Disease Control and Prevention.

AUTHOR CONTRIBUTIONS
CC, MM, TM, JM, FKT, OH, NW, NAM, KK, AvG and ST: conception or design of protocol. All co-authors: acquisition, analysis or interpretation of data for the work. FKT, OH, NW, MC, AB, LM and AvG: laboratory testing of samples. LL, MM, KM, FW and SN: enrolment and follow-up of participants. CC, MM and ST: drafting the work or revising it critically for important intellectual content. All co-authors: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DISCLAIMER
The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the funding agencies.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1111/irv.12881.

DATA AVAILABILITY STATEMENT
The study protocol including informed consent forms is available on the NICD website (https://www.nicd.ac.za/wp-content/uploads/2021/02/PHIRST-SARS-CoV-2-protocol-V1-amendment-Nov2020-incl-upd-consent.pdf). Analysis of the data for primary study objectives is planned to be completed by December 2023. Additional modelling and serologic studies will be concluded within one additional year, and primary de-identified data will be made publicly available no later than December 2025. The investigators welcome enquiries about possible collaborations and requests for access to the data set. Data will be shared after approval of a proposal and with a signed data access agreement. Investigators interested in more details about this study, or in accessing these resources, should contact the principle investigator, Prof Cheryl Cohen, at NICD (cheryl@cnicd.ac.za).

REFERENCES
1. Troeger C, Forouzanfar M, Rao PC, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis. 2017;17 (11):1133-1161. https://doi.org/10.1016/S1473-3099(17)30396-1
2. Iuliano AD, Roguski KM, Chang HH, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet. 2017;391(10127):1285-1300. https://doi.org/10.1016/ S0140-6736(17)33293-2
3. Roth GA, Abate D, Abate KH, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392(10159): 1736-1788. https://doi.org/10.1016/S0140-6736(18)32203-7
4. Shi T, McAllister DA, O’Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet. 2017;390(10098). https://doi. org/10.1016/S0140-6736(17)30938-8
5. Wahl B, Brien KLO, Greenbaum A, et al. Articles Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2010–15. Lancet Glob Heal. 2018;6(7):e744-e757. https://doi.org/10.1016/S2214-109X(18)30247-X
6. Tsang TK, Lau LL, Cauchemez S, Cowling BJ. Household transmission of influenza virus. Trends Microbiol. 2015;24(2):123-133. https://doi. org/10.1016/j.tim.2015.10.012
7. Kombe IK, Munywoki PK, Baguelin M, Nokes DJ, Medley GF. Model-based estimates of transmission of respiratory syncytial virus within households. Epidemics. December 2018;27:1-11. https://doi.org/10. 1016/j.epidem.2018.12.001
8. Whitney CG, Goldblatt D, O’Brien KL. Dosing schedules for pneumococcal conjugate vaccine. Pediatr Infect Dis J. 2014;33(SUPPL. 2): 172-181. https://doi.org/10.1097/INF.0000000000000076
9. WHO. Pertussis vaccines: WHO position paper. Wkly Epidemiol Rec. 2010;40(85):385-400.
10. Pobedy RG, Sinnathamy MA, Warburton F, et al. Uptake and impact of vaccinating primary school-age children against influenza: experiences of a live attenuated influenza vaccine programme, England, 2015/16. Eurosurveillance. 2018;23(25);pii=1700496. https://doi. org/10.2807/1560-7917.ES.2018.23.25.1700496
11. Munywoki PK, Koech DC, Agoti CN, 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. https://doi.org/10. 1093/infdis/jit820
12. Fregaszy EB, Warren-Gash C, White PJ, et al. Effects of seasonal and pandemic influenza on health-related quality of life, work and school absence in England: Results from the Flu Watch cohort study. Influenza Other Respi Viruses. 2018;12(1):171-182. https://doi.org/10. 1111/irv.12506
13. Statistics South Africa. Investigation into appropriate definitions of urban and rural areas for South Africa discussion document. Pretoria, South Africa; 2001.
14. Cohen C, Walaza S, Moyes J, et al. Epidemiology of viral-associated acute lower respiratory tract infection among children <5 years of age in a high HIV prevalence setting, South Africa, 2009–2012.
15. Kahn K, Collinson MA, Xavier Gómez-olivé F, et al. Profile: Agincourt health and socio-demographic surveillance system. Int J Epidemiol. 2012;41(4):988-1001. https://doi.org/10.1093/ije/dys115
16. Garenne M, Collinson MA, Kabudula CW, Gómez-Olivé FX, Kahn K, Tollman S. Completeness of birth and death registration in a rural area of South Africa: the Agincourt health and demographic surveillance, 1992–2014. Glob Health Action. 2016;9(1):32795. https://doi.org/10.3402/gha.v9.32795
17. World Bank. The World Bank. Overview of South Africa. http://www.worldbank.org/en/country/southafrica/research. 2016.
18. Wong KK-L, von Mollendorf C, Martinson N, et al. Healthcare utilization for common infectious disease syndromes in Soweto and Klerksdorp, South Africa. Pan Afr Med J. 2018;30(271). https://doi.org/10.11604/pamj.2018.30.271.14477
19. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: building an international community of software platform partners. J Biomed Inform. 2019;95:103208. https://doi.org/10.1016/j.jbi.2019.103208
20. Jernigan DB, Lindstrom SL, Johnson JR, et al. Detecting 2009 pandemic influenza A (H1N1) virus infection: availability of diagnostic testing led to rapid pandemic response. Clin Infect Dis. 2011;52 Suppl 1:S36–S43. https://doi.org/10.1093/cid/ciq020
21. Hu A, Colella M, Tam JS, Rappaport R, Cheng S-M. Simultaneous detection, subgrouping, and quantitation of respiratory syncytial virus A and B by real-time PCR. J Clin Microbiol. 2003;41(1):149-154. https://doi.org/10.1128/JCM.41.1.149-154.2003
22. van de Pol AC, Wolfs TF, van Loon AM, et al. Molecular quantification of respiratory syncytial virus in respiratory samples: reliable detection during the initial phase of infection. J Clin Microbiol. 2010;48(10):3569-3574. https://doi.org/10.1128/JCM.0097-10
23. Carvalho MG, Tondella ML, McCaustland K, et al. Evaluation and improvement of real-time PCR assays targeting lytA, ply, and psaA genes for detection of pneumococcal DNA. J Clin Microbiol. 2007;45(8):2460-2466.
24. Tatti KM, Sparks KN, Boney KO, Tondella ML. Novel multitarget real-time PCR assay for rapid detection of Bordetella species in clinical specimens. J Clin Microbiol. 2011;49(12):4059-4066. https://doi.org/10.1128/JCM.00601-11
25. World Health Organisation. Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza. Serological Diagnosis of Influenza by Haemagglutination Inhibition Testing; 2011.
26. Hacimustafaoglu M, Celebi S, Aynaci E, et al. The progression of maternal RSV antibodies in the offspring. Arch Dis Child. 2004;89(1):52-53. https://doi.org/10.1136/adc.2002.017780
27. Wu D-X, Chen Q, Yao K-H, et al. Pertussis detection in children with cough of any duration. BMC Pediatr. 2019;19(1):236. https://doi.org/10.1186/s12887-019-1615-3
28. National Department of Health: South African Ministry of H. National Consolidated Guidelines for the Prevention of Mother to Child Transmission of HIV (PMTCT) and the Management of HIV in Adolescents and Adults; 2015.
29. Statistics South A. Census 2011 Statistical release—P03014. Stat South Africa. 2012.
30. Human Sciences Research Council (HSRC). The Fifth South African National HIV Prevalence, Incidence, Behaviour and Communication Survey, 2017: HIV Impact Assessment Summary Report. Cape Town, South Africa; 2018. http://www.hsrc.ac.za/uploads/pageContent/9234/SABBSSMV_Impact_Assessment_Summary_ZA_ADS_cleared_PDFA4.pdf
31. Cohen C, Kleyhans J, Moyes J, et al. Asymptomatic transmission and high community burden of seasonal influenza in an urban and a rural community in South Africa, 2017–18 (PHIRST): a population cohort study. Lancet Glob Health. 2021;9(6):e863-e874. https://doi.org/10.1016/s2214-109x(21)00141-8
32. Fox J, Brandt C, Wassermann F, et al. The virus watch program: a continuing surveillance of viral infections in Metropolitan New York families. Am J Epidemiol. 1969;80(1):25-50. https://doi.org/10.1093/oxfordjournals.aje.a120913
33. Munywoki PK. Transmission of respiratory syncytial virus in households: who acquires infection from whom?. 2013. oro.open.ac.uk.

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