ARPHA Preprints

PENSOFT

PREPRINT

Author-formatted, not peer-reviewed document posted on 06/03/2025

DOI: https://doi.org/10.3897/arphapreprints.e152096

Enhancing Diagnostic Sensitivity: Investigating Molecular Mechanisms of Antigen Rapid Diagnostic Test (AgRDTs) Variability Across SARS-CoV-2 Variants

Frank Twum Aboagye, Mawutor Kwame Ahiabu, Maame Ekua Acquah, Queenstar Quarshie, Naa Kumah, Hannah Akahoho, Nfayem Imoro, Abena Enninful, Bill Egyam, Yvonne Ashong

ARPHA Preprints

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI: https://doi.org/10.3897/arphapreprints.e152096

Enhancing Diagnostic Sensitivity: Investigating Molecular Mechanisms of Antigen Rapid Diagnostic Test (AgRDTs) Variability Across SARS-CoV-2 Variants

Frank Twum Aboagye[‡], Mawutor Kwame Ahiabu[§], Maame Ekua Acquah[|], Queenstar Naa Dedei Quarshie[‡], Naa Adjeley Kumah[‡], Hannah Segbefiah Akahoho[¶], Nfayem Imoro[‡], Abena Konadu Owusu-Senyah Enninful[‡], Bill Clinton Egyam[#], Yvonne A. Ashong[¤]

‡ Biomedical and Public Health Research Unit, CSIR-Water Research Institute, Accra, Ghana
§ The Graduate School, Augusta University, Georgia, United States of America
| West African Centre for Cell Biology of Infectious Pathogens, College of Basic and Applied Sciences, University of Ghana, Legon, Accra, Ghana
¶ Department of Genetics and Biochemistry, Clemson University, South Carolina, United States of America
# Roche Product Ghana Limited, Accra, Ghana
¤ Department of Parasitology, Noguchi Memorial Institute of Medical Research, Accra, Ghana

Corresponding author: Frank Twum Aboagye (frankaboagye71@gmail.com), Yvonne A. Ashong (yashong@noguchi.ug.edu.gh)

Reviewable v 1

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 inform the development of next-generation AgRDTs with enhanced sensitivity across diverse viral variants, thereby supporting global efforts in pandemic surveillance and control.

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

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, 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 specialized laboratory setups 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 (Aboagye et al. 2024a, Dinnes et al. 2022, Lippi et al. 2023). 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, characterized 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

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

comprehensive understanding of the structural and functional implications of these mutations will inform 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 Organization (WHO) recommended diagnostic testing as one of the primary strategies to mitigate the spread of SARS-CoV-2 (WHO 2020). Among the array of diagnostic tools developed, antigen rapid diagnostic tests (AgRDTs) have been critical 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 scrutinized 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, Dong et al. 2022, Karon et al. 2021). 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

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

the binding efficiency of antibodies in certain AgRDTs (Rodrigues-da-Silva et al. 2023, Vecchio et al. 2021). 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 hypothesized 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 characterizing 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 identify 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. Ag-RDTs 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 (Aboagye et al. 2024b, Raïch-Regué et al. 2022), 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

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

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 recognized 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.

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

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 characterized to determine the variant present. These variants will include Alpha, 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 (Regev-Yochay et al. 2022, Stoira et al. 2021).

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

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 and Structural Analysis 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 characterization, 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

Structural Modelling

Using molecular modelling software, PyMOL, the identified mutations will be mapped onto 3D structures of the S and N proteins. The crystal structure of SARS-CoV-2 S glycoprotein and N glycoprotein will be used as templates. Structural analysis will focus on assessing how these mutations alter antigenic epitopes that are targeted by AgRDT antibodies. This will allow for a detailed understanding of how specific mutations affect the conformation of the viral proteins, which is key to predicting how these changes

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

impact antibody binding. This step provides a preliminary molecular-level explanation for reduced AgRDT sensitivity.

Protein Expression and Purification

Recombinant nucleocapsid and spike proteins from representative SARS-CoV-2 variants will be expressed in *E. coli* and mammalian cells. This dual expression strategy facilitates the production of proteins that accurately represent the variations in gene expression across diverse biological systems. This approach ensures that both isoforms are readily available for subsequent analyses. Purification will be performed using affinity chromatography techniques tailored to the specific tags employed during the expression process. This approach facilitates the selective isolation of target proteins based on their interactions with affinity ligands, ensuring high purity and yield. Following purification, the proteins will be assessed for purity and concentration using SDS-PAGE and UV spectrophotometry.

Immunological Assays: Molecular Mechanism of Antigen Detection

Antigen-antibody Binding Assays

Surface Plasmon Resonance (SPR) and Enzyme-Linked Immunosorbent Assay (ELISA) will be employed to assess the binding affinity of AgRDT antibodies to the expressed spike and nucleocapsid proteins from the different SARS-CoV-2 variants. The binding affinities of antibodies against proteins from different SARS-CoV-2 variants will be compared to determine the impact of specific mutations on diagnostic sensitivity. These assays will provide quantitative data on antigen-antibody interactions and the functional impact of structural changes and mutations, which are fundamental to understanding the diagnostic sensitivity of AgRDTs.

Structural Biology Analysis

The study proposes using X-ray crystallography and cryo-electron microscopy to visualize the conformational changes that occur after mutation of the S and N spike proteins of SARS-CoV-2. Using X-ray crystallography, high-resolution crystal structures of the nucleocapsid and spike proteins will be obtained to visualise how mutations affect protein conformation. Protein crystals will be grown, and diffraction data will be collected at synchrotron facilities. Cryo-electron microscopy will be used to visualize the larger, more dynamic regions of the spike protein (Zhu et al. 2023), such as the receptor-binding domain (RBD), particularly in mutated variants. This technique will complement crystallography by providing data on conformational flexibility.

Protein Expression, Molecular Dynamics Simulation and Post-Simulation Analysis

Molecular Dynamics Simulation and Protein Expression

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

The study will utilize the GROMACS 5.1.2 software in molecular dynamics (MD) simulations to investigate how mutations in the SARS-CoV-2 spike (S) and nucleocapsid (N) proteins impact their interactions with antibodies used in antigen rapid diagnostic tests (AgRDTs). Wild-type and mutant variants of these proteins will be sourced from the Protein Data Bank (PDB) or generated using homology modelling tools, if necessary. Additionally, recombinant nucleocapsid and spike proteins will be expressed in *E. coli* and mammalian cells, purified using affinity chromatography, and validated through SDS-PAGE and UV spectrophotometry.

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 minimization 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.

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 (PCA) will provide insights into molecular interactions and significant conformational changes. The results will be compared across wild-type and mutant variants to determine how specific mutations affect AgRDT sensitivity. Statistical tools will be applied to assess the significance of differences between the protein forms, and the findings will be visualized to highlight structural and binding variations. This integrated approach combines computational and experimental methods, offering a comprehensive understanding of the molecular mechanisms behind the variability in AgRDT sensitivity across SARS-CoV-2 variants.

Strategies for Improving AgRDTs Performance

Rational Antibody Design

Based on the structural and binding affinity data, conserved epitopes on the S and N proteins will be identified. New antibodies targeting these conserved regions will be engineered for enhanced binding affinity and tested against recombinant proteins from various SARS-CoV-2 variants. Developing antibodies that target conserved regions unaffected by mutations will help improve AgRDTs' resilience to new variants. AgRDTs can be adapted to maintain diagnostic accuracy even as SARS-CoV-2 evolves by designing antibodies with high affinity for these regions.

Enhanced AgRDTs Prototype Testing

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

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, and p-values less than 0.05 will be considered significant. The data will be analysed using software such as GraphPad Prism and R. Results will be presented in graphs and tables to provide clear visual and quantitative representations of the data, allowing for a detailed comparison of molecular mechanisms underlying the variability in AgRDT sensitivity across SARS-CoV-2 variants. Statistical models such as logistic regression will be applied to the sensitivity data to evaluate correlations between specific mutations and AgRDT performance.

Statistical analyses will evaluate the significance of observed differences between wild-type and mutant protein structures, binding affinities, and molecular dynamics metrics. Structural and binding affinity data will also be integrated to draw comprehensive conclusions about the molecular mechanisms affecting diagnostic sensitivity. The analysis of protein stability and flexibility will involve comparing Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) values for both wild-type and mutant forms of the spike (S) and nucleocapsid (N) proteins using paired t-tests or non-parametric-equivalents, depending on the data distribution. Binding affinities between antibodies and the spike or nucleocapsid proteins, calculated via MM-PBSA, will be compared across wild-type and mutant variants using ANOVA or Kruskal-Wallis tests if the data is not normally distributed. Furthermore, quantitative comparisons of the number and strength of hydrogen bonds and non-covalent interactions will be conducted using appropriate statistical tests, such as Chi-square or Fisher's exact tests, based on the distribution of categorical data. Principal Component Analysis (PCA) will also be employed to identify major conformational changes in the proteins, with differences between wild-type and mutant proteins assessed using multivariate analysis of variance (MANOVA).

Cross-validation with external datasets from other countries or regions will be performed to test the generalisability of the findings beyond the Ghanaian context. Data will also be visualized using tools like Matplotlib and PyMOL to present key structural and energetic differences.

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

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 optimize 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

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI: https://doi.org/10.3897/arphapreprints.e152096

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.

References

- Abdul-Mumin A, Abubakari A, Agbozo F, Abdul-Karim A, Nuertey BD, Mumuni K, Heuschen A, Hennig L, Denkinger C, Müller O, Jahn A (2021) Field evaluation of specificity and sensitivity of a standard SARS-CoV-2 antigen rapid diagnostic test: A prospective study at a teaching hospital in Northern Ghana. PLOS Global Public Health 1 (12). https://doi.org/10.1371/journal.pgph.0000040
- Aboagye FT, Acquah ME (2023) Isolation and Amplification of SARS-CoV-2 RNA from Nasopharyngeal Specimen. Protocol.io1-7. https://doi.org/https://dx.doi.org/10.17504/protocols.io.q26g7y32kgwz/v1
- Aboagye FT, Annison L, Hackman HK, Acquah ME, Ashong Y, Owusu-Frimpong I, Egyam B, Annison S, Osei-Adjei G, Antwi-Baffour S (2024a) Molecular Epidemiology of SARS-CoV-2 within Accra Metropolis Postlockdown. Advances in Virology 2024: 1-12. https://doi.org/10.1155/2024/2993144
- Aboagye FT, Annison L, Hackman HK, Acquah ME, Ashong Y, Owusu-Frimpong I, Egyam B, Annison S, Osei-Adjei G, Antwi-Baffour S (2024b) Comparative evaluation of RT-PCR and antigen-based rapid diagnostic tests (Ag-RDTs) for SARS-CoV-2 detection: performance, variant specificity, and clinical implications. Microbiology Spectrum 12 (6). https://doi.org/10.1128/spectrum.00073-24

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI: https://doi.org/10.3897/arphapreprints.e152096

- Alexander H, Alexander S, Getzoff ED, Tainer JA, Geysen HM, Lerner RA (1992) Altering the antigenicity of proteins. Proceedings of the National Academy of Sciences 89 (8): 3352-3356. https://doi.org/10.1073/pnas.89.8.3352
- Alhabbab R (2022) Lateral Flow Immunoassays for Detecting Viral Infectious Antigens and Antibodies. Micromachines 13 (11). https://doi.org/10.3390/mi13111901
- Alhamid G, Tombuloglu H, Rabaan A, Al-Suhaimi E (2022) SARS-CoV-2 detection methods: A comprehensive review. Saudi Journal of Biological Sciences 29 (11). https://doi.org/10.1016/j.sjbs.2022.103465
- Amadi JG (2024) Comparison of Lateral Flow Immunoassays with PCR for the Detection of SARS-CoV-2 Virus. SVOA Microbiology 5 (3): 57-63. https://doi.org/10.58624/SVOAMB.2024.05.044
- Andre M, Lau L, Pokharel M, Ramelow J, Owens F, Souchak J, Akkaoui J, Ales E, Brown H, Shil R (2023) From alpha to omicron: how different variants of concern of the SARS-Coronavirus-2 impacted the world. Biology 12 (9). URL: https://www.mdpi.com/2079-7737/12/9/1267
- Bayart J, Degosserie J, Favresse J, Gillot C, Didembourg M, Djokoto HP, Verbelen V, Roussel G, Maschietto C, Mullier F, Dogné J, Douxfils J (2022) Analytical Sensitivity of Six SARS-CoV-2 Rapid Antigen Tests for Omicron versus Delta Variant. Viruses 14 (4). https://doi.org/10.3390/v14040654
- Behera B, Mishra R, Thatoi H (2021) Recent biotechnological tools for diagnosis of corona virus disease: A review. Biotechnology Progress 37 (1). https://doi.org/10.1002/btpr.3078
- Bekliz M, Adea K, Essaidi-Laziosi M, Sacks JA, Escadafal C, Kaiser L, Eckerle I (2022) SARS-CoV-2 antigen-detecting rapid tests for the delta variant. The Lancet Microbe 3 (2). https://doi.org/10.1016/s2666-5247(21)00302-5
- Bodakuntla S, Kuhn CC, Biertümpfel C, Mizuno N (2023) Cryo-electron microscopy in the fight against COVID-19—mechanism of virus entry. Frontiers in Molecular Biosciences 10 https://doi.org/10.3389/fmolb.2023.1252529
- Cocherie T, Bastide M, Sakhi S, Zafilaza K, Flandre P, Leducq V, Jary A, Burrel S, Louet M, Calvez V, Marcelin A, Marot S (2022) Decreased Sensitivity of Rapid Antigen Test Is Associated with a Lower Viral Load of Omicron than Delta SARS-CoV-2 Variant. Microbiology Spectrum 10 (5). https://doi.org/10.1128/spectrum.01922-22
- Diao B, Wen K, Chen J, Liu Y, Yuan Z, Han C, Chen J, Pan Y, Chen L, Dan Y, Wang J, Chen Y, Deng G, Zhou H, Wu Y (2020) Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. medRxiv https://doi.org/10.1101/2020.03.07.20032524
- Dinnes J, Sharma P, Berhane S, van Wyk S, Nyaaba N, Domen J, Taylor M, Cunningham J, Davenport C, Dittrich S (2022) Rapid, point-of-care antigen tests for diagnosis of SARS-CoV-2 infection. Cochrane database of systematic reviews URL: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013705.pub3/abstract
- Dong L, Li W, Jiang Y (2022) Performance evaluation of antigen detection rapid diagnostic test (Ag-RDT) for COVID-19 diagnosis in a primary healthcare center during the Shanghai COVID-19 quarantine period. Virology Journal 19 (1). https://doi.org/10.1186/s12985-022-01871-6
- Ghasemi S, Harmooshi NN, Rahim F (2022) Diagnostic utility of antigen detection rapid diagnostic tests for Covid-19: a systematic review and meta-analysis. Diagnostic Pathology 17 (1). https://doi.org/10.1186/s13000-022-01215-6

- Goux H, Green J, Wilson A, Sozhamannan S, Richard S, Colombo R, Lindholm D, Jones M, Agan B, Larson D, Saunders D, Mody R, Cox J, Deans R, Walish J, Fries A, Simons M, Pollett S, Smith D (2024) Performance of rapid antigen tests to detect SARS-CoV-2 variant diversity and correlation with viral culture positivity: implication for diagnostic development and future public health strategies. mBio 15 (12). https://doi.org/10.1128/mbio.02737-24
- Harvey W, Carabelli A, Jackson B, Gupta R, Thomson E, Harrison E, Ludden C, Reeve R, Rambaut A, Peacock S (2021) SARS-CoV-2 variants, spike mutations and immune escape. Nature Reviews Microbiology 19 (7): 409-424. URL: https://www.nature.com/articles/s41579-021-00573-0/1000
- Joji RM, Shahid M (2021) The Role of Antigen Rapid Diagnostic Test in COVID-19 Diagnosis. The Open COVID Journal 1 (1): 108-111. https://doi.org/10.2174/2666958702101010108
- Kahn M, Schuierer L, Bartenschlager C, Zellmer S, Frey R, Freitag M, Dhillon C, Heier M, Ebigbo A, Denzel C, Temizel S, Messmann H, Wehler M, Hoffmann R, Kling E, Römmele C (2021) Performance of antigen testing for diagnosis of COVID-19: a direct comparison of a lateral flow device to nucleic acid amplification based tests. BMC Infectious Diseases 21 (1). https://doi.org/10.1186/s12879-021-06524-7
- Kapp MB (2006) Ethical and legal issues in research involving human subjects: do you want a piece of me? Journal of clinical pathology 59 (4): 335-339.
- Karon BS, Donato LJ, Bridgeman AR, Blommel JH, Kipp B, Maus A, Renuse S, Kemp J, Madugundu AK, Vanderboom PM, Chavan S, Dasari S, Singh RJ, Grebe SK, Pandey A (2021) Analytical Sensitivity and Specificity of Four Point of Care Rapid Antigen Diagnostic Tests for SARS-CoV-2 Using Real-Time Quantitative PCR, Quantitative Droplet Digital PCR, and a Mass Spectrometric Antigen Assay as Comparator Methods. Clinical Chemistry 67 (11): 1545-1553. https://doi.org/10.1093/clinchem/hvab138
- Khan MZI, Nazli A, Al-Furas H, Asad MI, Ajmal I, Khan D, Shah J, Farooq MA, Jiang W (2022) An overview of viral mutagenesis and the impact on pathogenesis of SARS-CoV-2 variants. Frontiers in immunology 13 URL: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1034444/full
- Krutova M, Brajerova M, Kepka Z, Briksi A, Hubacek P, Drevinek P (2022) The evaluation of an automated mariPOC SARS-CoV-2 antigen test compared to RT-qPCR SARS-CoV-2 assay and comparison of its sensitivity in Delta- and Omicron-variant samples. Influenza and Other Respiratory Viruses 16 (6): 1033-1039. https://doi.org/10.1111/irv.13048
- Liang Y, Guttman M, Davenport T, Hu S, Lee K (2016) Probing the Impact of Local Structural Dynamics of Conformational Epitopes on Antibody Recognition. Biochemistry 55 (15): 2197-2213. https://doi.org/https://doi.org/10.1021/acs.biochem.5b01354
- Liotti FM, Menchinelli G, Lalle E, Palucci I, Marchetti S, Colavita F, La Sorda M, Sberna G, Bordi L, Sanguinetti M, Cattani P, Capobianchi MR, Posteraro B (2021) Performance of a novel diagnostic assay for rapid SARS-CoV-2 antigen detection in nasopharynx samples. Clinical Microbiology and Infection 27 (3): 487-488. https://doi.org/10.1016/j.cmi.2020.09.030
- Lippi G, Henry B, Plebani M (2023) An overview of the most important preanalytical factors influencing the clinical performance of SARS-CoV-2 antigen rapid diagnostic tests (Ag-RDTs). Clinical Chemistry and Laboratory Medicine (CCLM) 61 (2): 196-204. https://doi.org/10.1515/cclm-2022-1058

Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI: https://doi.org/10.3897/arphapreprints.e152096

- Li Y, Guo Y, Cheng H, Zeng X, Zhang X, Sang P, Chen B, Yang L (2022) Deciphering gp120 sequence variation and structural dynamics in HIV neutralization phenotype by molecular dynamics simulations and graph machine learning. Proteins: Structure, Function, and Bioinformatics 90 (7): 1413-1424. https://doi.org/10.1002/prot.26322
- Luczo JM, Spackman E (2024) Epitopes in the HA and NA of H5 and H7 avian influenza viruses that are important for antigenic drift. FEMS Microbiology Reviews 48 (3). https://doi.org/10.1093/femsre/fuae014
- Lu X, Chen Y, Zhang G (2023) Functional evolution of SARS-CoV-2 spike protein: Maintaining wide host spectrum and enhancing infectivity via surface charge of spike protein. Computational and Structural Biotechnology Journal 21: 2068-2074. https://doi.org/10.1016/j.csbj.2023.03.010
- Miller B, McGee TD, Swails J, Homeyer N, Gohlke H, Roitberg A (2012) *MMPBSA.py* : An Efficient Program for End-State Free Energy Calculations. Journal of Chemical Theory and Computation 8 (9): 3314-3321. https://doi.org/10.1021/ct300418h
- Miller S, Lee T, Merritt A, Pryce T, Levy A, Speers D (2021) Single-Point Mutations in the N Gene of SARS-CoV-2 Adversely Impact Detection by a Commercial Dual Target Diagnostic Assay. Microbiology Spectrum 9 (3). https://doi.org/10.1128/Spectrum.01494-21
- Osterman A, Badell I, Basara E, Stern M, Kriesel F, Eletreby M, Öztan GN, Huber M, Autenrieth H, Knabe R, Späth P, Muenchhoff M, Graf A, Krebs S, Blum H, Durner J, Czibere L, Dächert C, Kaderali L, Baldauf H, Keppler O (2022) Impaired detection of omicron by SARS-CoV-2 rapid antigen tests. Medical Microbiology and Immunology 211 (2): 105-117. https://doi.org/10.1007/s00430-022-00730-z
- Peeling RW, Heymann DL (2021) Innovations in COVID-19 testing: the road from pandemic response to control. The Lancet. Infectious Diseases 21 (10): 1334-1335. https://doi.org/10.1016/S1473-3099(21)00291-7
- Perofsky AC, Huddleston J, Hansen CL, Barnes JR, Rowe T, Xu X, Kondor R, Wentworth DE, Lewis N, Whittaker L, Ermetal B, Harvey R, Galiano M, Daniels RS, McCauley JW, Fujisaki S, Nakamura K, Kishida N, Watanabe S, Hasegawa H, Sullivan SG, Barr IG, Subbarao K, Krammer F, Bedford T, Viboud C (2024) Antigenic drift and subtype interference shape A(H3N2) epidemic dynamics in the United States. eLife 13 https://doi.org/10.7554/eLife.91849
- Quick J (2020) nCoV-2019 sequencing protocol v1. https://doi.org/10.17504/protocols.io.bbmuik6w
- Raïch-Regué D, Muñoz-Basagoiti J, Perez-Zsolt D, Noguera-Julian M, Pradenas E, Riveira-Muñoz E, Giménez N, Carabaza A, Giménez F, Saludes V, Martró E, Robert N, Blanco I, Paredes R, Ruiz L, Ballana E, Clotet B, Blanco J, Izquierdo-Useros N (2022) Performance of SARS-CoV-2 Antigen-Detecting Rapid Diagnostic Tests for Omicron and Other Variants of Concern. Frontiers in Microbiology 13 https://doi.org/10.3389/fmicb.2022.810576
- Regev-Yochay G, Kriger O, Mina M, Beni S, Rubin C, Mechnik B, Hason S, Biber E, Nadaf B, Kreiss Y, Amit S (2022) Real World Performance of SARS-CoV-2 Antigen Rapid Diagnostic Tests in Various Clinical Settings. Infection Control & Hospital Epidemiology1-20. https://doi.org/10.1017/ice.2022.3
- Rodrigues-da-Silva R, Conte F, da Silva G, Carneiro-Alencar A, Gomes P, Kuriyama S, Neto AF, Lima-Junior J (2023) Identification of B-Cell Linear Epitopes in the Nucleocapsid

(N) Protein B-Cell Linear Epitopes Conserved among the Main SARS-CoV-2 Variants. Viruses 15 (4). https://doi.org/10.3390/v15040923

- Soni A, Herbert C, Filippaios A, Broach J, Colubri A, Fahey N, Woods K, Nanavati J, Wright C, Orwig T, Gilliam K, Kheterpal V, Suvarna T, Nowak C, Schrader S, Lin H, O'Connor L, Pretz C, Ayturk D, Orvek E, Flahive J, Lazar P, Shi Q, Achenbach C, Murphy R, Robinson M, Gibson L, Stamegna P, Hafer N, Luzuriaga K, Barton B, Heetderks W, Manabe Y, McManus D (2022) Comparison of Rapid Antigen Tests' Performance Between Delta and Omicron Variants of SARS-CoV-2. Annals of Internal Medicine 175 (12): 1685-1692. https://doi.org/10.7326/M22-0760
- Springer DN, Perkmann T, Jani CM, Mucher P, Prüger K, Marculescu R, Reuberger E, Camp JV, Graninger M, Borsodi C, Deutsch J, Lammel O, Aberle SW, Puchhammer-Stöckl E, Haslacher H, Höltl E, Aberle JH, Stiasny K, Weseslindtner L (2022) Reduced Sensitivity of Commercial Spike-Specific Antibody Assays after Primary Infection with the SARS-CoV-2 Omicron Variant. Microbiology Spectrum 10 (5). https://doi.org/10.1128/spectrum.02129-22
- Stoira E, Chiesa AF, Elzi L (2021) 'High prevalence of heterotopic ossification in critically ill patients with severe COVID-19' – Author's reply. Clinical Microbiology and Infection 27 (7). https://doi.org/10.1016/j.cmi.2021.02.004
- Thakur S, Sasi S, Pillai SG, Nag A, Shukla D, Singhal R, Phalke S, Velu GSK (2022) SARS-CoV-2 mutations and their impact on diagnostics, therapeutics and vaccines. Frontiers in medicine 9 URL: https://www.frontiersin.org/articles/10.3389/fmed.2022.815389/full
- Vecchio CD, Brancaccio G, Brazzale AR, Lavezzo E, Onelia F, Franchin E, Manuto L, Bianca F, Cianci V, Cattelan A, Toppo S, Crisanti A (2021) Emergence of N antigen SARS-CoV-2 genetic variants escaping detection of antigenic tests. medRxiv. Pages: 2021.03.25.21253802. https://doi.org/10.1101/2021.03.25.21253802
- WHO (2020) Advice on the use of point-of-care immunodiagnostic tests for COVID-19. URL: https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19
- Wijayanti NM, Widyananda MH, Muflikhah L, Widodo N (2023) Mutation-Induced Changes in the Stability, B-Cell Epitope, and Antigenicity of the Sars-Cov-2 Variant Spike Protein: A Comparative Computational Stud. Karbala International Journal of Modern Science 9 (3). https://doi.org/10.33640/2405-609X.3311
- Wu F, Zhao S, Yu B, Chen Y, Wang W, Song Z, Hu Y, Tao Z, Tian J, Pei Y, Yuan M, Zhang Y, Dai F, Liu Y, Wang Q, Zheng J, Xu L, Holmes E, Zhang Y (2020) Author Correction: A new coronavirus associated with human respiratory disease in China. Nature 580 (7803). https://doi.org/10.1038/s41586-020-2202-3
- Xue S, Han Y, Wu F, Wang Q (2024) Mutations in the SARS-CoV-2 spike receptor binding domain and their delicate balance between ACE2 affinity and antibody evasion. Protein & Cell 15 (6): 403-418. https://doi.org/10.1093/procel/pwae007
- Yao Z, Zhang L, Duan Y, Tang X, Lu J (2024) Molecular insights into the adaptive evolution of SARS-CoV-2 spike protein. Journal of Infection 88 (3). https://doi.org/10.1016/j.jinf.2024.106121
- Zhu K, Yuan C, Du Y, Sun K, Zhang X, Vogel H, Jia X, Gao Y, Zhang Q, Wang D, Zhang H (2023) Applications and prospects of cryo-EM in drug discovery. Military Medical Research 10 (1). https://doi.org/10.1186/s40779-023-00446-y

ARPHA Preprints
Author-formatted, not peer-reviewed document posted on 06/03/2025. DOI:
https://doi.org/10.3897/arphapreprints.e152096

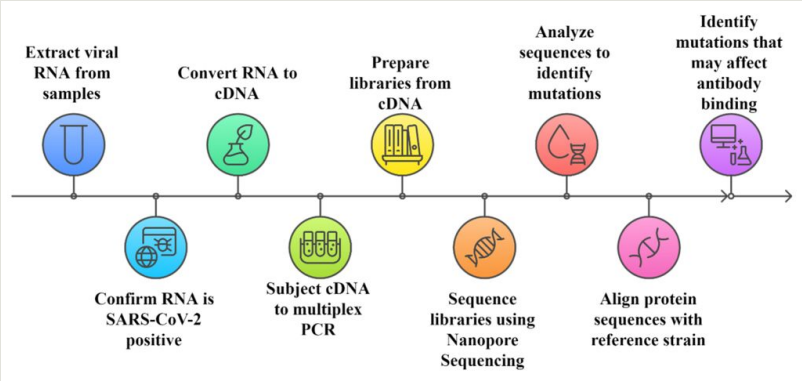

Figure 1.

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