BMJ Open

Feasibility of implementing a non-invasive self-sampling method for saliva specimens that can be used for the diagnosis of respiratory infections among paediatric patients in the Tshwane District, South Africa: a study protocol

Siphesihle Nxele ,[1,2] Thobeka Dlangalala ,[3,4] Nobuhle Vanessa Gxekwa,[2] Sebueng Ramatsokotla,[2] Alfred Musekiwa ,[5] Mankgopo Magdalene Kgatle,[6] Daniel B Hatchett,[7] Albert Shin,[7] Wan-chen Tu,[7] Ingrid H Robertson,[7] Xiaojing Su,[7] Erwin Berthier,[7] Sanitta Thongpang,[7] Ashleigh B Theberge,[8,9] Tivani Phosa Mashamba-Thompson[3]

To cite: Nxele S, Dlangalala T, Gxekwa NV, *et al*. Feasibility of implementing a non-invasive self-sampling method for saliva specimens that can be used for the diagnosis of respiratory infections among paediatric patients in the Tshwane District, South Africa: a study protocol. *BMJ Open* 2025;**15**:e099634. doi:10.1136/bmjopen-2025-099634

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2025-099634).

Received 18 February 2025
Accepted 19 August 2025

For numbered affiliations see end of article.

Correspondence to
Dr Siphesihle Nxele;
sr.nxele@up.ac.za

ABSTRACT

Introduction Effective community-based disease management is essential for public health. In low- and middle-income countries, sustainable strategies for timely diagnosis and treatment are a research priority. This study aims to assess the feasibility of a non-invasive saliva self-sampling method, paired with digitally linked molecular point-of-care diagnostics, for detecting respiratory infections among paediatric patients in the Tshwane District, South Africa.

Methods and analysis A field study will be conducted at Steve Biko Academic Hospital to compare saliva collection using the CandyCollect lollipop device and standard mouth swabs. The spiral groove of the lollipop device captures pathogens, which are stored in DNA/RNA preservation media and later analysed using quantitative PCR and commercially available rapid antigen tests. The multiplex respiratory pathogen panel, based on TaqMan real-time PCR technology, targets key paediatric pathogens including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, respiratory syncytial virus (RSV) and influenza A/B. Nucleic acids will be extracted using standard viral extraction kits and analysed following manufacturer protocols. Internal controls will be included in each qPCR run, and samples with CT values below defined thresholds will be considered positive. Rapid antigen tests will detect common pathogens such as influenza A/B, RSV and SARS-CoV-2 for comparative analysis. User experience and acceptability will be assessed via child-friendly and caregiver surveys following sample collection. The study will be implemented in two phases: diagnostic performance evaluation and user feedback assessment. The protocol is aligned with the Standard Protocol Items: Recommendations for Interventional Trials 2013 checklist.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The CandyCollect self-sampling method of saliva specimen collection is non-invasive, reducing anxiety and stress in paediatric patients, but may have limited sensitivity and specificity compared with invasive methods.
⇒ The self-sampling method is easy to use and can be performed by parents or caregivers, making it accessible in resource-limited settings, but may rely heavily on parent/caregiver compliance.
⇒ Saliva sampling is a cost-effective method, reducing the economic burden on the healthcare system and families, but the quality of saliva specimens may vary depending on factors such as age, diet and oral health.
⇒ Saliva specimens can be used to diagnose a wide range of respiratory infections, making them a valuable tool for paediatric healthcare, but may require further validation and standardisation to ensure reliability.
⇒ The self-sampling method can be easily scaled up for large-scale implementation in various settings, making it a promising tool for public health initiatives, but may need to be adapted for different paediatric populations and contexts.

Ethics and dissemination Ethical approval has been granted by the University of Pretoria (509/2023) and the Gauteng Department of Health (GP_202406_032). The study is registered in the Pan African Clinical Trial Registry (PACTR202411743094783). Findings will be disseminated through peer-reviewed journals, conferences and stakeholder briefings. The study complies with South Africa's Protection of Personal Information Act. Data

collection is scheduled from November 2024 to February 2025, with project completion expected within 1 year.

Trial registration number Pan African Clinical Trial Registry (PACTR202411743094783).

INTRODUCTION

Respiratory infections have become a common disease that has required consistent surveillance globally for better disease management. Some examples of such infections include influenza, respiratory syncytial virus (RSV) and COVID-19. Seasonal influenza virus is a common cause of acute lower respiratory infection (ALRI) in young children and a large proportion of the influenza-associated burden occurs among young infants in low-income and lower middle-income countries (LMICs).[1] RSV is reported to be the common cause of ALRI in young children.[2] It is estimated that 33.1 million episodes occurred in children aged 0–60 months in 2015, resulting in a total of 118 200 deaths worldwide.[2] Moreover, data have shown that the highest mortality rates are in sub-Saharan Africa where the HIV epidemic has increased morbidity of severe pneumonia, with RSV being one of the leading causes of pneumonia.[3] In South Africa specifically, information regarding the mortality burden of influenza and RSV is limited due to related deaths being attributed to secondary infections, which has made it difficult to directly relate child mortality rates to these respiratory infections.[4] The WHO gave RSV the highest ranking in terms of the burden of disease, which is measured by years lost through death or disability (disability-adjusted life-years). Lower respiratory tract infections are the leading cause of death in LMICs.[5]

The advent of COVID-19 showed the need to find ways to efficiently monitor the prevalence of such infectious diseases, especially in LMICs.[6] Respiratory infections commonly share similar transmission pathways such as droplets of saliva.[6] This calls for innovative specimen collection methods for more consistent diagnosis and respiratory infection surveillance that would reach all, including underserved populations, to ensure healthy lives and promote well-being for all at all ages as stated in the third Sustainable Development Goal (SDG) target. The development and implementation of methods that would promote the diagnosis of respiratory infections at point of care (POC) among under-fives will contribute to meeting SDG 3.2, which states that by 2030, preventable deaths of newborns and children under 5 years of age should end[7] as well as SDG Target 3.3, which specifically states that, among other diseases, communicable diseases should end.[8] The SDGs are a set of 17 global targets adopted by the United Nations in 2015 to promote peace, prosperity and environmental protection by 2030.[8] They address critical challenges such as poverty, inequality, climate change, education, health and economic growth. Achieving the SDGs is essential to ensure a more equitable, sustainable and resilient future for all people and the planet, fostering global cooperation and shared responsibility across nations and sectors. The National Development Plan of South Africa calls for quality care to be provided to communities with poor healthcare infrastructure,[9] which can be achieved through the implementation of simple and efficient non-invasive self-sampling methods that would promote easier diagnosis at the point of patient care. In addition, the National research priorities of South Africa's Framework in Research for Health highlight the need for prioritising respiratory disease management for vulnerable populations, emphasising the importance of the proposed research. Current practice in the hospitals and clinics in the Tshwane District when it comes to saliva sampling for respiratory infection determination involves sputum sampling and mouth swabs.

In this study, we define resource-limited settings as areas where there is a limitation in the capacity or capability to diagnose and treat life-threatening diseases. Young children and women remain some of the most vulnerable populations, and greater care needs to be taken in ensuring that they have a public health system that is functional, for better healthcare services.[10] The REASSURED criteria define the ideal features of diagnostic tools in resource-limited settings: real-time connectivity, ease of specimen collection, affordability, sensitivity, specificity, user-friendliness, rapid and robust performance, equipment-free operation and deliverability to end-users.[11] These criteria expand on the original ASSURED framework to emphasise digital integration and accessibility, ensuring diagnostics are effective, patient-centred and suitable for use at the POC. Implementation of innovative POC diagnostic approaches in line with the WHO's REASSURED criteria remains a challenge among vulnerable population groups. The proposed study of implementing non-invasive self-sampling methods of specimen collection will eliminate the need for complicated methods of collecting samples that can be analysed at the POC. Although this self-sampling tool is not a POC diagnostic tool on its own, it will introduce ease of sample collection, simplicity and user friendliness, which aligns with some of the REASURED criteria, and therefore will be implemented well with the guidance of these criteria and POC diagnostics especially in remote settings. We propose to conduct a pilot study to determine the feasibility of implementing a non-invasive self-sampling method that can be later combined with rapid molecular-based POC diagnostic technologies for respiratory infections among paediatric patients in the Tshwane District of South Africa. This non-invasive self-sampling method will be compared with standard care mouth swabs used in many clinics in underserved communities and hospitals. It is anticipated that results of the proposed study will show that the CandyCollect lollipop is a less-invasive, user-friendly self-sampling tool compared with mouth swabs. The results of the proposed study may show that there are more user-friendly ways of collecting saliva samples from children to determine if they have any respiratory infections or not. They also have the potential to guide

a future planned implementation study on the diagnosis of some of the most prevalent respiratory infections at the POC in the Tshwane District, using samples collected through the self-sampling method proposed herein.

Study aims and objectives

Study aim

The overarching aim of this study is to explore the feasibility of implementing a non-invasive self-sampling method of saliva specimen compared with the standard-of-care mouth swab that can be used for the diagnosis of respiratory infections among paediatric patients in Steve Biko Academic Hospital in the Tshwane District of Gauteng.

Study objectives

The objectives of the study are:

- To determine the potential impact of the CandyCollect non-invasive self-sampling lollipop for specimen collection among paediatric patients at Steve Biko Academic Hospital in the Tshwane District of Gauteng, in comparison with standard-of-care mouth swabs.
- To ascertain user experience on using the CandyCollect non-invasive self-sampling lollipop for specimen collection among paediatric patients at Steve Biko Academic Hospital in the Tshwane District of Gauteng, in comparison with mouth swabs.

METHODS AND ANALYSIS

Study design

We propose to conduct a field evaluation study for the feasibility of implementing a non-invasive self-sampling method of saliva specimen that can be used for the diagnosis of respiratory infections among population groups in Tshwane District, guided by the REASSURED criteria. The pilot study will be conducted to address two study objectives, with each phase addressing each objective. The first phase will determine the potential impact of a non-invasive self-sampling method (CandyCollect) for specimen collection among children from a variety of population groups in comparison with mouth swabs. The second phase will employ a qualitative study approach to ascertain user experience on using the non-invasive CandyCollect self-sampling method for specimen collection in comparison with mouth swabs. We used the Standard Protocol Items: Recommendations for Interventional Trials checklist to guide this protocol.[12] Although both the CandyCollect device and the standard mouth swab are clinically non-invasive, the CandyCollect is considered less invasive from a user experience perspective. Its lollipop-like design mimics familiar behaviours, such as sucking on a sweet, which may reduce discomfort and anxiety, particularly among paediatric users. In contrast, mouth swabs may be perceived as more intrusive due to their unfamiliar or clinical appearance. This study is designed to evaluate these differences by assessing user perceptions of comfort, ease of use and overall acceptability.

Study setting

This study will be conducted in the paediatric department of the Steve Biko Academic Hospital in Tshwane District of South Africa, which is situated in the northern parts of the Gauteng province (figure 1).[13] According to STATSSA, Tshwane district covers 6 368 km² of Gauteng's 19 055 km² with a population size of 2,9 million residents. One of the leading causes of death in this district is lower respiratory infections within a range of age groups.[14] The study is scheduled to commence at the end of November 2024 and be concluded in May 2025.

Intervention

The proposed study intervention includes the use of a CandyCollect self-sampling tool to collect saliva specimens from paediatric patients compared with mouth swabs to ascertain the feasibility of implementing the self-sampling tool prior to combining it with POC diagnostic technologies.

CandyCollect self-sampling kit

A self-sampling 'CandyCollect' technology has been developed and supplied by a research team from the University of Washington in collaboration with a team at the University of Wisconsin–Madison.[15–18] A study has been made available using this intervention in adults with a subsequent recent study in paediatric participants in Wisconsin, USA, performed by Tu *et al*.[18] The CandyCollect is a lollipop-shaped self-sampling device for non-invasive saliva collection. It features a plastic spoon-like head with an open spiral groove surrounded by candy (figure 2A). As the user sucks on it, saliva circulates through the groove, which is functionalised to capture bacterial and viral pathogens. Over time, pathogens accumulate in the groove (figure 2B). This technology enables easy, affordable and efficient sample collection. Samples are stored dry or in preservation media like Genotek DNA media for testing at a later stage via qPCR or rapid kits. Pathogen detection in saliva and mouth swab specimens will be conducted using a commercial multiplex respiratory pathogen panel, based on TaqMan real-time PCR technology. The rapid test kits, in this study, each user provides one CandyCollect and a mouth swab, which are biobanked for future analysis. For the mouth swab sample, the user is instructed to suck on the mouth swab as if it is a lollipop; this method of sample collection from children was introduced by DeMuri *et al* as a potential alternative to pharyngeal swabs for streptococcal pharyngitis.[19]

Pilot study objectives

Table 1 shows a summary of the methodology for determining the feasibility of implementing a non-invasive self-sampling method (CandyCollect) for specimen collection to be tested for respiratory pathogens and the objectives that will be addressed. The study consists of two phases: the first phase of the study will address

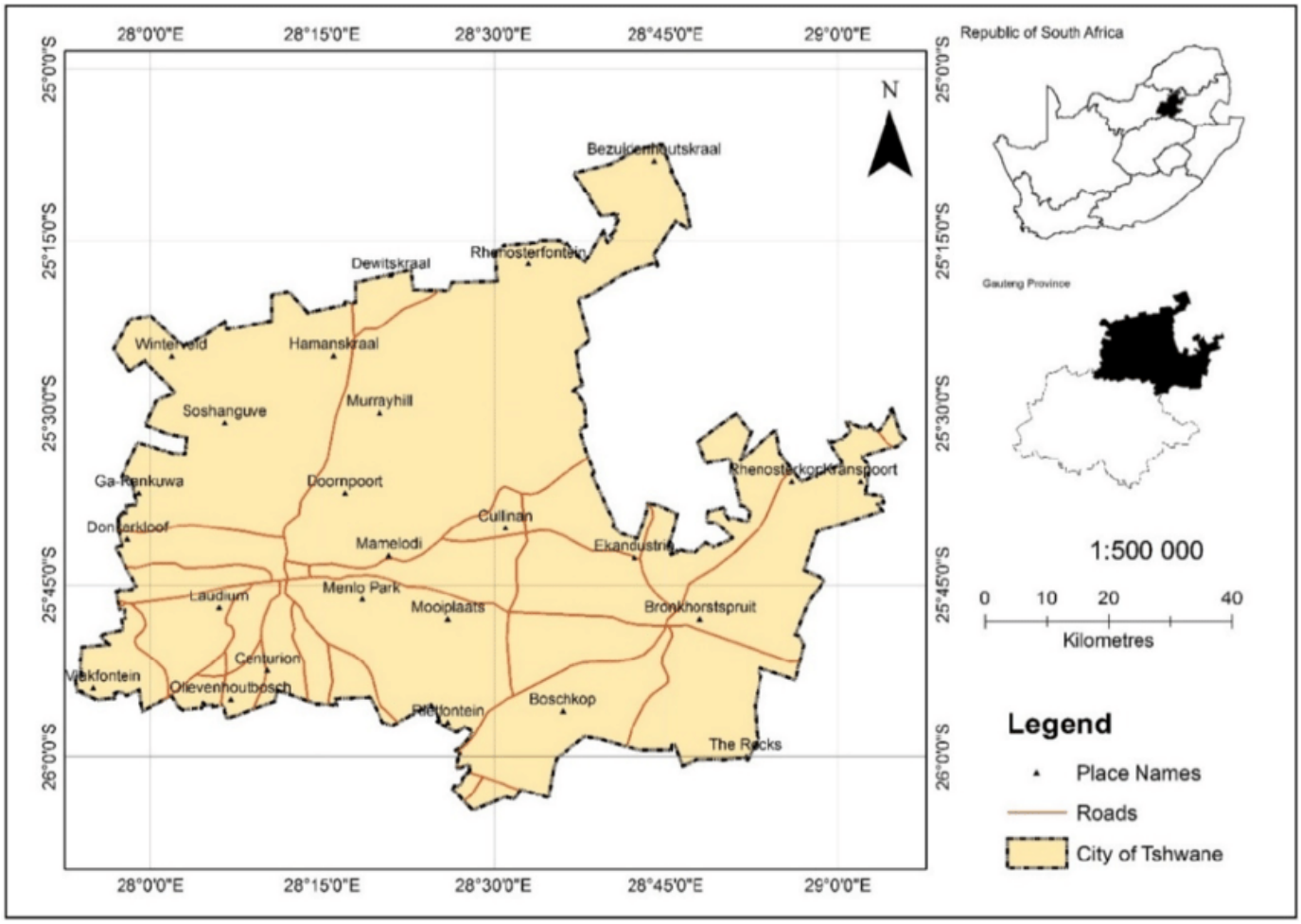

Figure 1 City of Tshwane map.[13]

the first research objective, which is to determine the potential impact of the CandyCollect non-invasive self-sampling method for specimen collection among a variety of population groups in comparison with mouth swabs. The second phase seeks to ascertain user experiences on using the non-invasive CandyCollect self-sampling method for specimen collection in comparison with mouth swabs. This should take less than an hour to complete from recruitment to completion of survey per participant. Figure 3 illustrates the process from sample collection to storage to analysis. This study involves the collection of saliva samples from paediatric participants using two non-invasive self-sampling methods: a standard mouth swab and the CandyCollect lollipop device. Sample collection is performed by the participants under the supervision of trained researchers and caregivers. Following collection, specimens are immediately placed in tubes containing DNA/RNA shield to preserve nucleic acid integrity. The samples will subsequently be analysed using a multiplex PCR panel for the detection of

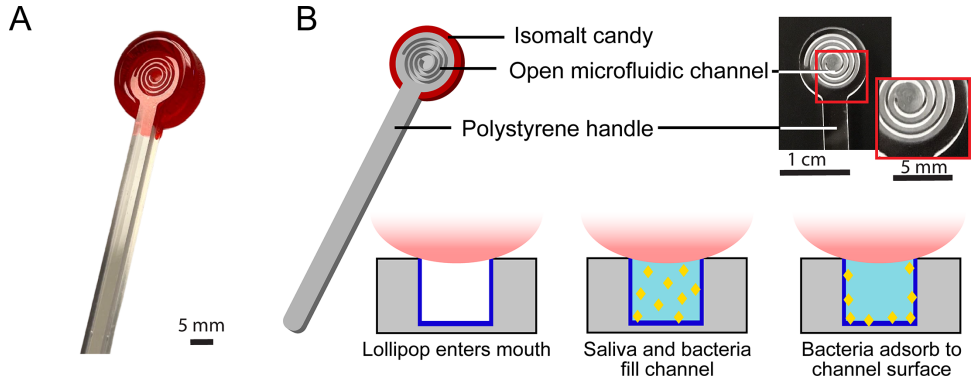

Figure 2 (A) CandyCollect lollipop and (B) CandyCollect mesofluidic channel (spiral groove) and step-by-step illustration of bacteria capture into microfluidic channel (reproduced from Lee, Su *et al* with permission).[15]

Table 1 Summary of methodology for determining the feasibility of implementing a non-invasive self-sampling method for respiratory pathogens

Objectives	Study population	Study design	Outcome measured (guided by REASSURED criteria)	Analysis	Tools of measurement
To determine the potential impact of a non-invasive self-sampling method (CandyCollect) for specimen collection among paediatric patients in comparison with mouth swabs	Paediatric patients at Steve Biko Academic Hospital in the Tshwane District and key stakeholders within those populations	Cross-sectional mixed-methods study	Disease prevalence, Specimen quality, Existing interventions, cost of current intervention, cost of intervention, cost of the POC diagnostic technologies, cost of analysis and reporting, time to completion, ease of use	Quantitative cost analysis, descriptive analysis	Mouth swabs, CandyCollect lollipop, PCR multiplex panel, POC rapid tests, Microsoft Excel, Stata 18
To ascertain user experiences on using the non-invasive CandyCollect self-sampling method for specimen collection in comparison with mouth swabs	Paediatric patients at Steve Biko Academic Hospital in the Tshwane District and key stakeholders within those populations who participated in the study	Mixed methods study (Observations, interviews, surveys)	Usability, ease of specimen collection, simplicity, acceptability, user satisfaction, acceptability, adherence, safety and tolerability, user preferences and barriers	Thematic analysis, descriptive analysis	Surveys/ questionnaires, observations

POC, point of care.

respiratory pathogens, alongside POC rapid diagnostic tests for selected targets.

Objective 1, phase 1

To determine the potential impact of a non-invasive self-sampling method (CandyCollect lollipop) for specimen collection among paediatric patients at Steve Biko Academic Hospital in the Tshwane district compared with using mouth swabs.

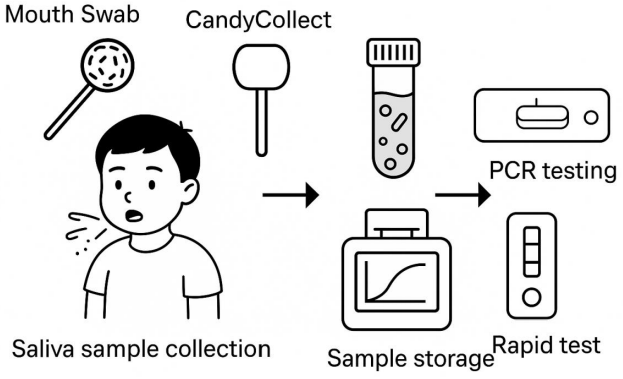

Figure 3 Schematic illustration of the study workflow: paediatric saliva samples are collected using either a mouth swab or the CandyCollect self-sampling device under supervision. Collected samples are stored in DNA/RNA shield tubes and later analysed using multiplex PCR panels for respiratory pathogens and point-of-care rapid antigen tests.

Study design

Observational study design.

Target population

Paediatric patients at Steve Biko Academic Hospital in the Tshwane District and key stakeholders will be recruited to participate. This includes key stakeholders who are the participants (paediatric patients), healthcare workers and parents or guardians of the paediatric patients at Steve Biko Academic Hospital in Tshwane District.

Sample size

A representative sample of the study population will be obtained using a simple random sampling technique guided by the Cochrane sample size formula.[20] The total sample size will be 50 participants. It was calculated using the G-Power 3.1 statistical software. A sample size of 50 participants will allow us to be relatively precise in our conclusions regarding feasibility outcomes. Besides, with this sample size, we can estimate a drop-out rate of 80% to within a 95% CI in our pilot study. The 80% drop-out rate was used as a conservative scenario to determine the precision of our feasibility estimates. As this is a pilot study primarily focused on assessing feasibility, a formal hypothesis test was not the primary objective.[21] A sample size of 50 participants was selected based on guidance for feasibility studies, which recommend a range of 24–50 participants to reliably estimate parameters such as recruitment rate, retention and acceptability.[22] Using G-Power 3.1,

we confirmed that a sample of 50 participants allows for the estimation of a single proportion (eg, adherence or dropout rate) with a 95% CI. This level of precision is considered acceptable for feasibility studies, as it provides sufficient information to inform the design and sample size of a future definitive trial.

Recruitment strategy

The participants will receive verbal invitations to participate. on their agreement to participate, the participants, parents and/or guardians of children will participate in an information session to introduce them to the intervention prior to piloting the study. Parental consent (online supplemental material: appendix A) and child assent (online supplemental materials: appendix B) forms will be distributed, filled and signed prior to the start of the study. Once participants have consented to participating in the study, the researchers will then run through the sampling and survey with each participant.

Eligibility criteria

Inclusion criteria

- Paediatric patients and guardians/parents (for the purpose of consent) at Steve Biko Academic Hospital in Tshwane District who agree to participate in the study as well as healthcare workers within that community.
- Children between the ages of 3–12 years.
- Teenagers (and their caregivers/parents for the purpose of consent) who are willing to participate.

Exclusion criteria

- Non-paediatric patients and guardians/parents at Steve Biko Academic Hospital in Tshwane District.
- Paediatric patients and guardians/parents at Steve Biko Academic Hospital in Tshwane District who decide not to complete the study.

Population groups not within the underserved communities of Tshwane District. ('Underserved communities' are operationally defined based on geographic location and healthcare access indicators.) Individuals residing in areas classified as rural, peri-urban or those designated by national data as having limited access to primary healthcare services were excluded. This geographic approach was used to ensure consistency in identifying participants with adequate access to standard healthcare services for the purposes of this feasibility study.

Sample collection

Caregivers/parents will be shown the two sampling tools (CandyCollect and mouth swab), and researchers will explain how the sampling needs to be conducted. The paediatric patients will also be present for the briefing. Thereafter, the mouth swab will first be provided, and the patients will be afforded the chance to swab themselves under supervision. Once they are done, they will be given the CandyCollect, and the stick will be collected once the candy has completely dissolved. The samples will immediately be transferred into DNA/RNA shield and transferred to storage in the laboratory for further analysis.

Outcome measures

Sample collection method performance

Description: measure of the effectiveness and reliability of the non-invasive CandyCollect self-sampling method and mouth swabs.

Key performance indicators include the success rate of sample collection, the quality of the samples collected (eg, adequacy for diagnostic purposes) and the time required to collect the sample. In this study, sample adequacy is defined as the successful collection of a saliva specimen that meets the requirements for processing using a multiplex PCR panel. Specifically, adequacy includes a minimum volume threshold (≥200 µL), the presence of human cellular material confirmed through amplification of an internal control gene (eg, RNase P) and the absence of PCR inhibitors that may interfere with target amplification.[23] This criterion applies to both CandyCollect and mouth swab samples and is used to assess their suitability for detecting respiratory pathogens through PCR-based analysis.

The goal is to assess whether this method can consistently yield usable samples for diagnostic or research purposes.

Analysis approach

- Sample adequacy rate (eg, percentage of usable samples): pathogen detection in saliva and mouth swab specimens will be conducted using a commercial multiplex respiratory pathogen panel, based on TaqMan real-time PCR technology. The panel targets a range of clinically relevant paediatric respiratory pathogens, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *RSV* and *influenza A/B*, among others. RNA and DNA will be extracted from samples using standard viral nucleic acid extraction kits, followed by qPCR analysis according to the manufacturer's instructions. Each run will include internal positive and negative controls, and samples will be classified as positive if the CT value falls below the specified threshold (typically CT <35). This method will be used to assess the diagnostic adequacy of samples collected with CandyCollect compared with standard mouth swabs. For comparison with PCR results obtained from CandyCollect and mouth swab saliva samples, commercially available rapid antigen tests will be used to detect common paediatric respiratory pathogens such as influenza A/B, RSV and SARS-CoV-2.
- Turnaround time for sample collection and processing will be measured.

Cost and resource analysis

Description: the evaluation of the economic feasibility of the self-sampling method by analysing the associated costs and resources required.

Table 2 Formulas and software to be used to determine the *sample collection method performance*

Metric	Calculation	Software
Adequacy rate	$\text{Adequacy } (\%) = \frac{\text{Number of adequate samples}}{\text{Total samples collected}} \times 100$	Excel
Mean time	$\text{Mean time} = \frac{\Sigma(\text{Time for each sample})}{\text{Number of samples}}$	Excel

It includes direct costs (eg, materials like swabs, lollipop, training of staff) and indirect costs (eg, time spent by healthcare workers or parents).

The analysis also considers potential cost savings compared with traditional sampling methods. The findings will help determine whether the method is affordable and sustainable in the study's healthcare setting.

Analysis approach: analyse existing expenditure on diagnostics and treatment for respiratory infections.

Estimate the total cost, including sample collection kits, transportation and storage.

Calculate both acquisition and ongoing maintenance costs of the POC devices.

Estimate the costs of lab work, data handling and final reporting for the self-sampling method.

Implementation

Description: focuses on system-level processes, feasibility and operational integration.

Analysis approach: feasibility (eg, ease of integration into existing workflows).

Acceptance rates among children and parents during sample collection.

Analysis approach

Sample collection method performance will be assessed by adequacy rates of the CandyCollect lollipop and mouth swabs,[24] as well as the time taken for sample collection and processing used.[25] Table 2 summarises the metrics to be measured, formulas for calculations and software for analysis of the data.

Cost and resource analysis focuses on estimating the financial impact of both current interventions and the proposed self-sampling method. This outcome measure will be assessed by calculating and comparing the direct (administration, distribution, manufacturing, marketing/education sample processing) and indirect costs (patient convenience, training, implementation), as well as the cost-effectiveness of both methods based on successful sample collection and processing. Training costs for CandyCollect will be calculated based on the time and hourly wage required to train staff and caregivers, while no training cost will be attributed to the mouth swabs due to existing staff proficiency. Patient convenience will be quantified using time-to-completion metrics and participant-reported ease-of-use scores. These data will be monetised using time valuation methods and willingness-to-pay benchmarks to estimate indirect costs related to user burden and accessibility. The collected data will be compared to determine which sampling method is more cost-effective. Table 3 summarises this and includes the formulas and software to be used to collect this information.

Implementation focuses on the practical aspects of integrating the CandyCollect lollipop method into clinical workflows. The evaluation examines operational feasibility and process efficiency. The metrics measured, calculations and analysis to be carried out are summarised in table 4:

Table 3 Formulas and software to be used to determine the cost and resource analysis

Metric	Calculation	Software
Direct cost per sample	$\text{Direct cost per sample} = \frac{\text{Total direct costs}}{\text{Number of samples collected}}$	Excel
Indirect cost per sample	$\text{Indirect cost per sample} = \frac{\text{Total indirect costs}}{\text{Number of samples collected}}$	Excel
Total cost per sample	Total cost per sample=direct cost per sample+lidirect cost per sample	Excel
Cost per successful sample	$\text{Cost per Successful Sample} = \frac{\text{Total cost}}{\text{Number of adequate samples collected}}$	Excel
Incremental cost-effectiveness ratio	$\text{ICER} = \frac{\Delta \text{ cost}}{\Delta \text{ Effectiveness}}\Delta$ Cost = Cost of CandyCollect−cost of mouth swab Δ Effectiveness = Effectiveness of CandyCollect − effectiveness of mouth swabs	Stata
Cost Comparison	Compare costs using **t-tests** (for normal distributions) or **Mann–Whitney U tests** (for non-normal data)	Stata

Table 4 Formulas and software to be used to determine feasibility of implementation

Metric	Calculation	Software
Feasibility	*Workflow efficiency:* Time savings (%) = $\frac{\text{Avg. time before}}{\text{Average time after}} \times 100$ *Bottleneck analysis:* Step proportion (%) = $\frac{\textit{Time spent on step}}{\textit{Total workflow time}} \times 100$	Excel
Acceptance rates	Acceptance rate (%) = $\frac{\textit{No. who accepted method}}{\textit{Total no. of participants}} \times 100$	Excel

Objective 2, phase 2

To ascertain user experiences on using the non-invasive self-sampling method for specimen collection among paediatric patients at Steve Biko Academic Hospital in Tshwane District compared with using mouth swabs.

Study design

Cross-sectional mixed-methods study.

Target population

Paediatric patients and guardians/parents at Steve Biko Academic Hospital in Tshwane District who complete phase 1 of the study. They will be engaged to achieve objective 2, phase 2. Although participants may sign up to participate, it is possible to have participants pull out of the study before completion; therefore, this group can only be determined once objective 1 is complete.

Sample size

A representative sample of the study population obtained under the first objective using a simple random sampling technique guided by the Cochrane sample size formula[20] will be used. The total sample size will be 50 participants in order for us to be relatively precise in our conclusions regarding feasibility outcomes. It was calculated using the G-Power 3.1 statistical software. As previously mentioned, with this sample size, we can estimate a drop-out rate of 80% to within a 95% CI in our pilot study. The sample size will also depend on the number of participants who remain in the study right through to the end.

Recruitment strategy

Key stakeholders, which include the study participants of objective 1, will be invited to answer and discuss survey questions to ascertain the feasibility of implementing this CandyCollect self-sampling tool and further determine the cost-effectiveness of implementing this intervention. They will be asked a number of questions in order to ascertain the user experience on using the non-invasive self-sampling method for specimen collection in comparison with mouth swabs.

Eligibility criteria

Inclusion criteria

- Paediatric patients and guardians/parents at Steve Biko Academic Hospital in Tshwane District who participated in objective 1 of the study to completion. Healthcare workers who agreed to this study being conducted within their clinics will also be engaged in a few questions and brief discussions.

Exclusion criteria

- Individuals who are not part of the underserved communities of Tshwane District.
- Participants who do not want to participate in the study.
- Individuals who are not healthcare workers or researchers associated with diagnosing of respiratory pathogens and infections within the underserved communities of Tshwane District.
- Paediatric patients who will not be able to complete the survey for developmental or medical reasons.

Outcome measures

The outcome measures with descriptions are summarised below as well as how these outcomes will be reported are shown in table 5.

Analysis approach

A caregiver survey (online supplemental materials: appendix C) and a child-friendly survey with illustrations (online supplemental materials: appendix D) will be discussed and completed with the participants after specimen collection. The survey used in the present work was adapted from the survey used in Tu *et al*[17]; notably, the Wong-Baker FACES Pain Rating Scale used in the survey has been previously described.[26] The analysis approaches for each outcome measure are summarised in table 2. To assess user experiences with the non-invasive self-sampling method for specimen collection, in comparison with traditional mouth swabs, a combination of multivariate and descriptive statistical analyses will be employed. An evaluation survey will be administered to gather data on user satisfaction, preferences and ease of use for both sampling methods. Descriptive statistics will be calculated to summarise the central tendencies and variability of the data. Specifically, means and SD will be used to describe the continuous variables related to user experience, such as ease of use and comfort level for each method. In addition, frequencies and percentages will be computed for categorical data, such as user preference and perceived effectiveness. These statistics will provide an overview of how respondents evaluate the two sampling methods.

Table 5 Summary of measured outcomes, description and analysis approach

Outcome measure	Description	Analysis approach
User satisfaction	Evaluate satisfaction with ease of use, comfort and convenience.	Descriptive statistics (mean, SD); visual representations (eg, bar charts)
Acceptability	Assess willingness to adopt the self-sampling method for regular healthcare. Measure perceived convenience compared with traditional methods.	Frequency, percentage, inferential statistics (eg, X^2) Descriptive statistics (mean, SD); frequency and percentage
Usability	Assess how easy and intuitive the self-sampling process is for users. Track physical discomfort or complications during/after sample collection.	Descriptive statistics (mean, SD); frequency; regression analysis if applicable Frequency, percentage; qualitative feedback if applicable
Safety and tolerability	Observe distress using visual scales or behavioural cues.	Descriptive statistics; frequency, percentage; inferential statistics
User preferences and barriers	Identify preferences on frequency, ease of sampling Explore concerns hindering adoption of the self-sampling method.	Descriptive statistics; qualitative feedback Qualitative feedback; frequency, percentage
Ease of specimen collection	Evaluate how simple and straightforward specimen collection is.	Descriptive statistics; frequency
Simplicity	Assess users' perception of how simple the sampling process is. Track the number of participants willing to use the method.	Descriptive statistics (mean, SD); frequency Frequency, percentage
Recruitment and adherence	Measure the proportion following sample collection instructions. Measure the proportion of participants who successfully complete the process.	Frequency, percentage

Visual representations, such as bar charts, will be created to illustrate the distribution of user responses and facilitate the comparison between the two methods. These charts will visually depict the proportion of users reporting positive or negative experiences with each method, helping to highlight key trends. Inferential statistics, including X^2 tests, will be conducted to examine the relationships between categorical variables, such as method preference and demographic factors (e.g, age, gender). This will allow for the identification of statistically significant differences or associations in user experiences across various groups. All analyses will be performed using STATA software, which will facilitate the efficient calculation of these descriptive and inferential statistics. The results will be interpreted to provide insights into the feasibility and acceptability of the non-invasive self-sampling method compared with traditional mouth swabs, informing future implementation strategies.

Thematic analysis will be performed on the qualitative data to identify relevant themes from the end-user perception evaluation. The following steps will be followed to determine relevant themes from the data collected:

- The questionnaire will undergo a comprehensive review to ensure data familiarisation.
- Data preparation will involve transcription and systematic organisation into relevant thematic categories.
- The findings will be documented in detail, incorporating direct participant quotations to support key insights.
- The results will be validated by comparing them with findings reported in the existing literature.
- Where applicable, key themes will be quantified by reporting the frequencies or percentages of recurring patterns.

ETHICS AND DISSEMINATION

The study protocol has been approved by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria (509/2023) and the South African Gauteng Province Department of Health (GP_202406_032). Changes to the protocol will be reported to the relevant committees. Any changes to this protocol will be reported to these committees. The trial is registered in the Pan African Clinical Trial Registry (PACTR202411743094783). Informed consent will be obtained from all participants, guardians and parents before data collection. For minors, child assent will be secured through age-appropriate forms (ages 7–18). Researchers will ensure participants understand the study before signing. All patient data will remain confidential and stored securely in password-protected electronic systems.

Data management

The study adheres to the Protection of Personal Information Act.[27] Personal data will be securely managed and not retained longer than necessary. Written and voice-recorded data will remain confidential, stored on a password-protected server with access limited to the research team. Backup data will be on Google Cloud, and data transfers to statisticians will occur via secure file transfer. Data will be stored confidentially for up to 2 years post-study, after which records will be de-identified to prevent reconstruction. Participants will receive a copy of the signed consent, and no personal identifiers will be used in questionnaires, which will be numbered according to sample numbers.

Patient and public involvement

It was not appropriate or possible to involve patients or the public in the design of this study. Participants will be engaged in data collection processes, including using CandyCollect self-sampling tool and providing feedback on their experiences. Participants will be acknowledged in the acknowledgement section of all three publications for their contributions to the study.

Risk of bias

To minimise bias in the study, a randomised sampling approach and standardised data collection protocol and sample size calculation will be employed prior to the study being conducted. Additionally, a pre-specified data analysis plan, multidisciplinary research team, piloting and quality control, and registration and publication of the study protocol will be implemented to reduce the risk of bias and ensure transparency and accountability.

Study timeline

Data collection is anticipated to be carried out between November 2024 and February 2025. The entire study is projected to be completed within 1 year.

Author affiliations
[1]School of Health Systems and Public Health, University of Pretoria, Pretoria, Gauteng, South Africa
[2]University of Pretoria Faculty of Health Sciences, Pretoria, Gauteng, South Africa
[3]Faculty of Health Sciences, University of Pretoria, Pretoria, Gauteng, South Africa
[4]Nuclear Medicine Research Infrastructure (NuMeRI) NPC, University of Pretoria, Pretoria, Gauteng, South Africa
[5]School of Health Systems, University of Pretoria, Pretoria, Gauteng, South Africa
[6]Nuclear Medicine, University of Pretoria, Pretoria, Gauteng, South Africa
[7]Chemistry, University of Washington - Seattle Campus, Seattle, Washington, USA
[8]Department of Chemistry, University of Washington, Seattle, Washington, USA
[9]Department of Urology, University of Washington, Seattle, Washington, USA

Acknowledgements SRN would like to thank TPMT, ABT and University of Washington for conceptualising this study, based in part on prior studies conducted with the CandyCollect device at the University of Washington and the University of Wisconsin–Madison, and the University of Washington team for providing the CandyCollect lollipops and mouth swabs that will be used to conduct this study. We would like to acknowledge Dr Gregory P. DeMuri, Dr Ellen R. Wald, Elena Alfaro, Andrea Blom, Dr Ulri Lee, Damielle L. Hieber, Anika M. McManamen, Victoria A. M. Shinkawa and Karen N. Adams for contributions to the development of the CandyCollect device and survey instrument used in this study. Thank you to the co-authors for contributions to study design and execution and assisting in refining and reviewing the protocol. Thank you to the University of Pretoria for hosting me as a post-doctoral fellow to be able to conduct this research.

Contributors Conceptualisation: SRN, ABT and TPM- T; methodology: SRN, MK, ABT, TPM- T, DBH, W-CT, IHR, XS. EB, ST, AS and XS; project administration: TD, NVS, SR and SRN. Supervision: ABT and TPM- T. Validation: TPM- T. Writing—original draft: SRN. Writing—review and editing: SRN, TPM- T, IHR and ABT. Statistical analysis: AM. Guarantor: SRN.

Funding This study at the University of Pretoria has not received funding. CandyCollect devices were provided by the collaborating team led by ABT at Washington University, which acknowledges funding from the Washington Research Foundation for initial CandyCollect development; further support was provided by Schmidt Sciences and the University of Washington.

Map disclaimer The depiction of boundaries on this map does not imply the expression of any opinion whatsoever on the part of BMJ (or any member of its group) concerning the legal status of any country, territory, jurisdiction or area or of its authorities. This map is provided without any warranty of any kind, either express or implied.

Competing interests ABT, XS, EB and ST filed patent 46 63/152,103 (International Publication Number: WO 2022/178291 Al) through the University of Washington on the CandyCollect oral sampling device. ABT reports filing multiple patents through the University of Washington and receiving a gift to support research outside the submitted work from Ionis Pharmaceuticals. EB is an inventor on multiple patents filed by Tasso, Inc., the University of Washington and the University of Wisconsin. ST has ownership in Salus Discovery, LLC and Tasso, Inc. EB has ownership in Salus Discovery, LLC and Tasso, Inc., and is employed by Tasso, Inc. However, this research is not related to these companies. ST, EB and ABT have ownership in Seabright, LLC, which will advance new tools for diagnostics and clinical research, potentially including the CandyCollect device. The terms of this arrangement have been reviewed and approved by the University of Washington in accordance with its policies governing outside work and financial conflicts of interest in research. The other authors declare no other conflicts of interest.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Siphesihle Nxele http://orcid.org/0000-0002-5621-0846
Thobeka Dlangalala http://orcid.org/0000-0003-1331-4247
Alfred Musekiwa http://orcid.org/0000-0001-5880-3680

REFERENCES

1 Wang X, Li Y, O'Brien KL, *et al*. Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study. *Lancet Glob Health* 2020;8:e497–510.
2 Li Y, Wang X, Blau DM, *et al*. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. *The Lancet* 2022;399:2047–64.
3 Cohen C, McMorrow ML, Martinson NA, *et al*. 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. *Influenza Resp Viruses* 2021;15:789–803.
4 Tempia S, Walaza S, Bhiman JN, *et al*. Decline of influenza and respiratory syncytial virus detection in facility-based surveillance during the COVID-19 pandemic, South Africa, January to October 2020. *Euro Surveill* 2021;26:2001600.
5 Avendaño CarvajalL, Perret Pérez C. Epidemiology of respiratory infections. In: *Pediatric respiratory diseases: a comprehensive textbook*. 2020: 263–72.
6 Chan KH, Lee P-W, Chan CY, *et al*. Monitoring respiratory infections in covid-19 epidemics. *BMJ* 2020;369:m1628.
7 Yovo E. Challenges on the road to achieving the SDG 3.2 targets in resource-limited settings. *Lancet Glob Health* 2022;10:e157–8.
8 General A. United nations transforming our world: the 2030 agenda for sustainable development. New York, NY, USA Division for Sustainable Development Goals; 2015.
9 Commission NP. National development plan vision 2030. 2013.
10 Govender K, Cowden RG, Nyamaruze P, *et al*. Beyond the Disease: Contextualized Implications of the COVID-19 Pandemic for Children and Young People Living in Eastern and Southern Africa. *Front Public Health* 2020;8.
11 Land KJ, Boeras DI, Chen X-S, *et al*. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol* 2019;4:46–54.
12 Chan A-W, Tetzlaff JM, Gøtzsche PC, *et al*. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586.
13 Magidi J, Ahmed F. Spatio-temporal variations of land surface temperature using Landsat and MODIS: case study of the City of Tshwane, South Africa. *SA J of Geomatics* 2020;9:379–96.
14 Dougan S. Mortality and causes of death in South Africa, 1997-2003: findings from death notifications. *Weekly Releases (1997–2007)* 2005;10:2674.
15 Lee UN, Su X, Hieber DL, *et al*. CandyCollect: at-home saliva sampling for capture of respiratory pathogens. *Lab Chip* 2022;22:3555–64.
16 Sanchez JC, Robertson IH, Shinkawa VAM, *et al*. Integration of Group A Streptococcus Rapid Tests with the Open Fluidic CandyCollect Device. *medRxiv* 2024.
17 Tu W-C, Robertson IH, Blom A, *et al*. Capture of Group A Streptococcus by Open-Microfluidic CandyCollect Device in Pediatric Patients. *medRxiv* 2024.
18 Tu W-C, McManamen AM, Su X, *et al*. At-Home Saliva Sampling in Healthy Adults Using CandyCollect, a Lollipop-Inspired Device. *Anal Chem* 2023;95:10211–20.
19 DeMuri G, Wald ER. Detection of Group A *Streptococcus* in the Saliva of Children Presenting With Pharyngitis Using the cobas Liat PCR System. *Clin Pediatr (Phila)* 2020;59:856–8.
20 Chaokromthong K, Sintao N. Sample size estimation using Yamane and Cochran and Krejcie and Morgan and green formulas and Cohen statistical power analysis by G* Power and comparisions. *Apheit International Journal* 2021;10:76–86.
21 Lewis M, Bromley K, Sutton CJ, *et al*. Determining sample size for progression criteria for pragmatic pilot RCTs: the hypothesis test strikes back! *Pilot Feasibility Stud* 2021;7:40.
22 Totton N, Lin J, Julious S, *et al*. A review of sample sizes for UK pilot and feasibility studies on the ISRCTN registry from 2013 to 2020. *Pilot Feasibility Stud* 2023;9.
23 Oughton M, Brukner I, Eintracht S, *et al*. Importance of Adequate qPCR Controls in Infection Control. *Diagnostics (Basel)* 2021;11:2373.
24 Baratloo A, Hosseini M, Negida A, *et al*. Part 1: Simple Definition and Calculation of Accuracy, Sensitivity and Specificity. *Emerg (Tehran)* 2015;3:48–9.
25 LLC S. StataCorp stata statistical software: release 15.1. College Station, TX; 2017.
26 Wong DL, Baker CM. Wong-Baker faces pain rating scale. *Pain Manag Nurs* 2012.
27 de Waal PJ. The protection of personal information act (POPIA) and the promotion of access to information act (PAIA): it is time to take note. *CACI* 2022;35:232–6.