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STUDY PROTOCOL

Surveillance, assessment and detection of influenza-associated respiratory infections in a high HIV burden setting in Lusaka, Zambia: A protocol for a cross-sectional surveillance study

[version 1; peer review: 1 approved with reservations]

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Abstract

Background

Respiratory viral infections including influenza, respiratory syncytial virus (RSV), and COVID-19 contribute substantially to morbidity and mortality across age groups. Influenza alone is associated with up to 650,000 deaths and approximately 5 million annual hospitalizations globally.

Although well documented in high-income countries, the epidemiology of respiratory viral infections remains limited in low-middle income countries, specifically southern Africa, including Zambia, where respiratory tract infections rank among the top causes of morbidity and mortality. While these infections frequently present mildly and may exhibit a self-limiting course, early diagnosis in routine care settings and strong surveillance systems are essential for transmission prevention, syndromic management of acute infection, improved diagnostic stewardship and emerging pathogen detection.

This study aims to characterise the influenza burden and other viral infections among adults with influenza-like illness (ILI) in Zambian

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outpatient clinics while evaluating the performance of a breath-based sample collection mask compared with nasopharyngeal swabbing for viral pathogen diagnosis.

Methods

This observational study is being conducted at two primary healthcare facilities, in Lusaka, Zambia, recruiting adults aged ≥18 years presenting with ILI for 2–7 days. Participants undergo clinical evaluation, chest x-ray, and nasopharyngeal swab sampling. Participants also wear facemasks for 30 minutes for breath-aerosol collection. Samples will be analysed using multiplex assays. They also complete a researcher-administered acceptability questionnaire and a daily symptom diary for two weeks. The primary outcome is influenza A/B prevalence; secondary outcomes include RSV & SARS-CoV-2 prevalence, seasonality, co-infection, clinical outcomes, diagnostic accuracy, and acceptability of facemask versus nasopharyngeal sampling. Heidelberg University, University of Zambia, and Zambia National Health Research Authority granted ethical approval.

Discussion

This study will strengthen epidemiological data on respiratory viral infections, and support evidence for local influenza vaccine implementation. It may inform future adoption of facemask-based sampling as a novel, preferable, potentially self-administered method for respiratory pathogen detection.

Trial registration

The study was retrospectively registered on www.clinicaltrials.gov on 19 June 2025 with number NCT07030075.

Plain Language summary

Respiratory viral infections caused by viruses such as influenza, respiratory syncytial virus (RSV), and COVID-19 cause illness and death worldwide. Influenza alone leads to millions of hospitalizations and thousands of deaths each year. While these infections are well studied in higher income countries, less is known about their impact in places like Zambia, where respiratory diseases are among the leading causes of sickness and death. Most infections are mild, but early diagnosis and good surveillance are important to stop spread, guide treatment, and detect new pathogens.

This study looks at how common influenza and other respiratory viruses are among adults with flu-like symptoms in outpatient clinics in Lusaka, Zambia. It also tests whether collecting breath samples using a facemask works as well as the standard nose and throat swab for detecting viruses.

Adults aged 18 and older with flu-like illness lasting 2–7 days are invited to take part in the study. They receive a clinical checkup, chest x-ray, and a swab. A subset of participants also wear a facemask for 30 minutes to collect breath samples. All samples are tested for viruses using advanced laboratory methods. Participants complete a short questionnaire about how acceptable the facemask method is and keep a symptom diary for two weeks. The main outcome is how often influenza A and B are found. Other outcomes include RSV and COVID-19 prevalence, seasonal patterns, coinfections, clinical outcomes, and how accurate and acceptable facemask sampling is compared with throat swabs.

By improving knowledge of respiratory viruses in Zambia, this study may support the introduction of influenza vaccines locally and explore facemask sampling as a simpler, more comfortable way to test for respiratory infections especially during global outbreaks when mask wearing may be mandatory.

Keywords
Acute respiratory illness, Influenza, COVID-19, RSV, Prevalence, Diagnostics, Breath-based sampling, Breath aerosol sampling, Zambia



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List of abbreviations

ARI: Acute respiratory infection
BMI: Basal metabolic index
COVID-19: Coronavirus disease 2019
FMS: Face mask sampling
Flu: Influenza
GISRS: Global Influenza Surveillance and Response System
HA: Hemagglutinin
HEF: Hemagglutinin esterase-fusion
IDEAS: Influenza disease evaluation and assessment
HIV: Human Immunodeficiency Virus
ICU: Intensive care unit
ILI: Influenza-like illness
IPD: Inpatient department
PLHIV: People living with HIV
RSV: Respiratory syncytial virus
SARI: Severe Acute Respiratory Infection
NIC: National influenza Centre
NA: Neuraminidase
OPD: Outpatient department
WHO: World Health Organisation
XBA: Exhaled breath aerosol

Introduction

Historically, respiratory pathogens have been responsible for some of the world's deadliest pandemics and the recent SARS-CoV-2 pandemic was a vivid reminder of this persistent threat.[1] The World Health Organization (WHO) anticipates that the next pandemic will likely be of a respiratory pathogen and this is reflected in their current list of priority pathogens for research and of public health concern which includes influenza and coronaviruses.[2–4] Influenza leads to considerable levels of morbidity and mortality in both the adult and paediatric populations, and its capacity for global spread has been well documented.[5,6] It is estimated that influenza is responsible for 290,000 to 650,000 global deaths and up to 5 million hospitalisations annually.[7,8] Influenza viruses notably pose a substantial risk to vulnerable groups including young children, the elderly, individuals with underlying health conditions, and those with compromised immune systems.[9,10]

Although the epidemiology of influenza has been comprehensively studied in many regions globally, there is still limited understanding of its epidemiology in Africa and its contribution to morbidity and mortality from lower and/or upper respiratory infections in the region.[11] In Zambia, although respiratory tract infections (RTIs) remain among the top five causes of morbidity and mortality, their aetiology – apart from tuberculosis (TB) and SARS-CoV-2 – is rarely established in routine clinical care due to the high cost of diagnostic testing.[12,13]

Rationale

Although several reports of influenza circulation have been made in Zambia in the past 10 years among both children and adults, the clinical epidemiology of the disease remains poorly described, especially in the post-COVID-19 era.[14–18] Estimates from a few local studies suggest that influenza accounts for 4–5% of all hospital admissions.[19,20] However, even fewer studies have reported on the burden and clinical outcomes of influenza, particularly among adults presenting with influenza-like illness.[21] This limited evidence on the burden of influenza-related disease has contributed to delayed decision-making concerning pandemic readiness and the use of existing and emerging vaccines, treatments, and diagnostic tools in the region.[11,22–27]

Sampling for respiratory pathogens in acute respiratory illness has long been done using nasopharyngeal swabs (NPS) as a standard due to its high sensitivity particularly in acute illness, suitability for a variety of test types, wide availability and standardisation, and collecting samples from the nasopharynx where respiratory viruses replicate most abundantly.[28–30] However, upper respiratory tract sampling using NPS has been noted to cause significant discomfort to patients and is prone to human sampling errors, limiting efficiency in diagnostic evaluations.[29,31,32] The usual samples such as sputum and NPS required for respiratory disease diagnosis can also be challenging to obtain for both patients and healthcare workers.[33–35]

To improve on the early detection of these viruses and to narrow the diagnostic gap among patients presenting to routine practice, we will compare the use of NPS to breath-based sample collection for testing on molecular platforms. As evidence supports airborne transmission of respiratory infections, we will aim to assess the potential of breath or aerosol-

based sampling using a face mask which is both more comfortable and easier to collect than traditional sampling for potentially earlier diagnosis of respiratory pathogens.[36–39]

The need for non-invasive alternatives cannot be over emphasised while at the same time looking to maintain diagnostic accuracy. Alternatives such as saliva have been explored but continue to show lower performance for pathogen identification to that of NPS on molecular platforms.[29,40] Breath-based diagnostic sampling tools are therefore an important alternative that is not only non-invasive but also may be easier to collect across age-groups and in various clinic settings while holding potential to detect early onset infection.[37,41] Specifically, diagnostics that aim for pathogen capture in exhaled breath aerosols may hold the promise of a non-inferior detection of specific respiratory pathogens to traditional options when tested on molecular platforms.[42,43]

Study objectives

Primary objectives

The primary objective is to determine the prevalence of influenza (A and/or B) infection among adult patients in Lusaka, Zambia, presenting with influenza-like illness in a high HIV burden setting, over one or more influenza seasons.

Secondary objectives

The secondary objectives are[1]: To determine the prevalence of RSV and SARS-CoV-2 among study participants, alongside seasonal variations[2] To determine the proportion of participants with influenza (A/B), RSV or COVID-19 with other co-infections[3] To determine the clinical outcomes of influenza (A/B), RSV and COVID-19 infection among adult outpatients by HIV and COVID-19 vaccination status. (4a) To evaluate the accuracy of breath-based sampling for diagnosis of respiratory viruses (influenza, SARS-CoV-2, and RSV) compared to NPS for rapid diagnosis of infection among symptomatic individuals in Zambia. (4b) To evaluate the acceptability of exhaled breath-based sampling for diagnosis and screening of respiratory infections of pandemic potential.

Protocol

Methods/design

Study type

This is a cross-sectional surveillance study with symptom follow-up assessments of up to 14-days.

Study location

The study will be conducted at two primary healthcare (PHC) facilities in Lusaka, Zambia which serve densely populated urban areas. Study participants will be recruited from various facility entry points including medical outpatient departments (OPDs), antiretroviral therapy (ART) or HIV clinics, maternal and child health (MCH) clinics and emergency or triage departments.

Study population

Adults over the age of 18 presenting with influenza-like illness (ILI) with duration of 2–7 days and willing to participate in the study.

Inclusion criteria

- age ≥ 18 years old
- presenting with ILI defined according to the Centers for Disease Control and Prevention (CDC) as fever (measured ≥ 38 °C or self-reported) with either cough or sore throat lasting 2 to 7 days.[21,44] Other symptoms may include sneezing, congestion, myalgia, fatigue, wheezing and shortness of breath)[45–47]
- willing to share their HIV status or be tested
- willing to share their COVID-19 vaccination status

- able and willing to give informed consent
- willing to provide two NPS samples: one as part of standard of care and one for the study purposes
- willing to provide an exhaled breath aerosol sample by wearing a face mask sample collection device
- willing to fill a symptoms diary card for symptom tracking for 7–14 days
- agree to be followed up and attend study visits up to two weeks after study entry

Exclusion criteria

- individuals who are unwilling to provide any reference standard samples such as the NPS
- those with symptoms for less than 2 days or greater than 7 days, or
- those unwilling or unable to provide written informed consent

Study procedures

Participant recruitment

Participants will be recruited at the health facility entry points and outpatient departments (OPDs). Study staff will conduct sensitization talks in these locations to introduce the study to potential participants. Interested individuals will be invited, consecutively, to the clinical research sites (or within the entry point, where possible) for a detailed discussion about possible participation, if eligible.

Informed consent

We will obtain informed consent from individuals who are symptomatic for ILI and are willing to join the study. The study staff will provide a brief summary of the study in facility entry points or waiting area where potential participants may be awaiting attendance. Interested individuals will be invited to the study site for further details on the study. At the study site, participants will be taken through the informed consent process with details of the study being shared in the language the participant prefers (English, Nyanja or Bemba). An impartial witness will be requested to be present during consenting of any illiterate participants who may be willing to participate. We will assess comprehension of study details using a set of affirmatory statements for which the participant must confirm as understood before signing consent.

Screening and sampling procedures

We will collect both qualitative and quantitative data to assess the burden of viral respiratory infections, the temporal distribution, diagnostic accuracy of exhaled breath aerosols (XBA) or breath-based sampling, and finally the acceptability of the XBA sample collection methods among end-users (Table 1).

1. Case report form (CRF): study staff will complete an electronic or paper-based structured CRF for collecting data related to sociodemographic characteristics, risk factors, medical history, HIV status, prior COVID-19 diagnoses and COVID-19 vaccination status. They will also undergo a systematic assessment of any current symptoms, including severity and duration. Paper-based forms will be utilized as back-up for electronic data capture, and they will be transcribed into the electronic data entry system, REDCap.

2. Vital signs: All participants will have routine vital signs and anthropometric measurements undertaken at enrolment and at both follow-up visits. These will help in evaluating the clinical severity of the episode.

3. Breath-based sampling: Some participants presenting with ILI and enrolled in the study, will be asked to wear a facemask for 30 minutes during their visit for sampling using a facemask (FMS).

4. Routine NPS sample collection: FMS sample collection will be followed by routine sample collection of two NPS samples using a polyester or nylon tipped swab (flocked) and placed in viral transport media (VTM). As per standard of

Table 1. Schedule of study activities.

Schedule of activities	Visits	V1							V2 (+/−1 day)		V3* (+/−1 day)
	Day	1	2	3	4	5	6	7	8	9–14	15
Study introduction		X									
Participant identification		X									
Informed consent obtained		X									
Vitals and anthropometrics		X									
Chest X-ray		X									
Case report form administration (history and physical examination)		X									
Sample collection using ▪ facemask x1, then ▪ nasopharyngeal swab x2		X									
Administer questionnaire on acceptability		X									
Symptoms diary card orientation		X									
Symptoms diary card completion		X	x	x	x	x	x	x	X	x	
Symptoms diary card review									X		X
Vital signs									X		X
Outcome assessment									X		X

Legend: This table shows the study schedule of activities and procedures conducted at each visit+/− 1 day indicates a one day window before and after the follow-up visit dateAbbreviations: V1 – visit 1(enrolment day), V2 – visit 2 (follow-up 1) and V3 – visit 3 (follow-up 2).
*Applicable only if symptoms had not resolved by V2

care, patients presenting with symptoms suggestive of TB (cough, fever, weight loss, night sweats) will undergo screening for TB using Xpert MTB/RIF Ultra and/or urine Alere LAM, as indicated.[48]

4. HIV testing: all participants who do not have a recent (less than 3 months) HIV test result will be tested for HIV through routine services using a finger prick HIV test kit.

5. Digital chest X-ray (CXR): As per routine screening guidelines for individuals presenting with a cough,[48] a CXR will be performed for all study participants and interpreted by study clinicians. When available, in addition to clinicians' review, computer-aided diagnosis (CAD) software will be utilized to detect heatmaps of chest abnormalities. We will utilize the CXR to assess the extent of lower respiratory tract involvement or infection spread at enrolment.

6. User experience questionnaire: To better understand participant experiences, preferences, and constraints of end-users, participants sampled with both a NPS and XBA will be requested to complete a researcher-administered structured questionnaire with a focus on assessing acceptability of the XBA sample collection device.

7. Symptom diary card: At enrolment, participants will be given a symptom diary card to complete for up to a week, documenting any persisting respiratory symptoms.

8. Participant Follow-up: A week after enrolment, the participants will be requested to return to the facility for review of their vital signs and the symptoms diary card. This will be followed by a close-out visit on day 15, which will involve a

review of vitals and may include the symptom diary card review if symptoms persisted beyond the first week after enrolment.

At the conclusion of the study visit, study participants will be reimbursed for their time in accordance with local ethical guidance.

Laboratory sample processing, testing and storage

Samples collected from participants will be secured by ensuring that screw caps are tightened and they will be labelled with a study-generated ID using a barcode. The samples will then be placed in biohazard bags and transported to a central laboratory at 2–8 °C in cooler boxes. At the laboratory, samples received will be accessioned and processed immediately (1 NPS) or stored at −20 °C (1 NPS & the facemask sample) for batched testing. Routine NPS samples transported in VTM will be tested in the routine care molecular laboratory where the samples received will first undergo nucleic acid extraction procedures, run using multiplex assays before being amplified on an open PCR platform as required. After sample testing, results will be made available electronically to clinicians within 24 to 48 hours. Results will then be printed, filed and shared with the patient at the next clinic visit. The second swab will be stored at minus 20 °C for later batch testing alongside the facemask samples.

Facemask samples will be processed as per manufacture instructions. In brief, the mask is removed from the bag and worn for 30 minutes. After removing from the participant, the mask filter is immediately pushed into the pre-filled buffer tubes using a probe on the tube cap and then labelled with a barcode to link the sample to the corresponding participant. The sample will then be transported to the laboratory at ambient temperature for processing. Upon accession, the facemask samples will be stored at −20° Celcius for later batched testing. When processing, the tube is removed from the storage, the sample details confirmed, thawed and processed using appropriate standard operating procedures (SOPs) and finally tested using a multiplex panel on an open PCR platform.

Stored sample testing: Stored NPS and facemask samples will be thawed before testing, undergo automatic extraction and amplification using a multiplex assay tested on open PCR platforms as per study SOPs.

Primary outcomes

Prevalence of influenza A and/or B detected in NPS samples collected among all participants.

Secondary outcomes

- Prevalence of RSV and SARS-CoV-2 in NPS samples collected among all participants
- Proportion of participants with influenza, RSV or SAR-CoV-2 who are co-infected with other respiratory pathogens.
- Seasonal distribution of influenza and other respiratory viruses across at least one completed influenza season.
- Quantify and compare the clinical outcomes associated with influenza A/B, RSV, and COVID-19 infections among adult outpatient populations, stratified by HIV status and COVID-19 vaccination status
- Diagnostic accuracy (sensitivity and specificity) of XBA sampling methods on PCR multiplex platforms compared with nasopharyngeal sampling.
- Acceptability of XBA devices among end-users using structured evaluation tools.

Statistical analysis

Sample size

To assess the primary outcome, the sample size calculation was based on estimating the prevalence (point estimate) of influenza A and/or B in OPD patients presenting with ILI to the clinic with a certain degree of precision. Therefore, assuming that between 4% to 17% of the subjects in the population have influenza A and/or B[16,20,49,50] and an expected indeterminate proportion of 10%, the study would require a sample size of 540 for estimating the expected proportion

with 5% absolute precision and 95% confidence. To account for possible loss to follow-up in objectives 1 and 2, the final sample size will be increased by ~10% to 594 participants to be enrolled.

The study is powered for the primary objective while the secondary objectives are dependent on the successful conduct of the first objective. With the above assumptions for positivity, we expect that about 65 to 100 individuals will test positive for influenza with a few others testing positive for other respiratory infections. To ensure that we screen adequate numbers of participants to meet the secondary objectives, we will target equal numbers of enrolments from the ART clinic to those from other entry points such as OPD and MCH.

For objectives 3 and 4, a consecutive subset of participants enrolled in the study (starting with availability of the mask) will be sampled with both the standard of care NPS and the novel breath-based sample collection tools. For these diagnostic accuracy objectives, assuming that the novel breath sampling device sensitivity is 90%, a sample of 35 patients with influenza, RSV or COVID-19 would allow us to calculate sensitivity with 10% confidence interval (CI) and specificity with <5% CI width. The total sample size will be 35 patients with infection and a minimum of 300 patients with or without infection.

Analysis

Data will be analysed using statistical programming language such as STATA Statistical Software (Stata Corporation Version 18.5 College Station, Texas 77845, USA) or R software 4.5.1 (Copyright 2025 The R Foundation for Statistical Computing Platform). Descriptive statistics will be used to describe characteristics of participants enrolled. We will quantify and report the prevalence with the 95% CI for influenza A and/or B amongst the study participants. The prevalence will also be reported using the same 95% CI stratified by sex, age group, HIV status, and presence of other respiratory viruses, and symptomology. Acknowledging that results outputs may include indeterminates (non-positive non-negative results), we will plan for repeat testing and exclude any persisting indeterminate from the numerator and denominator during analysis. Alternately, we will conduct sensitivity analysis assuming indeterminates as positive or negative to ascertain their impact on prevalence. Chi-squared tests of association will also be used to ascertain relationships between sociodemographic and clinical characteristics with influenza A and/or B infection. We will also undertake multivariable logistic regression to identify independent predictors of influenza positivity and that of co-infection with other viruses such as RSV and SARS-CoV-2.

For the seasonal variation of influenza, we will plot weekly proportions of positives on a line graph plotted over at least 1 whole season. Apart from inspecting the fitted curve, we will conduct a descriptive temporal analysis of the trends in influenza positivity over the duration of the study.

We will use Kaplan-Meier (KM) survival methods to analyse the time to symptom resolution. Each participant's time to resolution will be defined from the date of enrolment to the first day of sustained symptom-free days according to their diary entries. KM estimates will be used to estimate the median time to resolution and ascertain the probability of remaining symptomatic at beyond the follow-up duration of 14 days (computation of the survival function). Additionally comparative survival analyses will be conducted to evaluate the differenced in time-to-event subgroups including type of pathogen detected (Influenza, RSV, COVID-19), symptom severity at presentation (severe or non-severe), HIV status (PLHIV or not) and history of COVID-19 vaccination (vaccinated or not).

Additionally, we will perform comparative analysis on the accuracy of the breath-based sampling methods for respiratory virus detection while comparing it to NPS as a reference. We will construct two-by-two tables for the breath-based samples against the NPS samples to ascertain the sensitivity/specificity, predictive values, and likelihood ratios. We will then use McNemar's test or t-test to measure the difference between the paired proportions assessing for statistical significance, as appropriate. To minimise biasing our results, we will ensure that all participants will be sampled using both tools and regardless of their clinical presentation. Additionally, repeat testing will be done for any indeterminate or invalid results using any left-over samples.

For the structured questionnaire adapted from Sekhon et al. questionnaire (2022) on acceptability of healthcare interventions,[51] we will analyse the proportion of participants who find the XBA sampling methods to be acceptable and more preferred compared to standard NPS samples. We will conduct multistep analysis including exploratory factor analysis (EFA) to confirm that the items group together as expected based on the theoretical framework of acceptability (TFA) constructs, reliability analysis to assess internal consistency of responses and comparison analysis to compare responses across the various patient groups. Conclusions on the responses will be drawn based on the differences in proportion of participants by their preferences which will be estimated using Chi- squared test or McNemar's test as appropriate.

Data safety and monitoring

Adequate measures will be put in place to ensure that the study is implemented as pre-described in the protocol and standard operating procedures. Data will be collected on paper case report forms (CRFs) and later transcribed into an electronic database. Aside from real-time and regular interval quality checks of paper forms, a second person will review the electronic database regularly for data accuracy and completeness confirmation.

Study monitoring of the study will be conducted by an internal monitoring team from the centre for infectious disease research in Zambia (CIDRZ) research regulatory department and the university of Zambia biomedical research ethics committee (UNZABREC).

Any protocol deviations or adverse events occurring on the study will be reported as per local ethical and regulatory guidance.

Ethics considerations

The study protocol was reviewed and received approvals from ethics committees from the Medical Faculty of Heidelberg University Hospital – study number S-718/2024, the University of Zambia biomedical research ethics committee (UNZABREC) – reg No. 5955–2024 (local IRB) and by the national regulator, the National health research authority in Zambia (NHRA) – 1760/03/12/2024. Initial approval was received on 8 November 2024. The study was retrospectively registered on www.clinicaltrials.gov on 19 June 2025 with number NCT07030075. Because this study did not meet the formal definition of a clinical trial, it was not initially subject to mandatory registration on ClinicalTrials.gov. However, following updated guidance and a local regulatory recommendation requiring registration of all clinical studies regardless of type, the study was subsequently registered. This resulted in a retrospective entry.

Risk/benefit

Participants enrolled in the study will be exposed to risks that are no greater than those encountered in routine respiratory health services.

The facemask sample collection is a non-invasive procedure that does not cause the participant harm; however, it may cause mild discomfort in some participants.

nasopharyngeal swabbing which is part of routine care may cause discomfort and rarely can result in nosebleeds in some participants. We will ensure that only trained health workers with a good knowledge of anatomy and the procedure conduct it on the study participants.

HIV testing will be requested for all individuals with no test result that is less than 3 months as per national recommendations. HIV testing may cause psychological distress for participants and so we will ensure that standard counselling is done before and after testing and result sharing. The test will also be done within routine care settings.

CXR exposes participants to radiation. We will discuss these risks with the participants and aim to use digital x-ray machines (where possible) as they are reported to have a lower dose of radiation. We will take care to not conduct CXRs on pregnant women, following standard care radiological practice.

Participants may be exposed to potential breach of confidentiality or inadvertent disclosure of personal information while participating in the study. However, the study team will ensure that all team members are trained in upholding Good Clinical Practice (GCP) standards and data security measures are put in place for both paper and digital participant records.

Dissemination of results

During the study we will share progress updates with the community at scheduled monthly community advisory board (CAB) meetings and biannual progress reports with local ethical and research regulatory authorities. Further dissemination will be done at the end of the study with the community leaders including facility, district, provincial and national health offices. Lastly, the study results will be submitted as abstracts to local or international conferences and as a manuscript for publication to high-impact open access journal s for publication.

Conclusions/Discussion

The local prevalence of influenza and other respiratory pathogens among adults remains poorly documented limiting appropriate care and contributing to perpetuated use of antibiotics for nearly all respiratory complaints in outpatient settings. Further, limited laboratory diagnosis of the causes of acute respiratory illness driven both by uncomfortable sampling options and lack of routine testing capacity continue to undermine policy decisions around testing, vaccination, local treatment guidelines and public health pandemic preparedness efforts. This study aims to contribute to the local epidemiological data on respiratory infections while also assessing a potentially preferable sample collection tool.

The study has the potential to facilitate background screening for multiple respiratory diseases of concern, including tuberculosis (TB). Integrated symptom assessment with CXR may support clinical management and prompt further diagnostic evaluation for TB within routine services.[48] Facemask samples may be utilized for molecular detection of tuberculosis (TB); however, differences in mask-wearing protocols (e.g., 45 minutes for TB versus 30 minutes for viral pathogens) may reduce sensitivity for TB detection.

Operationally, seasonal fluctuations in clinic attendance could slow participant enrolment, particularly during periods of lower symptomatic presentation. To mitigate sampling bias, recruitment schedules have been standardized across sites, ensuring consistency in data collection despite variable patient flow.

Study status

The study began recruitment on 20 May 2025 and will continue until December 2026. As of April 2026, a total of 451 participants had been screened and 285 enrolled on the study. Recruitments had initially been steady but slowed down from October 2025, which we attribute to the hot rainy season, also aligning with historical data expectations. Due to resource constraints and delay in facemask acquisition the first 116 participants were only sampled using a NPS and this was communicated with the local ethics committees.

Many of our study participants have been returning for Visit 2 and slightly fewer for Visit 3 clinic reviews as per expectation. They have reported no difficulties completing their symptom diary cards. Continued care is received under routine services for those still presenting with symptoms after Visit 3 (close-out) clinic review.

Ethics approval and consent to participate

This study is in agreement with the Helsinki declaration and Zambian national guidelines for ethics in research as outlined by the national health research authority (NHRA). The study was approved by the Medical Faculty of UKHD at Heidelberg University, the University of Zambia biomedical research ethics committee (UNZABREC) and by the national research regulator, National Health Research Authority (NHRA). All enrolled participants provide written informed consent obtained in either English, Bemba or Nyanja.

Data and software availability

This protocol is yet to include study results. The study datasets will be made available in recommended repositories alongside the publication of study results in the future, once the study is accepted for publication. Extended data inclusive of participant information sheets, Informed consent forms, study surveys and interview guides are included here https://doi.org/10.5281/zenodo.20310534[52] for further reference.

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This protocol addresses an important public health issue, particularly in a country where information on influenza and other respiratory viral infections is still limited. The study is timely and has the potential to provide valuable evidence on the burden of respiratory viruses in adults while also evaluating an innovative, non-invasive sampling method. The manuscript is generally well organized, and the objectives are easy to follow.

The background provides sufficient justification for conducting the study, and the rationale for comparing facemask sampling with nasopharyngeal swabs is convincing. The study design is appropriate for answering the research questions, and the planned laboratory and clinical procedures are described in reasonable detail.
However, there are a few areas that would benefit from clarification before indexing.

First, the protocol mentions that only a subset of participants will undergo facemask sampling, but it is not entirely clear how these participants will be selected. A clearer explanation of the selection process would help readers understand whether any selection bias could be introduced.

Second, although the statistical analysis section is generally adequate, it would be helpful if the authors briefly described how missing data and invalid laboratory results will be handled.
Similarly, more detail on the analysis of diagnostic accuracy would strengthen the protocol.
The sample size calculation for estimating influenza prevalence is appropriate, but it is less clear whether the study will have sufficient power for some of the planned subgroup analyses and diagnostic accuracy objectives. A short justification would improve the manuscript.
I also suggest adding a simple participant flow diagram summarizing enrolment, specimen collection, follow-up visits, and analysis. This would make the protocol easier to understand.

Finally, the manuscript would benefit from careful proofreading, as there are several minor grammatical errors and formatting inconsistencies throughout the text. These do not affect the scientific quality but should be corrected before indexing.

Overall, I believe this is a well-planned and relevant study. The suggested revisions are relatively minor and are intended to improve the clarity and reproducibility of the protocol rather than to change its scientific approach.

Recommendation: Minor Revision.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Maternal Child Health ,Public Health HIV,STD

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.