Assessing SARS-CoV-2 Seroprevalence during Routine Antenatal Care Visits in Zambia

Protocol Version 1.1

13 June 2021

Ministry of Health

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Principle and Co-Investigators

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CDC's role: CDC will not engage in data collection or have access to identifiable data; rather, CDC will provide technical leadership on protocol development, data collection procedures, data analysis, interpretation of results, and their dissemination. All data will be owned by the Zambia Ministry of Health.

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Conflict of interest: The primary investigators and co-investigators have no personal, financial, or other relationships that might pose a conflict of interest (or the appearance of a conflict) in their role in this activity. The contents in this protocol are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Differences from CDC umbrella protocol

This protocol was adapted from a generic protocol approved by CDC entitled, "Assessing COVID-19 seroprevalence from routine antenatal care visits," approved on 12/17/2020 (eClearance ID: 0900f3eb81c55548). No major methodological changes were made. Minor differences made in response to local feedback include:

- Prevalence estimates at district level rather than health facility level
- Twenty women each will be recruited from 40 health facilities among 4 districts each month compared to 30 women from 30 health facilities
- First visit ANC routine lab tests (HIV, syphilis, Hepatitis B, and/or malaria) will be recorded on the questionnaire
- Per guidance from the CDC-Atlanta team, several questions on potential exposure were removed from the questionnaire and COVID-19 vaccination history was added (compared to the generic protocol questionnaire)

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Acronyms

Acronym	Explanation
ANC	Antenatal Care
CIDRZ	Centre for Infectious Disease Research in Zambia
CDC	U.S. Centers for Disease Control and Prevention
CHWs	Community Health Workers
COVID-19	Coronavirus Disease
DHO	District Health Office
DBS	Dry Blood Spot
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
MNCH	Maternal, Newborn and Child Health
MOH	Ministry of Health
MTA	Materials Transfer Agreement
NAAT	Nucleic Acid Amplification Test
NHRA	National Health Research Authority
ODK	Open Data Kit
PEPFAR	U.S. Presidential Emergency Plan for AIDS Relief
PHO	Provincial Health Office
PMTCT_STAT	Prevention of Mother to Child Transmission of HIV
PPE	Personal Protective Equipment
QA/QC	Quality Assurance/ Quality Control
SARS-CoV-2	Severe Acute Respiratory Syndrome-Related Coronavirus-2
SOPs	Standard Operating Procedures
UNZA BREC	University of Zambia Biomedical Research Ethics Committee
VTM	Viral Transport Media
ZNPHI	Zambia National Public Health Institute

1.0 Introduction

1.1 General information on research topic

The novel coronavirus SARS-CoV-2, the cause of COVID-19, was first detected in Wuhan city, China in December 2019 and the first cases were confirmed in Zambia in March 2020. Current data suggest that as many as 50% of cases are asymptomatic [1]. Initial surveillance has focused primarily on patients with symptoms or severe disease, and, as such, the full spectrum of the disease, including the extent and fraction of mild or asymptomatic infections that do not require medical attention are not clear. Estimates of the case fatality ratio, and other epidemiological parameters, will likely be lower than current estimates once the full spectrum of disease is able to be included in the denominator. With any novel virus, such as SARS-CoV-2, initial population seroprevalence is assumed to be negligible. Thus, antibody seropositivity in a population can allow inferences to be made about the extent of infection, the cumulative incidence of infection in the population, and trends in infection over time.

This protocol proposes to investigate the extent of SARS-CoV-2 infection, as determined by seropositivity, among pregnant women attending 1st antenatal care (ANC) visits, as a surrogate for the prevalence in the general population. The utility of ANC-based surveillance has been established for malaria [2-5], and there is no evidence to date suggesting that pregnant women are at higher risk of contracting SARS-CoV-2 than non-pregnant adults, suggesting that ANC-based surveillance could provide useful information for monitoring the spread of SARS-CoV-2 over time [6]. This will be a non-randomized assessment of the potential to leverage pregnant women attending 1st ANC as a pragmatic sentinel population to monitor prevalence of SARS-CoV-2 and to detect changes in transmission in selected areas.

As the Zambian Ministry of Health (MOH) continues to focus on controlling and mitigating further spread of the SARS-CoV-2 through active surveillance for case detection, contact tracing, physical distancing and isolation while strengthening case-based management, it is important to build a national SARS-CoV-2 surveillance system that is crucial for containing transmission of the virus and preparing for future waves of the infection.

1.2 Overview of research and research gaps

This surveillance platform may be able to provide key information on the spread of the SARS-CoV-2 virus through the general population. By using serologic testing to understand trends in the proportion of pregnant women over time who were exposed to SARS-CoV-2, we will be able to understand not only the ultimate proportion of pregnant women who were ever infected, but also be able to track and potentially detect outbreaks (e.g. in areas with increasing positivity rates), unlike data that would be available from a single cross-sectional survey, which could give the overall prevalence at a single point in time. In most places to date, testing has focused on symptomatic people, their contacts or others considered at high risk for infection, but there is limited understanding of the true proportion of asymptomatic cases.

There are gaps in information about the prevalence and clinical characteristics of SARS-CoV-2 in Zambia. We propose to conduct SARS-CoV-2 sentinel surveillance among first-time ANC attendees in selected Maternal, Newborn and Child Health (MNCH) departments in four districts (Lusaka, Chongwe, Chadiza and Chipata) of Lusaka and Eastern provinces to assess SARS-CoV-2 seroprevalence. Pregnant women will serve as a proxy for the healthy adult population of these districts, much as they have in sentinel surveillance for HIV and malaria. Because we will use serologic testing from blood and not nasal swabs, this collection will not substantially alter the risk for either the health worker nor the woman and will not deplete the supplies of SARS-CoV-2 Polymerase chain reaction (PCR) reagents used for diagnosis.

1.3 Overall purpose of the research

The purpose of this project is to provide information on the seroprevalence of SARS-CoV-2 antibodies in the community with pregnant women as the sentinel population. CIDRZ will work with facility in-charges in the selected sentinel sites to provide sensitization and SARS-CoV-2 antibody testing to pregnant women presenting for their first ANC visit.

2.0 Statement of the Problem

Zambia is experiencing a SARS-CoV-2 epidemic. From March 18, 2020 to February 19, 2021, at total of 73,203 confirmed cases have been reported in Zambia. However, the true number is likely much larger, because surveillance systems for SARS-CoV-2 in Zambia are incomplete [7]. ANC surveillance of SARS-CoV-2 antibodies could provide needed insights into transmission trends in the general population. This surveillance will provide a means to systematically detect SARS-CoV-2 antibodies in pregnant women and notify relevant authorities about the prevalence.

3.0 Rationale/ Justification

Since 2020, the coronavirus disease has become a major public health concern and sustainable and efficient surveillance platforms are urgently needed; assessing women attending 1st ANC should provide insights into transmission trends in the general population. This activity will determine what proportion of pregnant women demonstrate evidence of SARS-CoV-2 infection over time. The findings of this research may be used to change the way health system monitors the epidemic and ensures health services are made available with minimal disruptions.

4.0 Literature Review

ANC surveillance has not been routinely utilized for SARS-CoV-2 in any country. A cross-sectional study from Cape Town, South Africa found a seroprevalence of 38% among residual serum from antenatal clinic attendees in July and August 2020 [8]. Six African countries including Zambia (i.e., Kenya, Malawi, Mozambique, Nigeria, Uganda, and Zambia) are piloting ANC surveillance based on experience for surveillance of other infectious diseases (HIV, syphilis, malaria) [9,10]. ANC surveillance has been a key strategy for HIV for many years, because women attending ANC clinics represent an accessible healthy population [11-13].

Prevalence estimates of SARS-CoV-2 infections have varied widely throughout Africa. This is likely a result of varying time, study population, and study design [14-20]. Nevertheless, SARS-CoV-2 infections are under-reported because of the large asymptomatic proportion of

infections, testing limitations, and surveillance gaps [21,22]. Africa is no exception, where several prevalence surveys (including in Zambia) have shown under-ascertainment of SARS-CoV-2 infections [7,23]. For instance, during a population-based study in six districts in Zambia in July 2020, the ratio of reported cases to estimated infections ranged from 1:1012 (Livingstone District) to 1:21 (Lusaka District) [7]; overall, only 1 case was reported for every 92 infections. In Cape Town, 5% of residual serum specimens from persons living with HIV and women attending ANC clinics testing positive for SARS-CoV-2 antibodies had positive PCR test results in the public health surveillance system [24]. Other studies from other African countries have revealed substantial under-ascertainment of SARS-CoV-2 infections [18,25-27]. Strategies for improvement in surveillance have been called for by the Africa CDC and others [28,29].

5.0 Research questions

This study will assess the seroprevalence of SARS-CoV-2 in pregnant women aged 15-49 years who present to the health facility for their first antenatal care visit. This surveillance will answer the following research question: "What is the seroprevalence of antibodies to SARS-CoV-2 among pregnant women attending first ANC visit in Zambia?"

6.0 Research Aims and Objectives

6.1 Research Aim

The primary aim of this activity is to determine SARS-CoV-2 seroprevalence from women attending antenatal clinics for their first ANC visit as an easy-access population for routine monitoring.

6.2 Primary Objectives

6.2.1 To estimate on a monthly basis the seroprevalence of SARS-CoV-2 among pregnant women aged 15-49 years attending their first ANC visit at selected health facilities in Chadiza, Chipata, Chongwe, and Lusaka districts of Eastern and Lusaka Provinces.

6.2.2 To monitor trends in SARS-CoV-2 seroprevalence in the selected districts.

6.3 Secondary Objectives

6.3.1 To determine the feasibility and acceptability of SARS-CoV-2 antibody tests among pregnant women

6.3.2 To estimate the prevalence of HIV-COVID 19 co-infection among pregnant women aged 15-49 years

6.3.3 To determine sociodemographic factors associated with SARS-CoV-2 seropositivity among pregnant women

6.3.4 To compare trends in seroprevalence among pregnant women with routine surveillance data to determine if ANC sentinel surveillance is a viable surveillance strategy for SARS-CoV-2

7.0 Methodology

7.1 Study Design

This will be a passive surveillance of pregnant women attending their first antenatal care visit at the selected study sites in the four selected districts. Pregnant women attending 1st ANC will be used as a pragmatic sentinel population to monitor the seroprevalence of SARS-CoV-2 over time. Women will be informed of this activity during group health education sessions and will be consented during individual HIV testing and counselling. To minimize discomfort to the participant, spots of blood will be collected from all women consenting to participate (up to 20 women per study site per month) on filter paper for a SARS-CoV-2 serologic test using a finger prick or a venous blood draw, depending on the method being used to obtain blood for routine HIV and syphilis testing during first ANC visits. In addition, women will be asked to respond to a short questionnaire. The woman's name will not be included on the sample or on the form; rather, a unique study ID will be used to link the questionnaire to the sample. Seroprevalence data will be aggregated at the district level on a monthly basis.

7.2 Study Site and Population

7.2.1 Study Sites

Study sites will be selected in each of four purposively selected districts using a probability-proportional-to-size selection of 10 eligible facilities within each district. Eligible facilities will

have averaged at least 19 first ANC visits per month over the course of the previous year. Surveillance activities will be implemented in two urban and rural districts as follows:

Table 1: Study sites and sampling interval for SARS-CoV-2 seroprevalence surveillance in ANC clinics in Chadiza, Chipata, Chongwe, and Lusaka Districts - Zambia

District		Facility	Average monthly ANC attendance (2020)*	Sampling interval
Chadiza	1	Chanida Rural Health Centre	19	1
	2	Nsadzu Rural Health Centre	21	1
	3	Madzaela Health Post	21	1
	4	Bwanunkha Rural Health Centre	23	1
	5	Chanjowe Health Post	24	1
	6	John Rural Health Centre	24	1
	7	Mkumbuzi Rural Health Centre	28	1
	8	Zemba Rural Health Centre	36	1
	9	Chadiza Rural Health Centre	44	2
	10	Miti Rural Health Centre	46	2
Chipata	1	Champhande Rural Health Centre	21	1
	2	Chikando Rural Health Centre	23	1
	3	Lunkwakwa Urban Health Centre	25	1
	4	Mwami Hospital Affiliated Health Centre	28	1
	5	Chipata Hospital Affiliated Health Centre	34	1
	6	Jerusalem Rural Health Centre	35	1
	7	Walela Health Post	56	2
	8	Mchini Health Post	69	3
	9	Namseche Rural Health Centre	92	4
	10	Kapata Urban Health Centre	162	8
Chongwe	1	Nchute Health Post	19	1
	2	Chikumbi Health Post	22	1
	3	Kasisi Rural Health Centre	23	1
	4	Palabana Rural Health Centre	24	1
	5	Kanakantapa Rural Health Centre	27	1
	6	Water Falls Rural Health Centre	28	1
	7	Katoba Rural Health Centre	32	1
	8	Chainda Rural Health Centre	35	1
	9	Ngwerere Main Rural Health Centre	109	5
	10	Chongwe Referral Rural Health Centre	137	6
Lusaka	1	Coptic Hospital	21	1
	2	Kuku Health Post	98	4
	3	Chainda Urban Health Centre	99	4
	4	Matero Main Urban Health Centre	228	11

	5	Chaisa Urban Health Centre	231	11
	6	Kanyama West Health Post	246	12
	7	George Urban Health Centre	371	18
	8	Matero First Level Hospital	407	20
	9	Kanyama First Level Hospital	572	28
	10	University Teaching Hospital - Adult	606	30
* Based upon PMTCT_STAT indicator from PEPFAR MER				

7.2.2 Study Population

A sample of 20 pregnant women attending their first ANC visit at the selected study sites will be enrolled into the study until the monthly sample size is reached.

7.3 Selection of Participants, Sampling and Sample Size

7.3.1 Selection of Participants

Women presenting to the selected study sites for their first ANC visit at selected health facilities in the four districts will be eligible to participate in the study. A facility-specific interval for systematic sampling will be defined based on each facility's monthly mean number of women attending their first ANC visit based on the historic monthly ANC attendance during fiscal year 2020. until the monthly sample size is met at a given facility. Data will be collected over a 12-15 months period of implementation to ensure that the number of required samples are met in all implementing sites.

7.3.1.1 Inclusion criteria

All women presenting to the selected study sites will be considered for inclusion into the study if they are:

- Confirmed pregnant and are registered for their first ANC visit
- Aged 15 to 49 years
- Able to provide consent to participate and have blood sample collected for the SARS-CoV-2 antibody test

7.3.1.2 Exclusion criteria

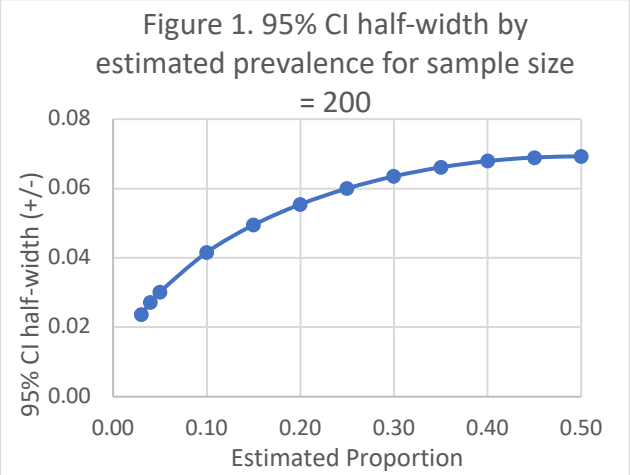
- Not first ANC visit for the pregnant woman
- Women less than 15 years or above 49 years old
- Women not able to provide consent for study participation

7.3.2 Sample Size

All pregnant women who meet the eligibility criteria presenting at the study sites will be approached for participation into the study. Monthly ANC attendance varies by district, as shown in Table 2. Study districts will be expected to recruit 200 pregnant women per month. The estimated seroprevalence is expected to vary by study site, as prevalence has been demonstrated to be different between urban and rural locations in Zambia [7]. A sample size of 200 women per district per month will be sufficient to detect a range of estimated proportions of SARS-CoV-2 seroprevalence (Figure 1). The confidence interval (CI) reflects the bounds of the true seroprevalence of COVID-19 among pregnant women attending ANC (and not the overall pregnant women population or general population). Where prevalence is expected to be low (i.e. rural districts), a sample size of 200 women produces a 95% CI half-width of 2.4% for 3% seroprevalence (lowest estimate). Where prevalence is expected to be higher (i.e. urban districts), a sample size of 200 women produces a 95% CI half-width of 7.0% for 50% seroprevalence. Women will be recruited evenly throughout the month from study sites selected for each district. If the monthly recruitment target is met, study sites will be expected to pause sample collection and reconvene at the start of the next month.

Table 2: Average monthly number of first ANC attendees using PMTCT_STAT denominator

District	**Average monthly ANC attendance**
Chadiza	424
Chipata	759
Chongwe	653
Lusaka	6,378



7.4 Implementation of Procedures

7.4.1 Implementation of pre-survey activities

7.4.1.1 Engage and mobilize stakeholders

At the beginning of the study, we will systematically identify and engage stakeholders (Provincial and District Health Offices (PHO/DHOs) and other implementing partners) to help build involvement and allow them to participate in the planning of activities for implementation and ensure that their expectations are met.

7.4.1.2 Community engagement and sensitization

Information about the study will be cascaded to various levels of the community through sensitizations meetings with community leaders such as headmen, teachers and political leaders. Health care providers at all the sentinel sites will be part of the sensitization meetings with the community. Additionally, information about the study will be incorporated in routine health talks which are given to pregnant women as they come to register for ANC.

7.4.1.3 Train DHO staff from sentinel sites

Health facility in-charges and healthcare workers from the MNCH departments and laboratory hubs will be oriented in the approved study protocol by MOH/CIDRZ research teams and supported throughout the study time. They will also be trained in phlebotomy/specimen collection, labeling and storage, biohazard waste disposal, transfer of specimens, and quality assurance and control. Study staff will also be trained in data collection, documentation, storage and safety, reporting and monitoring. All staff participating in implementation of study activities will be required to obtain good clinical practice and human research protection training. Laboratory staff will be reoriented on running of assays to be used in the study. Additionally, on-site orientation will be conducted to enhance hands-on experience of study procedures. Lay healthcare providers and Peer Educators who have been providing support at the study sites will be selected and trained to provide support for study specific tasks such as participant identification and provision of health education. All staff will be provided with SOPs which will provide them step-by-step instructions on how to perform study procedures.

7.4.1.4 Develop data collection and management tools

The following standard operating procedures will be developed to provide written step-by-step instructions to help study staff to perform routine tasks at the study sites: screening and eligibility, informed consent, study enrolment, sample collection, handling and testing, data collection and management (questionnaire completion, data entry, security, QA/QC), roles and responsibilities SOP, etc).

In addition, a basic questionnaire will be used to collect information from consented ANC attendees. This questionnaire contains information about symptoms of COVID-19, possible exposures to SARS-CoV-2, and risk mitigation behaviors. Additionally, the questionnaire will collect information on sociodemographic characteristics and routine ANC lab results (HIV, syphilis, Hepatitis B, and malaria).

7.4.1.5 Procure study supplies

Study sites will be supplied with all required commodities (i.e., assays, lancets, handwarmers, microtainers, dry blood spot (DBS) cards, protective clothing, polyester fiber-tipped applicator, paper towel, waste segregation polythene bag, depressors, cool box, ice packs, bleach, hand sanitizer, scissors, cryovial with virus transport media (VTM), a fine tipped permanent marker, barcodes, bar code scanner, specimen collection and transportation log forms, etc.) to enable them to collect DBS samples for the SARS-CoV-2 serologic tests. The study will outsource tablets for data entry purposes. Laboratories will be equipped with all necessary materials to process and run DBS samples.

7.4.2 Implementation of survey activities

7.4.2.1 Recruitment of study participants

All study processes will be undertaken as part of routine ANC so as not to disrupt patient flow. Women will be informed of this activity during group health education at the beginning of the first ANC visit. Health education on SARS-CoV-2 will cover symptoms of active infection, testing, and prevention methods, but will emphasize that this study looks for antibodies to SARS-CoV-2 in the blood which are NOT an indicator of current infection.

Women experiencing symptoms of COVID-19 will be managed according to national guidelines (such women will not be recruited in the study because they will not proceed with routine ANC given their acute complaints). Women will be informed that the quality of their antenatal care will not be impacted by their decision whether or not to participate in the study. If they do participate, they will not receive their test results as this study aims to measure population level seroprevalence and does not impact individual clinical care. Women will be approached for consent during individual counseling and testing sessions and blood will be collected on filter paper for a SARS-CoV-2 serologic test using blood from the same finger prick as that for HIV and syphilis testing. While waiting for HIV test results, consenting women will be asked to respond to a short questionnaire. Study participants will then continue with the remainder of the ANC visit as usual.

Sites should have regular access to serological and other routine tests required for care of pregnant women. As a test of past infection, serology results will not be communicated to the facility or participants because they do not affect clinical care (i.e. vaccine eligibility). Any woman that has symptoms of COVID-19 will be referred to relevant testing facility and will follow the national SARS-CoV-2 testing and treatment guidelines. Because they will receive evaluation for their acute complaints per national guidelines, they will not undergo routine ANC services, and, thus, will not be eligible for participation in this study.

7.4.2.2 Sample collection, labelling, storage and courier

All staff (i.e., study and health facility staff) involved in the collection and transportation of specimen will be trained in safe handling practices and spill decontamination procedures. After obtaining consent from the participant and completing the study questionnaire and while maintaining rapport with the participant, the research nurse or phlebotomy staff will collect blood samples onto a dry-blood spot (DBS) card using finger stick procedures by trained staff. DBS samples collected on filter paper using a standard operating procedure will be used for SARS-CoV-2 antibody detection. Specimen and questionnaires will be labeled at time of collection with an identification sticker that has a pre-printed unique study ID and laboratory specimen ID, and current date. A paper log will be completed by study staff that contains the date, study staff ID, study ID, and a lab label to create a paper

trail in case of mislabeling. This paper log will be stored at the health facility and collected by the study team on a monthly basis. DBS cards will be placed in a bag prior to transportation to district hub laboratories for storage and testing using the existing viral load sample courier system supported by PEPFAR.

DBS cards will be tested for anti-SARS-CoV-2 antibodies using a serologic assay. The specific assay has not been determined yet, as potential cross-reactivity is an emerging issue in Africa [30,31]. Ongoing studies at CDC in Atlanta will inform assay selection. Analyses will be done at a hub or central laboratory. Samples will be kept until the study procedures outlined in the protocol are completed or for up to three years, at which time any remaining samples will be destroyed. The samples will not be used for another purpose than what is stated in the protocol.

Sample analysis will be batched as appropriate; total antibodies or IgG antibodies to SARS-CoV-2 will be assessed using laboratory-based serological assays such as enzyme linked immunosorbent assay (ELISA), immunofluorescence (IFA), chemiluminescent assays (CLIA), multiplexed bead-based assays (the specific assay to be utilized in the study in pending guidance from CDC in Atlanta). All testing will be conducted under appropriate and recommended biosafety conditions per CDC guidance.

7.5 Quality control and Assurance activities (supervisory, data and logistics monitoring)

Project supervisors will review the data collection forms and aggregate results on a monthly basis to assess for completeness, accuracy, and consistency. Periodic site visits will be conducted by the research team to assess for adherence to protocol procedures. Deviation from study protocols will be documented in an incident report and reported to the IRB within three working days. Additionally, up to 20% of samples may be run in duplicate at a central laboratory in Lusaka for the purposes of quality assurance.

7.6 Study staff roles and responsibilities

CIDRZ will support the Ministry of Health/Zambia National Public Health Institute to undertake this surveillance by providing technical assistance, training and mentoring of staff based in the health facilities and DHOs, project oversight, and supplies procurement. The project may be supplemented by PEPFAR funded activities as well as with additional technical support from CDC Zambia. The roles and responsibilities of CIDRZ supported are as outlined:

7.6.1 Technical Lead

The technical lead will be responsible for technical oversight throughout the study period. Before implementation, the technical lead will lead protocol development and submission for ethical approval. The technical lead will participate in developing SOPs and training study staff as well as monitoring data reporting and quality control activities. Upon completion of the study, the technical lead will coordinate preparation and dissemination of the final report.

7.6.2 Study Coordinator

The study coordinator will support study implementation by ensuring that all study activities are being implemented as laid out in the approved protocol. He/she will prepare the ethics application and ensure that study staff are working in compliance with, and be responsible for, tracking the project milestones. The study coordinator will manage the study budget, provide support for recruiting and training of staff. Additionally, he/she will be the point of contact between the principal investigator and staff at study sites.

7.6.3 Research Nurse

One research nurse will be employed for each of the study sites. Their role will be to ensure site compliance with the approved protocol by reviewing regulatory requirements. They will be responsible for communicating information about the study to potential participants and ensuring that staff involved with study implementation have the correct credentials and study related training. The research nurse will draw a blood sample onto filter paper via finger prick for SARS CoV-2 antibody testing and will adhere to sample storage and

transportation procedures. They will also ensure that correct source documents are used and that data is accurately captured in the questionnaires. They will act as the point of contact between the study coordinator and site staff.

7.6.4 Community Health Workers

Community health workers advocate for underserved communities to receive services and will be engaged from the already existing cadres known at the facility to supplement efforts of MOH HCWs and research nurses. For this study, they will conduct health education to pregnant women attending ANC visits. They will also be trained on how to collect information from the ANC attendees and administer the questionnaire.

7.6.5 Data Coordinator

The data coordinator will provide support for data management. He/she will ensure that ANC COVID folder on the central server is updated routinely and resolve any problems with the database. He will conduct quality improvement activities and liaise with the study coordinator about any identified quality problems with the data.

7.6.6 Program Assistant

The program assistant will provide administrative support for the study. He/she will submit request for supply purchases, make regular follow ups about delivery timelines and keep stock of supplies. In addition, he/she will submit cash requests for various activities and ensure that receipts are submitted to the Finance office as per stipulated guidelines. The program assistant will identify venues for trainings and communicate with the vendor about scheduled trainings.

7.7 Data collection and management

The study questionnaire and database will be designed using Open Data Kit (ODK)–a free, open-source suite of tools that will allow collection of data using android mobile devices without Internet connectivity. CHWs will be trained to administer the electronic questionnaire that will be uploaded onto password protected android devices (see appendix IV) which will be piloted to ensure that data is correctly captured and stored when data

collection begins. Individual respondents will be assigned a unique identifier to assist with data confidentiality. Respondents' names and other personally identifying information will not be recorded in the questionnaire. Data will be uploaded to a server after each day of ANC data collection. Data collection devices (tablets, storage card) will be handled only by facility or study staff and kept in a locked office, in a lockable cabinet. Data will be exported to MS Excel for cleaning once every week. Any missing variables will be communicated to the research nurse for follow up and action with the CHW.

7.8 Data analysis

Data will be analyzed on a monthly basis using a statistical program. Basic descriptive statistics, by district, including prevalence point estimates, confidence intervals, and relative standard errors will be tabulated. Variance and confidence intervals will be adjusted for health facility clustering. Prevalence estimates will be disaggregated by sociodemographic variables, routine ANC lab results, and risk factors.

7.9 Data Ownership

All data collected through this protocol are the property of the Zambian Ministry of Health. All reviews and decisions regarding data sharing, for example at the request of a journal at the time of publication, will be made by the MoH as the owners of the primary data according to NHRA policies and procedures.

7.10 Sponsor monitoring

CDC is the primary sponsor for the activities described herein. As the sponsor, the CDC may conduct monitoring or auditing of activities to ensure the integrity of the data collected and to ensure the rights and protection of individuals. Monitoring and auditing activities may be conducted by:

7.10.1 CDC staff ("internal")

7.10.2 Authorized representatives of CDC (e.g., a contracted party considered to be "external")

7.10.3 Both internal and external parties.

Monitoring or auditing may be performed by means of on-site visits to government facilities or through other communications such as telephone calls or written correspondence. The visits will be scheduled at mutually agreeable times, and the frequency of visits will be at the discretion of CDC. During the visit, any related materials may be reviewed and the CoAg along with the relevant staff should be available for discussion of findings. To ensure compliance with national and international regulatory guidelines, the activities outlined in this proposal may be subject to inspection or review by authorized Zambian or international regulatory authorities, including the UNZA BREC, NHRA, and US IRBs.

7.11 Dissemination of findings

Monthly summary data from the ANC visits at all participating facilities will be monitored to ensure that data are being collected correctly, however, no formal interim analyses will be conducted. Results will be presented by CIDRZ through tables and disseminated through reports to the Ministry of Health, ZNPHI, CDC, and other stakeholders. Results will also be disseminated through conference presentations and through publications in peer reviewed journals.

8.0 Ethical Considerations

8.1 Ethical committee approvals

The study protocol will be reviewed by the local IRB (UNZA BREC) and CDC before data collection starts for appropriate input and approvals. An information sheet with details about the study will be offered to eligible participants in their preferred language (i.e., (Chewa/Nyanja, Bemba) while study staff provide detailed explanation about the study procedures Participants will be told the general purpose, the possible risks, and benefits in the local language (Nyanja/Chewa or Bemba). Individual written consent will be obtained before sample collection and administration of the questionnaire. Pregnant and recently pregnant women are considered emancipated minors for the purpose of providing their own informed consent. Women will be offered a copy of the consent form if they wish to take it. Participation is voluntary and refusal will not affect a participant's health care. Additionally, participants may refuse to answer specific questions during the questionnaire.

8.2 Risks & Benefits for Subjects

This investigation poses minimal risk to participants, involving the collection of a small amount of blood. Every attempt will be made to utilize the finger prick being made for routine HIV and syphilis testing during the first ANC visit. During the blood collection, women may experience temporary discomfort, and there is a very small risk of infection. The area will be cleaned thoroughly prior to drawing blood to prevent infection. There are no economic risks associated with this study. Pregnant women will not be asked to make any additional visits to the health facility specifically for the purposes of the study. However, the participants may spend additional time (estimated 10 minutes) at the facility during the first ANC visit to complete the questionnaire. The primary benefit of the study is indirect in that data collected will help improve and guide efforts to understand the extent of SARS-CoV-2 infection, which might help contribute to efforts to prevent further transmission of the virus in Zambia. There is no specific direct benefit to participants.

Of note, this activity will NOT contribute to clinical care of the participants in real-time. Among other reasons, this is because serologic conversion in general does not occur until after acute illness; because serologic tests are not diagnostic for current SARS-CoV-2 infection, they are inappropriate for clinical decision making; and because lab analysis would take place remotely and results would not be available until at best days or weeks after the clinic visit. Further, as we will not record any identifiers on the filter paper samples, it will not be possible to link the results of SARS-CoV-2 serologic testing back to the woman from whom the sample was taken.

8.3 Unexpected/Adverse Events

This study does not entail substantial risk to any participants. However, any major deviations from the protocol will be treated as an unanticipated problem/event and will be reported to all IRBs of record within the timeframe specified by the IRB. This also includes any problem or event that: 1) poses harm or risk of harm to subjects or others; 2) is unanticipated (not described in research plan or consent form); and 3) related to the study procedures.

8.4 Prevention of SARS-CoV-2 Infection in Investigation Personnel

All personnel involved in the investigation will be trained in infection prevention and control procedures for SARS-CoV-2 as detailed by the Zambian Ministry of Health, including proper correct use of masks, physical distancing, limiting time in enclosed and/or crowded spaces, and hand hygiene. Personnel who develop symptoms will be isolated, tested, and referred appropriately. They will be allowed to resume involvement after resolution of symptoms and completion of recommended days in isolation.

9.0 Timeline

Activities	Responsible Person	Duration in Months														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Adaptation of protocol to UNZA BREC	PI/Technical Lead	X														
Submission and approval	PI/ Technical Lead		X													
Training for all DHO staff and facility in charges from sentinel sites	Technical Lead/ Study Coordinator			X												
On site orientation for all MCH staff in the sentinel sites	Study Coordinator/ Study Nurses			X												
Enrolment of participants	Facility Staff/ Study Nurses			X	X	X	X	X	X	X	X	X	X	X	X	
Supervisory visits to sentinel sites	All			X	X	X	X	X	X	X	X	X	X	X	X	X
Monitoring availability of all logistics and surveillance activities	Study Coordinator/ Study Nurses/ Program Admin			X	X	X	X	X	X	X	X	X	X	X	X	X
Monthly data reporting and monitoring	Study Nurses			X	X	X	X	X	X	X	X	X	X	X	X	X
Quality control and assurance activities	Technical Lead/ Study Coordinator			X	X	X	X	X	X	X	X	X	X	X	X	X
Data analysis and preparation of the final report	Technical Lead/ SI team														X	X
Presentation of final report by CIDRZ to MoH Senior staff, ZNPHI and CDC	PI/ Technical Lead															X
Presentation of the results by MoH/ZPHI to various stakeholders	MoH/All															X
Submission of the copy of report to Scientific and Ethical Committee	Technical Lead															X
Dissemination of findings (Journal publication, abstract and poster)	Technical Lead															X
Posting findings on Government and CIDRZ websites	Technical Lead															X

10.0 Budget

We are requesting a total of US$300,992.02 which will be allocated to personnel, travel, supplies and other costs. The budget has been broken down as follows:

Category		Cost (US$)	%
Personnel		60,897.93	20.23
Equipment		-	-
Supplies		125,366.00	41.66
Travel		42,120.00	13.99
Other			24.12
	Trainings	26,508.07	
	Stakeholder meetings	1,500.00	
	Communication	3,600.00	
	Administrative costs	9,000.00	
	Stipends for ANC TS and transport refunds	35,000.00	
CIDRZ IDC		-	
Total		**300,992.01**	**100**

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