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variability across SARS-CoV-2 variants**

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# Enhancing diagnostic sensitivity: Investigating molecular mechanisms of Antigen Rapid Diagnostic Test (AgRDTs) variability across SARS-CoV-2 variants

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## Abstract

The emergence of COVID-19, caused by SARS-CoV-2, led to the widespread use of antigen rapid diagnostic tests (AgRDTs) due to their speed, affordability and ease of use. However, the diagnostic sensitivity of AgRDTs has been inconsistent across emerging SARS-CoV-2 variants, with some variants exhibiting reduced detection rates. Thus, AgRDTs have been unreliable in detecting the different variants of SARS-CoV-2. This study explores the molecular mechanisms responsible for this variability, focusing on structural changes in the viral spike (S) and nucleocapsid (N) proteins and how these changes affect antigen-antibody interactions. Using structural biology techniques, such as X-ray crystallography and cryo-electron microscopy, molecular virology approaches like whole genome sequencing, immunoassays including ELISA and surface plasmon resonance (SPR) and computational modelling tools for molecular dynamics simulations, this research will uncover specific mutations that impact diagnostic sensitivity. The results of this study will provide information for the development of next-generation AgRDTs with enhanced sensitivity across diverse viral variants, thereby supporting global efforts in pandemic surveillance and control.

## Keywords

Antigen Rapid Diagnostic Tests (AgRDTs), SARS-CoV-2 variants, diagnostic sensitivity variability, molecular mechanisms, structural biology

## Introduction

The COVID-19 pandemic, now classified by the WHO as an epidemic, has swept across the globe, transforming public health paradigms and highlighting the crucial need for rapid and accurate diagnostic testing. Since SARS-CoV-2 was identified as the causative agent of COVID-19 in December 2019 (Wu et al. 2020), diagnostic methods have been at the forefront of pandemic control measures.

The real-time reverse transcription-polymerase chain reaction (RT-PCR) is the gold standard for SARS-CoV-2 detection because of its high sensitivity and specificity (Behera et al. 2021). However, RT-PCR testing is costly and time-consuming, requiring specialised laboratory set-ups and technical expertise (Alhamid et al. 2022), limiting its accessibility in remote or resource-constrained regions. In the later stages of the pandemic, antigen rapid diagnostic tests (AgRDTs) became an attractive alternative for population-level screening due to their ease of use, low cost and quick results turnaround time (Peeling and Heymann 2021).

Most antigen-detection rapid diagnostic tests (Ag-RDTs) rely on viral nucleocapsid recognition to detect SARS-CoV-2 infection because it is the most abundant viral protein (Dinnes et al. 2022, Lippi et al. 2023, Aboagye et al. 2024a). These tests offer numerous advantages, particularly in settings that demand quick results, such as airports, schools and healthcare facilities, where large-scale screening is essential to curb viral transmission. However, the rapid evolution of SARS-CoV-2, characterised by mutations in the viral genome, has raised significant concerns about the reliability of these tests, particularly as new variants of concern (VOCs) continue to emerge. Variants like Alpha (B.1.1.7), Delta (B.1.617.2) and Omicron (B.1.1.529) have presented unique challenges for AgRDTs, as mutations in key structural proteins could potentially alter antigenicity (Wijayanti et al. 2023) and, by extension, test performance.

The sensitivity of AgRDTs, defined as their ability to identify infected individuals correctly, has shown variability across different SARS-CoV-2 variants. This variability can have serious public health consequences, leading to missed diagnoses (false negatives) and subsequent uncontrolled virus transmission. As a result, understanding the molecular underpinnings of this variability is critical. Specifically, it is necessary to elucidate how mutations in the S and N proteins affect antigen-antibody binding interactions, a key determinant of AgRDT performance.

This study aims to address these critical gaps by investigating the molecular mechanisms underlying the variable sensitivity of AgRDTs across SARS-CoV-2 variants. A

comprehensive understanding of the structural and functional implications of these mutations will provide information for the development of more robust diagnostic tools, ensuring accurate detection regardless of viral evolution. Furthermore, this study could have far-reaching implications for the design of diagnostic tests for other rapidly mutating viral pathogens, thus contributing to global preparedness for future pandemics.

## Background Information

The development of diagnostic tests is fundamental to controlling infectious disease outbreaks. During the early stages of the COVID-19 pandemic, the World Health Organisation (WHO) recommended diagnostic testing as one of the primary strategies to mitigate the spread of SARS-CoV-2 (WHO 2020). Amongst the array of diagnostic tools developed, antigen rapid diagnostic tests (AgRDTs) have been crucial in facilitating large-scale COVID-19 testing (Joji and Shahid 2021). Unlike RT-PCR, which detects viral RNA, AgRDTs target viral proteins, specifically the spike (S) or nucleocapsid (N) proteins, using antigen-antibody interactions on lateral flow assay platforms (Alhabbab 2022). These tests are designed to deliver results within 15–30 minutes, making them invaluable for prompt preliminary real-time decision-making in clinical and public health settings (Amadi 2024).

Despite their advantages, AgRDTs have been scrutinised for their variable performance, particularly their reduced sensitivity compared to RT-PCR (Ghasemi et al. 2022). The sensitivity of AgRDTs has been reported to range between 50% and 80%, depending on factors such as the viral load, stage of infection and the specific test used (Abdul-Mumin et al. 2021, Karon et al. 2021, Dong et al. 2022). Several studies have shown that AgRDT sensitivity is highest when viral loads are high, typically during the early phase of infection when viral replication peaks (Diao et al. 2020, Kahn et al. 2021, Liotti et al. 2021). However, evolving mutations in SARS-CoV-2 variants affecting viral gene expression have resulted in alterations in viral proteins targeted by these tests, complicating the detection process (Harvey et al. 2021, Khan et al. 2022, Thakur et al. 2022). The emergence of SARS-CoV-2 variants has added a new layer of complexity to diagnostic testing. Variants such as Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2) and Omicron (B.1.1.529) harbour mutations in the viral genome, particularly in the genes encoding the S and N proteins (Andre et al. 2023). The S protein, which facilitates viral entry into host cells via the angiotensin-converting enzyme 2 (ACE2) receptor, is the most rapidly evolving region of the virus due to the selection pressures exerted by the host immune system and vaccine interventions (Lu et al. 2023, Yao et al. 2024). Mutations in the S protein, particularly in the receptor-binding domain (RBD), can alter the structural conformation of the protein, potentially affecting the binding of antibodies used in AgRDTs (Xue et al. 2024). Similarly, mutations in the N protein, a highly conserved and abundant structural protein responsible for packaging the viral RNA (Miller et al. 2021), may also impact AgRDT sensitivity. Although the N protein is considered a more stable target for antigen detection due to its lower mutation rate compared to the S protein, recent variants have exhibited changes in key epitopes of the N protein that could reduce

the binding efficiency of antibodies in certain AgRDTs (Vecchio et al. 2021, Rodrigues-da-Silva et al. 2023). Bekliz et al. (2022) showed that some commercially available AgRDTs had diminished performance when detecting the Delta variant, which harbours mutations in both the S and N proteins.

The molecular mechanisms by which these mutations impact AgRDT sensitivity remain poorly understood. It is hypothesised that changes in protein conformation, antigenicity or protein stability may alter epitope recognition, reducing the affinity of AgRDT antibodies for their target antigens (Alexander et al. 1992, Liang et al. 2016). Structural biology techniques, such as X-ray crystallography and cryo-electron microscopy (cryo-EM), can provide insights into how specific mutations affect protein structure and antibody binding (Bodakuntla et al. 2023). In addition, molecular dynamics simulations and immunoassays, such as surface plasmon resonance (SPR), can be used to quantify changes in antigen-antibody interactions caused by these mutations.

This study will combine some techniques and concepts in virology, structural biology and immunology to investigate how mutations in the S and N proteins of SARS-CoV-2 variants affect the diagnostic sensitivity of AgRDTs. By systematically characterising the structural and functional impacts of these mutations, this study aims to uncover the molecular mechanisms that drive variability in AgRDT performance. The results will provide crucial information for improving current diagnostic tools and developing new tests that are resilient to viral evolution, as well as identifying new and/or more conserved or stable diagnostic targets.

## Problem Statement

Antigen rapid diagnostic tests (AgRDTs) have been pivotal in COVID-19 control efforts globally, including in Ghana, due to their accessibility and speed. AgRDTs were initially developed to detect the original Wuhan strain of SARS-CoV-2 (Goux et al. 2024). With the rise of numerous mutations in different variants of concern (VOCs), there is a growing concern that these tests may now have either reduced or compromised antigen recognition capabilities (Osterman et al. 2022, Raïch-Regué et al. 2022). Numerous studies have demonstrated that diagnostic sensitivity varies across different emerging SARS-CoV-2 variants (Raïch-Regué et al. 2022, Aboagye et al. 2024b), with strains like Delta and Omicron exhibiting reduced detection rates in some AgRDTs (Bayart et al. 2022, Cocherie et al. 2022, Krutova et al. 2022, Soni et al. 2022). This inconsistency raises concerns about false-negative results, which can exacerbate viral transmission, especially in low-resource settings like Ghana, where testing infrastructure is already limited. Globally, the issue poses a broader threat to public health, as these undetected cases may fuel new outbreaks. Structural mutations in the spike (S) and nucleocapsid (N) proteins are suspected to disrupt antigen-antibody interactions, thereby reducing test sensitivity (Springer et al. 2022). Despite these concerns, the molecular mechanisms driving this variability remain poorly understood. As the virus continues to evolve, a lack of insight into these interactions could undermine future diagnostic efforts, both in Ghana and worldwide. This study aims to fill this critical knowledge gap by investigating how

mutations in these viral proteins affect AgRDT performance, providing data essential for enhancing diagnostic reliability across diverse settings.

## Significance of Study

Rapid and accurate diagnosis is fundamental to controlling the spread of infectious diseases like COVID-19. AgRDTs offer a practical solution for large-scale testing, particularly in regions where access to PCR testing is limited. However, the reduced sensitivity of AgRDTs against certain SARS-CoV-2 variants poses a significant challenge to public health efforts. By uncovering the molecular mechanisms behind the variability in AgRDT sensitivity, this study will provide critical insights that could lead to the development of more reliable diagnostic tools. The study underscores the hypothesis of Raïch-Regué et al. (2022) that “the performance of AgRDTs for various VOCs depends on the specific antibodies used by each test and viral mutations alone cannot accurately predict their performance”. As a result, understanding the viral epitopes recognised by the capture antibodies in each commercial test is essential for ensuring the efficacy of AgRDTs in detecting different SARS-CoV-2 variants. The findings from this study will contribute to overcoming current diagnostic limitations, providing important information for improving test performance in varied public health settings.

The findings from this study on SARS-CoV-2 antigen rapid diagnostic tests (AgRDTs) hold significant potential for advancing diagnostics for other rapidly evolving viruses, such as influenza, HIV and various respiratory pathogens. Similar to SARS-CoV-2, these viruses experience frequent mutations that can alter antigen-antibody interactions which, in turn, can reduce diagnostic sensitivity. By elucidating the molecular mechanisms that lead to variability in AgRDT performance for SARS-CoV-2, this research establishes a foundation for improving diagnostic accuracy across other viruses.

## Aim and Objectives

The study seeks to investigate the molecular mechanisms responsible for the variability in diagnostic sensitivity of antigen rapid diagnostic tests (AgRDTs) across different SARS-CoV-2 variants.

### Specific Objectives

The study specifically seeks to:

1. Evaluate the diagnostic sensitivity of commercially available AgRDTs across SARS-CoV-2 variants, including Alpha, Beta, Delta and Omicron.
2. Identify structural changes in the spike and nucleocapsid proteins of SARS-CoV-2 variants that may influence AgRDT performance.

3. Investigate the effect of specific mutations on antigen-antibody binding affinity using structural biology techniques, such as X-ray crystallography and cryo-electron microscopy (cryo-EM).

## Research Questions

1. How does the diagnostic sensitivity of AgRDTs vary across different SARS-CoV-2 variants?
2. What are the structural changes in the viral nucleocapsid and spike proteins that may affect AgRDT sensitivity?
3. How do specific mutations in these viral proteins alter antigen-antibody interactions?

## Hypothesis

Variability in the diagnostic sensitivity of AgRDTs across SARS-CoV-2 variants is driven by specific mutations in the viral nucleocapsid and spike proteins, which alter antigen-antibody interactions and reduce binding affinity in some variants.

## Proposed Research Methodology

This study will use a combination of virological, structural and immunological techniques to investigate the impact of SARS-CoV-2 variants on the sensitivity of AgRDTs. AgRDTs will be tested against clinical samples containing different SARS-CoV-2 variants to determine sensitivity thresholds. Structural biology techniques, such as X-ray crystallography and cryo-EM, will be employed to examine the conformational changes in viral proteins. Molecular dynamics simulations and immunoassays, including surface plasmon resonance (SPR) and enzyme-linked immunosorbent assay (ELISA), will quantify the impact of mutations on antigen-antibody binding affinity.

## Study Design and Sample Collection

This cross-sectional and exploratory study will collect archived nasopharyngeal swab specimens from COVID-19-positive individuals in Ghana across multiple testing centres (hospitals, health centres and accredited laboratories). The sample population will be characterised to determine the variant present. These variants will include Alpha, Beta, Delta, Omicron and any emerging variants during the study period. This approach ensures the representation of different SARS-CoV-2 variants circulating in Ghana, which is crucial for assessing the variability in AgRDT sensitivity across these variants. The inclusion of both symptomatic and asymptomatic individuals reflects real-world clinical settings where AgRDTs are widely used for mass screening of COVID-19 (Stoira et al. 2021, Regev-Yochay et al. 2022).

## Sample Size Determination

A sample size of 324 is proposed, based on the previous prevalence of SARS-CoV-2 (30.2%) reported by Aboagye et al. (2024a), a 5% precision level and a confidence level of 95% (z-score: 1.96) using the Cochrane's formula for sample size determination. This sample size has enough statistical power to detect a true effect or difference in establishing the molecular mechanism that underlies the variability in diagnostic sensitivity of AgRDTs across different SARS-CoV-2 variants.

## AgRDT Diagnostic Sensitivity Testing

Each sample will be tested using FDA-approved and commercially available COVID-19 AgRDTs. Sensitivity will be evaluated by determining the limit of detection (LoD) for each test using serial dilutions of viral load. RT-PCR will serve as the gold standard reference for viral detection. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) will be calculated for each AgRDT across different SARS-CoV-2 variants.

## Genetic Characterisation SARS-CoV-2 Variants

### Whole Genome Sequencing (WGS) and Analysis

Viral RNA from each sample will be extracted and confirmed as SARS-CoV-2 positive, as described by Aboagye and Acquah (2023). The SARS-CoV-2 positive RNA will be converted into cDNA using the LunaScript® RT SuperMix Kit (New England Biolabs, UK). The cDNA will be subjected to a multiplex PCR using the ARTIC nCoV-2019/V3 (second batch) primers following the protocol of Quick (2020). Libraries will be prepared from the cDNA and subjected to high-throughput sequencing technology such as Nanopore Sequencing. Data from WGS will allow for comprehensive variant identification and characterisation, which are essential for understanding how specific mutations impact diagnostic sensitivity. Sequences will be analysed using bioinformatics tools to identify mutations in key protein-coding regions, particularly the spike (S) and nucleocapsid (N) proteins. The protein sequences will be aligned with the reference Wuhan-Hu-1 strain to identify specific mutations, particularly those that may affect antibody binding.

Fig. 1

## Protein Expression and Purification

Recombinant nucleocapsid and spike proteins representing wild-type and mutant SARS-CoV-2 forms will be expressed in *Escherichia coli* and mammalian cells to ensure proper folding and post-translational modifications affecting antigenicity. Proteins will be purified by affinity chromatography using tag-specific ligands and quality assessed via SDS-

PAGE and UV spectrophotometry (Stocks et al. 2022). This approach ensures structurally and functionally relevant protein isoforms across variants.

The recombinant proteins will be used in antigen-antibody binding assays (ELISA and SPR) to evaluate the impact of specific mutations on antibody affinity as described by Tapela et al. (2023), addressing questions on diagnostic sensitivity. Where applicable, proteins will also support structural studies (X-ray crystallography). Combined with computational tools like homology modelling and molecular dynamics, this strategy enables a comprehensive analysis of how variant mutations influence AgRDT performance via molecular recognition and conformational effects.

## Immunological Assays: Molecular Mechanism of Antigen Detection

### Antigen-antibody Binding Assays

Surface Plasmon Resonance (SPR) and ELISA will be used to evaluate the binding affinity of AgRDT antibodies to spike and nucleocapsid proteins from various SARS-CoV-2 variants, as has been previously reported (Tapela et al. 2023). The comparison of binding affinities will help determine how specific mutations influence diagnostic sensitivity. Immunoassays will be used to generate quantitative data on antigen-antibody binding, providing insight into the functional effects of structural changes. To be consistent, monoclonal antibodies targeting the spike and nucleocapsid proteins will be sourced from commercial suppliers based on their diagnostic relevance and epitope specificity. Antibodies will not be extracted from antigen rapid diagnostic test kits to avoid technical variability and ensure standardised assay conditions. Using well-characterised monoclonals ensures reliable comparisons of antigenic differences across variants (Mykytyn et al. 2023).

### Structural Biology Analysis

This study proposes to use X-ray crystallography and cryo-electron microscopy (cryo-EM) to examine conformational changes in mutated SARS-CoV-2 spike (S) and nucleocapsid (N) proteins. High-resolution crystal structures will be obtained from recombinant proteins expressed in *E. coli* or mammalian systems, with diffraction data collected at synchrotron facilities. Cryo-EM will complement crystallography by visualising flexible regions of the spike protein, particularly the receptor-binding domain, in mutant variants (Zhu et al. 2023), offering insight into structural heterogeneity affecting antibody recognition. Where experimental methods are limited by crystallisation or stability issues, homology models (AlphaFold) will be used to predict protein structures. These models, alongside public PDB entries, will provide information for molecular docking and dynamics simulations to assess the impact of mutations on antigen-antibody interactions. Using AlphaFold and PyMOL, mutations will be mapped on to 3D structures of the S and N proteins. Analyses will focus on alterations in antigenic epitopes relevant to AgRDT binding, providing a molecular basis for potential changes in diagnostic sensitivity. PyMOL will be used for

visualisation and structural annotation. This step provides a preliminary molecular-level explanation for reduced AgRDT sensitivity.

## **Molecular Dynamics Simulation and Post-Simulation Analysis**

### **Molecular Dynamics Simulation and Protein Expression**

The study will utilise GROMACS 5.1.2 for molecular dynamics (MD) simulations to investigate how mutations in the SARS-CoV-2 spike (S) and nucleocapsid (N) proteins affect their interactions with antibodies used in antigen rapid diagnostic tests (AgRDTs) using a similar approach described by Choi et al. (2022). Wild-type and mutant structures of these proteins will be obtained from the Protein Data Bank (PDB) where available or generated using homology modelling tools such as AlphaFold, particularly for variants lacking experimental structural data.

Although X-ray crystallography and cryo-electron microscopy (cryo-EM) are included in the study design for high-resolution structural characterisation of selected antibody-antigen complexes, their application is contingent on experimental feasibility, specifically the successful expression, purification and crystallisation or grid preparation of stable antigen-antibody complexes. Given these limitations, homology models will be used as the primary structural input for MD simulations to enable consistent comparison across variants. Recombinant spike and nucleocapsid proteins from representative variants will be expressed in *E. coli* and mammalian cells, purified by affinity chromatography and validated using SDS-PAGE and UV spectrophotometry. These proteins will be used for antigen-antibody binding assays and as material for crystallographic and cryo-EM studies where applicable. This integrated strategy ensures structural and functional insights are achieved through both in vitro and in silico approaches.

Molecular docking will predict the most favourable binding orientations between the spike or nucleocapsid proteins and AgRDT antibodies and the protein-antibody complex will be solvated to mimic physiological conditions. Energy minimisation and equilibration under constant pressure and temperature will ensure stability before MD simulations. These simulations will run for 100 to 500 nanoseconds under the NPT ensemble, recording atomic trajectories for subsequent analysis (Al-Karmalawy et al. 2021).

### **Post-Simulation Analysis and Comparative Study**

Post-simulation analysis will involve Root Mean Square Deviation (RMSD) to assess protein stability, Root Mean Square Fluctuation (RMSF) to evaluate residue flexibility and binding free energy calculations using Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) (Miller et al. 2012), to quantify antigen-antibody interactions. Hydrogen bond analysis and principal component analysis will be used to assess molecular interactions and conformational changes. Comparisons between wild-type and mutant variants will help determine the impact of specific mutations on AgRDT sensitivity. Statistical analysis will be conducted to evaluate the significance of observed differences, with visualisation of structural and binding variations. This combined computational and

experimental approach aims to explain the molecular basis of variability in AgRDT sensitivity across SARS-CoV-2 variants.

## Strategies for Improving AgRDTs Performance

### Rational Antibody Design

Based on structural modelling of antibody-antigen complexes and binding affinity data from ELISA and SPR assays, candidate epitopes on the spike (S) and nucleocapsid (N) proteins will be identified. Structural epitope prediction tools such as DiscoTope and ElliPro will be used to map conformational B-cell epitopes on regions of the antigen that engage directly with monoclonal antibodies (Pacheco-Olvera et al. 2022). These predicted epitopes will then be assessed for sequence conservation using multiple sequence alignment (MSA) of viral protein sequences from a diverse set of SARS-CoV-2 variants. Epitopes that are both structurally accessible and conserved across variants will be selected for rational antibody design.

New monoclonal antibodies targeting these conserved regions will be engineered to enhance binding affinity and specificity. These antibodies will be tested against recombinant spike and nucleocapsid proteins from different SARS-CoV-2 variants. Developing antibodies that bind conserved, mutation-resistant regions will improve the robustness of AgRDTs and help ensure their diagnostic performance remains consistent as the virus continues to evolve.

### Enhanced AgRDTs Prototype Testing

Prototypes of AgRDTs incorporating the newly-designed antibodies will be developed. These prototypes will be tested on the validation cohort of samples collected earlier and their performance will be compared to existing commercial AgRDTs while using RT-PCR as the gold standard for evaluating their diagnostic performance. Testing the enhanced AgRDTs will demonstrate whether the new antibody designs improve sensitivity across all variants. This step is critical for translating the molecular and structural insights into practical diagnostic tools that can be deployed in real-world settings.

### Data and Statistical Analysis

All statistical tests will be two-tailed, with  $p < 0.05$  considered significant. Data will be analysed using GraphPad Prism 9.0 (GraphPad Software, USA) and R 4.3.2 (R Foundation, Austria). Results will be presented in tables and figures to compare molecular differences across variants. Logistic regression will assess links between mutations and AgRDT performance. RMSD and RMSF will be used to evaluate protein stability and flexibility, with paired t-tests or non-parametric tests applied, based on distribution. MM-PBSA-derived binding affinities will be compared using either ANOVA or the Kruskal–Wallis test. Differences in hydrogen bonding and non-covalent contacts will be tested using Chi-square or Fisher's exact tests. PCA will detect major conformational shifts, with MANOVA used for group comparisons. External datasets will support cross-

validation. Structural changes and energy profiles will be visualised using Matplotlib and PyMOL.

## Expected Outcomes

1. A detailed evaluation of the diagnostic sensitivity of commercially available AgRDTs across SARS-CoV-2 variants, identifying those that exhibit reduced sensitivity for specific variants.
2. Identification of critical structural modifications in the nucleocapsid and spike proteins that influence antigen-antibody interactions in antigen rapid diagnostic tests (AgRDTs).
3. Understanding how specific mutations in viral proteins alter diagnostic performance, providing a molecular basis for variability in sensitivity.
4. Recommendations for improving the design of AgRDTs to enhance their detection capabilities for emerging SARS-CoV-2 variants and future pathogens with similar mutation profiles.

## Research Impact

The findings from this study on SARS-CoV-2 AgRDTs have significant potential to advance diagnostic strategies for other rapidly evolving viruses, such as influenza, HIV and various respiratory pathogens. Like SARS-CoV-2, these viruses undergo frequent mutations that can alter antigen-antibody interactions, which can reduce diagnostic sensitivity. By elucidating the molecular mechanisms behind the variability in AgRDT performance for SARS-CoV-2, this research can serve as a foundation for improving diagnostic accuracy for other viruses.

In the case of influenza viruses, which frequently undergo antigenic drift and shift leading to mutations in hemagglutinin (HA) and neuraminidase (NA) (Luczo and Spackman 2024 , Perofsky et al. 2024) that may result in false negatives in rapid diagnostic tests. The insights from this study could help design influenza diagnostics that are more resistant to such mutations. In the case of HIV, where the envelope glycoprotein (gp120) mutates rapidly and affects antigen-based detection (Li et al. 2022), the methods used in this research can be adapted to optimise AgRDT performance by predicting how these mutations impact antigen-antibody interactions. Additionally, the structural and computational strategies applied here could support the development of more reliable diagnostics for respiratory viruses, such as respiratory syncytial virus (RSV) and adenoviruses, by focusing on conserved antigenic regions that are less susceptible to mutation, thereby maintaining consistent diagnostic accuracy across viral strains.

In the context of global health, the study's findings will be especially relevant in low-resource settings, where access to PCR-based diagnostics may be limited and rapid tests are the mainstay for controlling infectious disease outbreaks. By developing more mutation-resistant diagnostics for viruses like influenza and HIV, the findings could help ensure that high-sensitivity tests remain available and effective in regions that experience

high viral mutation rates and strain diversity. This would strengthen diagnostic capacity for future pandemics, ensuring rapid identification of cases even as viruses continue to evolve.

## Ethics and security

Ethical clearance would be obtained from the Institutional Review Board of the Council for Scientific and Industrial Research (CSIR-IRB) and the Ghana Health Service Ethics Review Committee in accordance with the declaration of the Helsinki Protocols, which requires researchers to seek ethical approval for studies involving human participants (Kapp 2006).

## Author contributions

The research idea was conceptualised by FTA, MEA and YAA. The manuscript was written by FTA, MKA, MEA and QNDQ, with contributions to methodology by FTA, MKA, QNDQ and YAA. Visualisation was carried out by FTA, while validation was performed by MEA and YAA. The manuscript was reviewed and edited by FTA, MKA, MEA, QNDQ, NAK, HSA, NIM, AKOSE, BCE and YAA. All authors have reviewed the final draft and approved the manuscript for submission.

## Conflicts of interest

The authors have declared that no competing interests exist.

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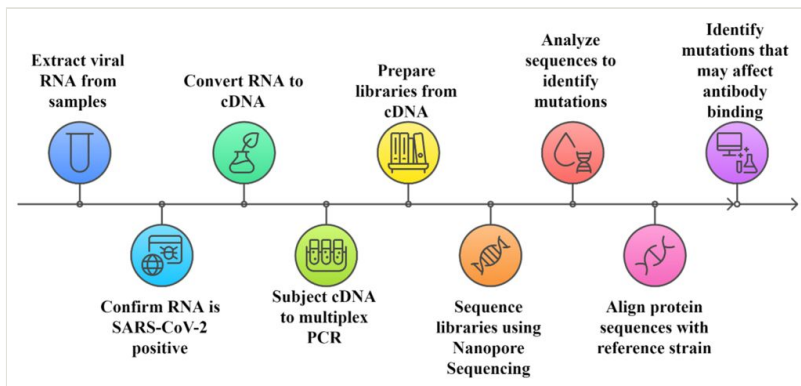


Figure 1.

Schematic diagram of workflow from viral RNA isolation through Whole Genome Sequencing to detection of mutations using bioinformatics tools.