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A Field Evaluation of the Covid-19 Antigen Testing Regimen at a Primary Hospital in Ghana

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Abstract **Background:** Accurate, reliable and rapid diagnostic tools for COVID-19 are essential for tackling the ongoing pandemic, especially in the developing world. This study aimed to assess the performance and viability of the testing regimen at a district-level hospital testing centre where both nasopharyngeal and oropharyngeal samples are taken simultaneously but the samples are tested separately. The nasopharyngeal samples are tested using the SD Biosensor Standard F Covid-19 FIA antigen test kit whiles RT-PCR is conducted on the oropharyngeal samples. **Methods:** A retrospective analysis was conducted on 314 paired samples taken from March to August 2021 whereby the dual testing scheme was applied. The field diagnostic performance of the Standard F Antigen test was compared with Reverse Transcription Polymerase Chain Reaction (RT-PCR) results. The sensitivity, specificity and expected predictive values were then evaluated. The RT-PCR is the gold standard for Covid-19 testing. **Results:** A total of 314 matched Covid-19 Antigen Rapid Diagnostic Test (Ag-RDT) and RT-PCR samples were analysed retrospectively. The sensitivity and specificity of the Ag-RDT was 80.85% (95% Confidence interval of 66.74% – 90.85%) and 98.9% (95% Confidence interval of 96.74% – 99.77%) respectively. The overall accuracy was determined to be 96.2% with a Cohen's Kappa score of 0.84 indicating an almost perfect agreement. **Conclusion:** The results of samples taken from the different anatomical sites have an almost perfect agreement. Therefore, the performance of the Standard F Covid-19 antigen test kit is optimal and the testing regimen at the hospital is adequate. It can thus be replicated at other testing sites. However, adequate training in the sampling procedure, storage, transport as well as stringent monitoring and supervision is required.

Keywords: *Covid-19, antigen testing, Sars-Cov 2*

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1. Introduction

The Covid-19 pandemic has brought about a heavy burden and extraordinary pressure on healthcare systems [1,2,3], the developing world has not been spared either. Testing is one of the key pillars in managing this pandemic. Accessibility and availability of rapid and accurate diagnostic tools for COVID-19 are very crucial for disease surveillance and contact tracing to curtail the spread of the virus [1,3,4].

Testing for SARS-CoV-2 infections is conducted through both direct and indirect methods. The direct method involves the identification of viral RNA or Antigens whiles the indirect method involves the identification of specific antibodies to Covid-19. Examples of direct methods of Covid-19 detection are; real-time reverse transcription-polymerase chain reaction (RT-PCR), reverse transcription-loop-mediated isothermal amplification (RT-LAMP), and antigen-based tests. Examples of Indirect Methods are serological tests, enzyme-linked immunoassay (ELISA), chemiluminescence immunoassays, these are conducted for diagnostic purposes, establishing prior exposure to the virus and/or vaccine, [4,5].

RT-PCR is the "gold standard" assay for the laboratory diagnosis of both symptomatic and asymptomatic COVID-19 cases [5]. The RT-PCR however has its limitations, including the relatively high cost, need for qualified personnel and sophisticated equipment and a suboptimal turnaround time [5]. To help address RT-PCR shortcomings, antigen-detecting rapid diagnostic

tests (Ag-RDTs) have been developed and are increasingly common in the clinical setting with many brands available [6,7]. If Ag-RDTs are accurate, they may have a greater public health impact than RT-PCR [8]. In Ghana, only a few Covid-19 Antigen test kits have been approved by the Food and Drugs Authority (FDA) [6]. The approved Antigen kits include Standard F and the Standard Q test kits and indeed, few healthcare facilities have the capability and approval for Covid-19 testing in Ghana.

According to Dinnes and ECDC [3,5], the Covid-19 Antigen test have several advantages over the RT-PCR including;

(i) No need for high-level technical expertise and laboratory capacity;

(ii) May be performed locally in a decentralized locality with the associated logistic advantages;

(iii) May facilitate timely decisions regarding quarantine and/or treatment regimens and epidemiological investigations of new clusters

Conversely, Ag-RDTs are less sensitive than RT-PCR [6]. A recent Cochrane review [2,3] underlined significant differences between study inconsistencies in sensitivity and specificity estimates between Ag-RDT's as well as viral load. The WHO [10] recommends that Covid-19 Ag- RDTs have a minimum of 80% sensitivity and 97% specificity. Whiles for low-incidence settings, the ECDC [5] proposes a more conservative threshold of ≥ 90% for sensitivity. In Ghana, it is currently unclear as to which minimum threshold is being used in terms of specificity and sensitivity since it is not stated in the emergency authorisation guidelines for the emergency use of Antigen/Antibody diagnostic test kit for Sars-Cov 2, which was developed and published by the FDA in 2020. It should however be noted that, in a correspondence posted on their website and a news article responding to the Ag-RDT validation process, the FDA stated that their criteria were 99% for both sensitivity and specificity [6,7,9]. This requirement although admirable it is very ambitious, it is even higher than that of WHO and ECDC.

The Ghana Ports and Harbours Authority (GPHA) Hospital is located in the Western Region of Ghana. It provides healthcare services to the 445,205 population of the Sekondi-Takoradi Metropolis and beyond. Only nasopharyngeal samples placed in Copan UTM, Standard, BD VTM and Standard F Covid-19 FIA Buffer solutions are acceptable by the SD Biosensor F200 analyser for the antigen test. Because of the unavailability of the three compatible viral transport media (i.e. Copan, Standard and BD VTM) for the Standard F antigen test, and the inconvenience of taking two nasopharyngeal samples, the Medical Laboratory Scientists take one nasopharyngeal swab into the Standard buffer solution for the antigen test. Because the buffer solution is finished after the antigen test, an oropharyngeal sample is taken for the RT-PCR confirmation simultaneously.

The RT-PCR results turnaround time was between 5 – 14 days, dependant on the workload at the testing centre in the region. The Antigen tests, therefore, provided quick and actionable results. This study, therefore, wanted to ascertain how reliable and accurate the results from the Covid-19 antigen tests at the facility was.

This research was mainly driven by the scarcity in obtaining data especially from the African Continent on the performance of independent evaluation approved Antigen test kits on the field. Additionally, the correlation of results taken from different anatomical sites is of keen interest to us since factors like the anatomical site, sampling technique, specimen storage and transport can all influence the final output. Accordingly, the key aim of this study was to assess the diagnostic accuracy of the Standard F Ag-RDT testing regimen at a facility in a real-world setting.

2. Methods

2.1. Study Design and Setting

A retrospective analysis was conducted on samples from GPHA Hospital from the 8th of March to the 1st of August, 2021. The inclusion criteria were that both RT-PCR (for confirmation of Ag-RDT output) and Antigen tests have been conducted on the samples, which were taken simultaneously. The Covid-19 Antigen tests are conducted immediately at the hospital whiles the oropharyngeal samples are temporarily stored and transported to the Public Health Reference Lab for onward submission to the Western Region Veterinary Service laboratory, which is the only centre currently in the region where the RT-PCR test can be conducted.

A total of 314 samples RT-PCR and Ag-RDT results were all that were available, matched and analysed. These involved samples from both symptomatic and asymptomatic patients. All antigen tests were performed within 30 minutes after sample collection, whiles the samples for RT-PCR were stored and transported within 24 hours after collection.

Because the dual testing regime was conducted as part of Covid-19 routine testing at the facility, no formal approval was required from individuals for the retrospective study. However, institutional approval was sort from the GPHA hospital to utilise the data.

STANDARD F COVID-19 Ag FIA (Biosensor, Republic of Korea) tests were performed by following the manufacturer's instructions whiles standard protocols were followed in the performance of the RT-PCR test at the regional testing centre. With the Standard F antigen test, if the cut-off Index (which is a numerical expression of the measured fluorescence signal from the formation of an antigen-antibody complex) is <1.00, it indicates a Negative reaction. However, if it is ≥ 1.0 it means it is Positive. It should however be noted that the results from the testing centre only indicated whether samples were positive or negative. The ct values were therefore not included in this study since it was not immediately available.

2.2. Data Analysis

The Gold Standard for Covid-19 testing is the RT-PCR. It was therefore considered as the reference standard against the Standard F COVID-19 Ag FIA [11]. The accuracy and diagnostic performance of the antigen test

were assessed through the calculation of sensitivity and specificity. The positive and negative predictive values (PPV and NPV) were calculated from the overall sensitivity and specificity and hypothetical positivity prevalence.

The analysis was performed using Microsoft Excel, Medcalc and Mede calculator software.

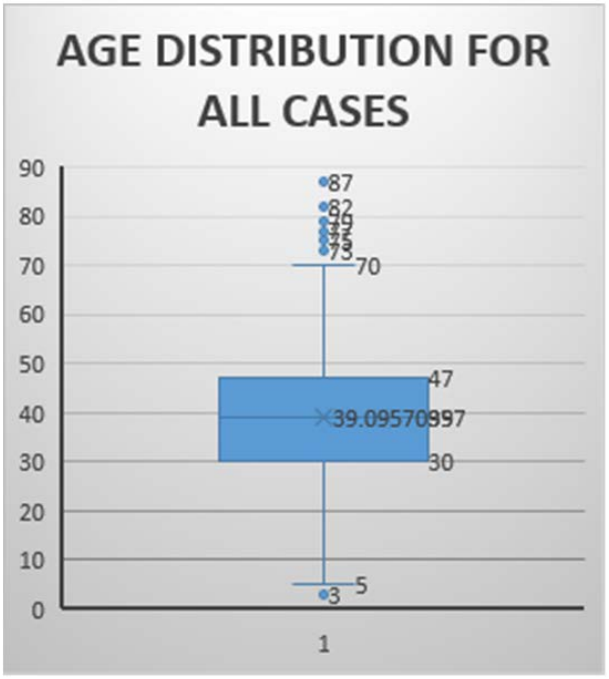

Figure 1. The ages of participants from 3 to 87 years

3. Results

A total of 314 samples were analysed. Of these, 85.0% (n = 267) were found negative and the remaining 15.0% (n = 47) tests were positive by RT-PCR. On the other hand, 86.9% (n=273) were determined to be negative and 13.1% (n=41) were also determined to be positive by the Standard F Covid-19 Antigen FIA test kit.

Overall diagnostic performance of the testing regimen.

Table 1 and Figure 2 reports the overall data on the performance of the testing regimen at the hospital. The proportion of false-negative results was 2.9% (n =9) with 0.9% (n=3) false-positive specimens revealed.

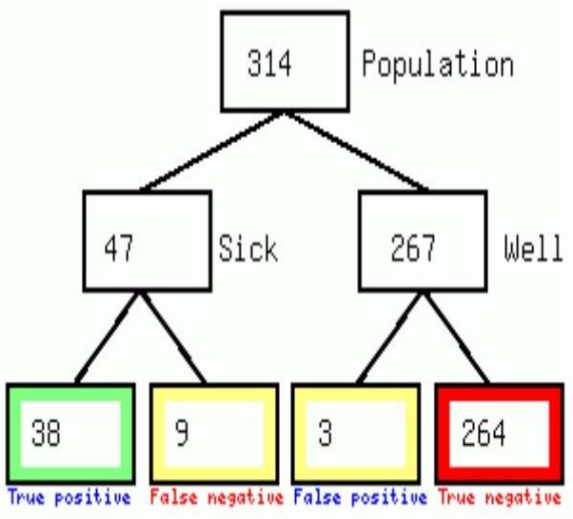

Figure 2. Overall Findings (**Sick*- Only Represents those confirmed by RT-PCR as infected. **Well*- Only Represents those confirmed by RT-PCR as not having covid-19)

From Table 2, the resultant sensitivity and specificity were therefore 80.85% (95% CI: 66.74%– 90.85%) and 98.88% (95% CI: 96.75%–99.77%), respectively. The Negative Predictive Value (NPV) was also determined to be 96.70% (95% CI: 94.22%- 98.14%) whiles the Positive Predictive Value (PPV) was 92.68% (95% CI: 80.30%-97.52%).

The overall accuracy and Cohen Kappa was determined to be 96.18 % (n=302 agreements) (95% CI: 93.42% - 98.01%) and 0.842 (95% CI: 0.754 – 0.929). The kappa score of 0.84 means there is an almost perfect agreement between the two tests results.

Table 1. Overall data performance of the test

	RT-PCR POSITIVE	% Age	RT-PCR NEGATIVE	% Age	TOTAL
Standard F POSITIVE	38 *(True POSITIVE)*	12.1%	3 *(False POSITIVE)*	0.9%	41
Standard F NEGATIVE	9 *(False NEGATIVE)*	2.9%	264 *(True NEGATIVE)*	84.1%	273
TOTAL	47		267		314

Table 2. Regimen performance

Statistic	Value	95% CI
Sensitivity	80.85%	66.74% to 90.85%
Specificity	98.88%	96.75% to 99.77%
Positive Likelihood Ratio	71.96	23.16 to 223.60
Negative Likelihood Ratio	0.19	0.11 to 0.35
Disease Prevalence (*)	14.97%	11.21% to 19.40%
Positive Predictive Value (*)	92.68%	80.30% to 97.52%
Negative Predictive Value (*)	96.70%	94.22% to 98.14%
Accuracy (*)	96.18%	93.42% to 98.01%

4. Discussion and Conclusions

This study critically assesses the dual testing regimen in this hospital to determine its reliability and suitability especially because of the sampling and testing from two different anatomical sites using different methods. Nasopharyngeal samples are the first choice followed by oropharyngeal samples for RT-PCR testing [12,13]. The oropharyngeal sample taken is, therefore, the second choice. It is, however, more tolerated than the nasopharyngeal sample and provides reliable results. Overall, the dual testing would have served a better purpose with the provision of the Copan, Standard and BD VTM.

However, with the absence of these three VTM's, the testing regimen implemented by the Lab Scientist is fit for purpose with high accuracy of 96.18%. The kappa score was also 0.84 indicating that there is an almost perfect agreement between the results from the antigen and the RT-PCR tests. The dual testing regimen is therefore very reliable and can be continued in the absence of the three VTM's. The strong correlation of results is also an indicator of the proficiency of the sample taking, storage and transport techniques used by the scientists. Continuous training and monitoring are however required to ensure that performance standards are maintained or even improved.

The diagnostic performance of the Standard F Covid-19 Antigen FIA was also assessed in a real-world hospital setting, specifically a developing country primary hospital setting. The sensitivity was approximately 81%, whiles the specificity was 99%. The Sensitivity and specificity derived fall within the acceptable range set by the WHO (i.e minimum sensitivity of 80% and minimum specificity of 95%) but falls below that purported range set by the Ghanaian FDA of minimum 99% sensitivity and specificity. Overall, the Standard F test Kit meets WHO requirements and thus it was approved and listed the same. This field performance further lays credence to the fact that it meets requirements both in the real world hospital and assessment settings. However, it should be noted that sensitivity generally increases with an increase in viral load (ct<25) [8], and because of the lack of ct values from the RT-PCR tests, a stratified sensitivity value cannot be calculated. Its significance, however, cannot be ignored.

In some studies for example [8,14,15], no false-positive tests were recorded. However, in this study, there were 3. From Figure 3, all the false positive cases had COI below well above 2.0 (COI ≥ 1.0 = Positive). Their values were well within the range for Positive cases. It is the position of the researcher that the difference in sampling site and the storage and transport chain contributed to this observation, and that these cases may be true positive cases. Fortunately, Ghana Health Service Protocol for Antigen Testing indicates that all symptomatic antigen-positive cases should be managed as such while asymptomatic cases may be confirmed with RT-PCR. This, therefore, allows for immediate intervention to be delivered to symptomatic patients whiles awaiting confirmatory results. This is in line with ECDC [5], which indicates that in high incidence settings, positive results will most probably identify true positives with no need for subsequent RT- PCR confirmation, while negative results for strongly suspected cases require immediate RT-PCR validation.

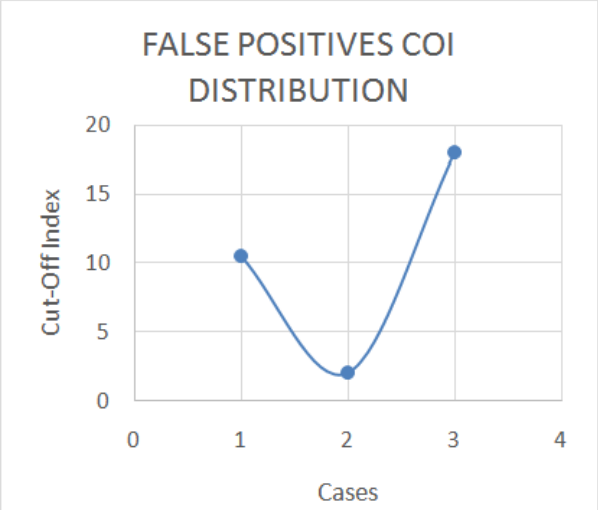

Figure 3. The false-positive distribution curve

The use of FIA tests was associated with a lower number of false negatives and therefore they perform better than Lateral Flow Test Kits (LFT's). In this study, sensitivity was affected by the 9 false-negative results recorded. The stated clinical Specificity and Sensitivity of the Standard F Antigen Test Kit is 99.63% and 93.70% respectively for ct values ≤ 30. From Figure 4, only 1 of the false Negative result had a Cut-Off Index (COI) of approximately 0.7, all the remaining had a COI below 0.4. Therefore, their values were well within the Normal COI range of <1.0 for Negative cases.

The occurrence of some false positive and false negative cases can therefore be because of the high specificity of the Standard F antigen test kit which can lead to an increased frequency of false-negative, variation in anatomical sites, specimen storage and transport, and challenges in sample taking. Additionally, patients are generally more cooperative with oropharyngeal samples than nasopharyngeal samples because of their tolerability. Also, Ag-RDTs may be more useful in screening positive patients with high viral loads [8], therefore a low viral load is another causal factor for the variation.

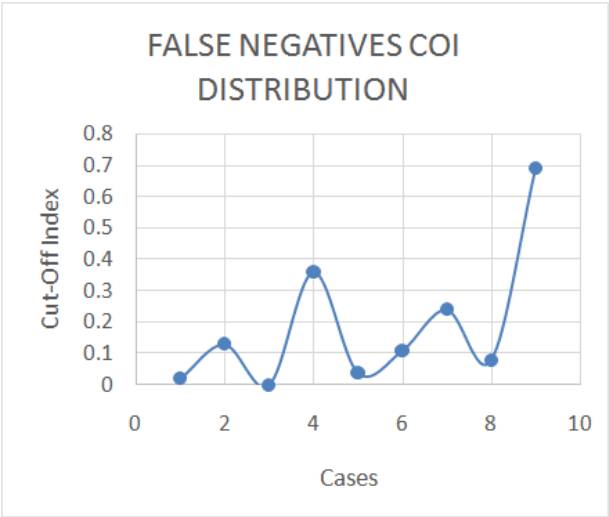

Figure 4. False-negative distribution curve

A methodical review on the diagnostic accuracy of some Ag-RDTs against RT-PCR performed by ECDC [5] documented a high variability in sensitivity (ranging from 29%–93.9%), while the specificity was constantly high (ranging from 98.8%–100%). The latest Cochrane review [2] has reported a wider range for both sensitivity (0%–94%) and specificity (90%–100%). Based on these findings, the field performance of the Standard F kits are high end and is therefore optimum for testing.

This study however has a few limitations. Firstly, although the total sample size is relatively large (the median sample size of 77 studies included in the Cochrane review by Dinnes et al. [2,3] was 182), our sample size of 314 is therefore adequate. Despite this, it is however skewed to RT-PCR Negative samples. FIND [8] suggests that in retrospective validation studies of SARS-CoV-2 Ag-RDTs, the minimum number of both RT-PCR positive and negative samples should be 100. In this study, however, we had only 38 positive samples. We however hope that the difficulty in the availability of such data and resources in this research setting will also be considered.

Secondly, there is no doubt that results from two different anatomical sites although they were taken at the same time may naturally differ. Test kit diagnostic performance estimation in such a situation will certainly be affected. This study, however, seeks to focus more on the strength in having a high correlation of results from varying anatomical sites rather than what could have been but is currently inexistent on site.

This study took place from March 2021 when the Sars-Cov2 incidence rate was very low to August 2021 when there was an active third wave and other variants were also recorded in Ghana. The rapid dispersion of novel SARS-CoV-2 variants [17,18] may interfere with the accuracy of Ag-RDTs available; therefore, continuous monitoring of the performance of Ag-RDTs are necessary.

In conclusion, this study has established that the Standard F Covid-19 antigen test kit meets the WHO [7,10] criteria. The dual testing regimen is very useful and can be replicated in other facilities, however, it will be ideal if the same sample is used for both testing. An arrangement should therefore be made by the appropriate authorities to procure compatible Viral Transport Media. The rapid diagnostic test kit is a very powerful tool in the fight against Covid-19 since it can assuage the pressure of RT-PCR testing sites. The challenges of Ag-RDT's in cases of low viral load (ct values from 30 to 35) should also be considered [8]. The Positive Predictive Value of 92.68%, a Negative Predictive Value of 96.7% and an overall accuracy of 96.18% are all indicative of an optimum testing scheme.

Statement of Ethics

Due to the retrospective nature of this study, ethical review and approval were waived because it was based on routine COVID-19 testing.

Conflicts of Interest

The authors declare no conflict of interest since the data used for this study are independently verifiable and accessible.

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