Annals of Medical Laboratory Science (2022) **2**(2): 23 - 30
https://www.annalsmls.org
https://doi.org/10.51374/annalsmls.2022.2.2.0062

West African Postgraduate College of Medical Laboratory Science Advisory Position Paper on Covid-19 Testing and Containment in Ecowas Region

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How to cite this paper: Adu-Gyasi, D., Nkrumah, B., Raheem. T. Y., Umukoro, S., Okara, G. C., and Mirabeau, T. (2022). West African Postgraduate College of Medical Laboratory Science Advisory Position Paper on Covid-19 Testing and Containment in Ecowas Region. *Annals of Medical Laboratory Science* **2**(2): 23 - 30

Received: September 12, 2021
Accepted: April 30, 2022
Published: June 01, 2022

ISSN No: 2805-4024

ABSTRACT
Obviously, medical laboratory testing remains one of the tools successfully used in managing the coronavirus disease 2019 (COVID-19) pandemic globally. Due to the novel nature of the virus responsible for COVID-19, many countries adopted their own approaches to mitigate the impact the pandemic was having on the respective countries. The West Africa Postgraduate College of Medical Laboratory Science in contributing to managing the situation set up an ad-hoc committee to review and advise on acceptable diagnostics approaches that could be used in fighting the COVID-19 pandemic. The committee of experts reviewed the scientific and professional approaches that could be used to test for COVID-19 to identify cases for isolation, treatment, management, and subsequent control of transmission. Again, the committee made recommendations of what method should be used depending on the objective one seeks to achieve not neglecting patient safety.
Annals of Medical Laboratory Science (2022) 2(2), 23 - 30

Keywords: COVID-19 pandemic, laboratory testing, diagnostics approaches, West Africa Postgraduate College of Medical Laboratory Science

INTRODUCTION

Preamble

Several coronaviruses can infect humans including Severe Acute Respiratory Syndrome Coronavirus Virus-2 (SARS-CoV-2) which causes the disease named coronavirus disease 2019 (COVID-19) (World Health Organization, 2020). The ongoing pandemic of the recently-emerged SARS-CoV-2 is critically challenging health systems worldwide (Yelin *et al.*, 2020).

One of the key strategies for containing the disease across the world, is to undertake widespread testing for COVID-19 followed by isolation and treatment of confirmed cases and containment measures for clusters of confirmed cases (Gupta *et al.*, 2020). Diagnostic testing is an essential response strategy to interrupt the transmission for the COVID-19 pandemic by identifying positive cases, isolating them and informing patient management (World Health Organization, 2020).

Delays in testing can lead to large disease cluster forming, unchecked progression of severe cases and overburdening of the health system with critically ill patients (Gupta *et al.*, 2020).

COVID-19 Diagnostic Platforms or Approaches.

Every laboratory test developed must undergo rigorous approval processes to be suitable for

https://doi.org/10.51374/annalsmls.2022.2.2.0062

diagnostic purposes. Where it is novel, it must go through a process to demonstrate adequate performance against "gold standard tests" to be accepted as a new diagnostic method.

Testing for patients will have to be prompt and immediate since possible recollection of samples and retesting may be required to ascertain COVID-19 (Fang *et al.*, 2020; Wikramaratna *et al.*, 2020). Prompt testing for COVID-19 is more necessary to enable testing for other diseases that maybe necessary to manage patients.

The choice of testing methods and procedures will have to be selected for patient safety and care. For testing, it is important to know the needs of the request. Is it to identify infected patients or for the discharge of convalescing patients who are potentially still infectious (Wikramaratna *et al.*, 2020)? To ensure prompt detection and containment of SARS CoV-2 infection, rapid, reliable, and accurate testing platforms are needed in West Africa**.**

Serological Testing

Rapid Diagnostic Test (RDT) Kits

A few RDTs based on detection of antibody to SARS CoV-2 in the infected persons are being introduced (Prazuck *et al.*, 2020; Drain, 2022). False negative or false positive tests results with RDTs are possible. Development of antibodies to SARS COV-2 infection need more work to understand the immune presentation (Long *et al.*, 2020). In immuno senescence/immunocompromised individuals, which often happens in the elderly, HIV/AIDS patients, diabetics, cancer patients, and other conditions, RDT based on antibody detection may give false negative results (Mouliou and Gourgoulianis, 2021).

Immunofluorescence Assay (IFA), Enzyme-Linked Immunosorbent Assay (ELISA) and other Serological Assays

Serological assays target antigens and antibodies in the fluids and tissues of patients depending on the interest of manufacturers and users. It can recognize recombinant SARS-CoV Nucleoprotein/NP Protein, but not react with recombinant MERS-CoV Nucleoprotein/NP protein (To *et al.*, 2020).

ELISA is more convenient and economical than reverse transcription-PCR (RT-qPCR), and can be used as an alternative tool for the early diagnosis of SARS CoV-2 infection in laboratories with limited resources and expertise and for mass screening for the reservoir of SARS CoV-2 (Lau *et al.*, 2004; Imai *et al.*, 2020; Long *et al.*, 2020).

Recommendations on serological testing

Use of antibody-based test kits should be avoided until scientific evidence proves their accuracy and usefulness. Extensive validation of SARS COV-2 RDT platforms are required by respective regulatory authorities in the West Africa region to assess and determine their performance before use.

Serological test kits including ELISA must be validated and approved for patient testing due to the complexity of natural targets and unpredictable interference.

Duplicate testing is one of the procedures for ensuring reliability and accuracy of laboratory assays. In view of the current RT-qPCR technology where internal control such as the detection of normal DNA trend in the assayed sample is possible, duplicate testing may be a waste of limited resources. However, when assessing treatment follow-up or second opinion on previously tested individual persons, it should be described as re-tests and not duplicate tests. There are instances where a test could even be re-tested three times as tie breakers, as practiced in HIV testing algorithm.

RT-qPCR testing platform

Background

Molecular based testing platform using the RT-qPCR remains the gold standard because of its ability to detect minute quantity of the virus even when antibody production may not be detectable (Dorlass *et al.*, 2020). However, it is important to know that the platform requires properly trained Medical Laboratory Scientists and other laboratory personnel, adequate laboratory space that will allow

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uni-directional movement within the laboratory space and ensure infection prevention and control (Humphreys, 2021). A minimum of four rooms (for inactivation, RNA extraction, PCR reagent preparation, and running the PCR/detection of amplicons) would ideally be appropriate to prevent contamination during processing of samples with the PCR procedures. Separate automatic pipettes, certified biosafety cabinets, fridges, freezers are recommended in each room. Adequate human, financial, infrastructural resources are required as priorities in West Africa region in the containment of COVID-19 and any other pandemics in the future. This is because future pandemics might occur (Drain, 2022), and we must therefore be well prepared to contain them. Professionals are always advised to adhere strictly to manufacturers' instructions.

Presently, RT-qPCR testing regimes vary significantly between countries, determined both by policy decisions and testing capacity (Wikramaratna *et al.*, 2020). Some opt (or, rather, are able to) test large portions of the population, including those who are asymptomatic or self-isolating with mild symptoms (Wikramaratna *et al.*, 2020).

Rapid collection and testing of appropriate specimens from patients meeting the suspect case definition for COVID-19 is a priority for clinical management and outbreak control and should be guided by a medical laboratory expert. Suspected cases should be tested for the virus with nucleic acid amplification tests (NAAT), such as RT-qPCR (World Health Organization, 2020).

Nucleic acid amplification tests (NAAT) for COVID -19 routine confirmation is based on the detection of unique sequences of viral RNA by NAAT such as reverse transcription polymerase chain reaction (RT-qPCR) with confirmation by nucleic acid sequencing when necessary. The viral genes targeted so far include the N, E, S and RdRP genes (World Health Organization, 2020).

A test with good analytical sensitivity and specificity does not necessarily have good clinical sensitivity and specificity (Saah and Hoover, 1997). "Diagnostic sensitivity often has more to do with the ability to obtain the target substance in a processed sample from a person who has the condition than with the ability to detect very low concentrations of a substance" (Saah and Hoover, 1997). If the target substance is not in the processed sample because of vagaries of sampling or processing, an assay with perfect analytical sensitivity still fails to give a positive result (Saah and Hoover, 1997).

Guidelines from the World Health Organization (WHO) and the European Centre for Disease Control (ECDC) assert that a single negative test is insufficient to rule out infection (Wikramaratna *et al.*, 2020).

With reagent shortage challenges, it has become important to come up with innovative ways to conserve reagents used for diagnostic tests. At the same time, as the disease is novel, it is of value to validate any modifications to the testing process before universal adoption (Yelin *et al.*, 2020) since it has implications for clinical decisions about treatment, and decisions about who needs to be quarantined or can be released safely into the community (Wikramaratna *et al.*, 2020).

The clinical course of COVID-19 and the viral load (whether viral RNA or mRNA for potential replication), and development of antibodies vary from the time of infection and onset of symptoms from Day 4, 7, 9 up to Day 14 (Wikramaratna *et al.*, 2020; Wölfel *et al.*, 2020; Zou *et al.*, 2020). The probability of a positive test decreases with time after onset of symptoms (Wikramaratna *et al.*, 2020; Zou *et al.*, 2020) and therefore testing must be done with caution bearing in mind patient safety and timeliness of releasing results.

To consider a case as laboratory-confirmed by NAAT in an area with no COVID-19 virus circulation, one of the following conditions needs to be met as described by the WHO (World Health Organization, 2020):

A positive NAAT result for at least two different

https://doi.org/10.51374/annalsmls.2022.2.2.0062

targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus using a validated assay (as at present no other SARS-like coronaviruses are circulating in the human population it can be debated whether it has to be COVID-19 or SARS-like coronavirus specific); or One positive NAAT result for the presence of betacoronavirus, and COVID-19 virus further identified by sequencing partial.

Limit of Detection (LOD)
Overall, the clinical sensitivity of the tests, varies depending on the commercial vendor, how the LOD estimate was determined (some companies used intact virus, which better simulates real samples, whereas others used just nucleotide sequence) and the specimen type submitted (some vendors only validated their assays for certain specimen types).

NB: It is better to stick to what the manufacturer instructs from method validation and approval for use in patient testing.

False Negative
False Negative from diagnosing COVID-19 with RT-qPCR tests result from low level of viral RNA copies as mentioned by the manufacturers of the reagents (World Health Organization, 2020).

When the appropriate sample is not used for testing, false negative results could be obtained (World Health Organization, 2020; Wang *et al.*, 2020).

All potentially identified samples must be adequately evaluated before used for patient testing (Xu *et al.*, 2020).

Precautions for sample processing and analysis
RNA extraction should be done in a certified biosafety cabinet BSL-2 or equivalent facility. Heat treatment of samples prior to RNA extraction is not recommended.

Reagents and equipment
Sites can choose reagents and equipment that best suit their budget and setting, and has been verified or validated depending on the circumstances, in accordance with international standard(s) like The Joint Commission and Clinical and Laboratory Standards Institute (CLSI), among others.

Safety Precaution
During specimen collection and testing, authorities should ensure that adequate SOPs are in use and that staff are trained for appropriate specimen collection, documentation, storage, packaging and transport, testing and waste management/disposal (World Health Organization, 2020). All specimen collected for laboratory investigations should be regarded as potentially infectious. Additionally, all health care workers who collect specimens should adhere rigorously to infection prevention and control guidelines. Specific WHO interim guidance on this has been published: "Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected, interim guidance, January 2020" and "WHO interim guidance for laboratory biosafety related to 2019nCoV" (World Health Organization, 2020).

Reception, management, and processing of specimen from patients with or suspected of having an Airborne High Consequence Infectious Disease (eg COVID-19)
For patients to be effectively managed, routine laboratory tests like Full Blood Count (FBC), Liver Function Test (LFT), Blood culture and clotology may be required. Designated laboratories must develop separate protocols in line with local and international standards for this process, and a comprehensive risk assessment conducted and approved before such samples are analyzed in such laboratories for the safety of the personnel (World Health Organization, 2020).

Recommendation on RT-qPCR testing
RT-qPCR which is the "gold standard" or reference testing method must be used as guided by the WHO (Organization 2020) and users must strictly adhered to manufacturers' instructions particularly for patient testing.

"**Pooling of samples" or Group testing model.**
Background
Pooling is an old technique that is used in

https://doi.org/10.51374/annalsmls.2022.2.2.0062

infectious disease units but as SARS-CoV-2 is a novel pathogen, it is unclear how diluting a sample containing its RNA would affect the sensitivity of this assay and the false-negative rate (Yelin *et al.*, 2020). Especially a virus that is highly contagious and a day with a positive case on the loose can hamper the progress of the battle against COVID-19 pandemic.

Group testing will result in the saving of reagents and personnel time with an overall increase in testing capability of at least 69% (Abdalhamid *et al.*, 2020). In practice, it is important to acknowledge that assay performance may depend on a variety of factors, including those related to implementation and perhaps even the population being tested (Warasi *et al.*, 2016).

While the development of clinical prediction rules and non-testing screening are critical to any epidemiological response, dealing with a novel disease for which data is still sparse and testing capabilities are limited means that maximizing the impact of each individual test can benefit the continued refinement of our strategy (Noriega and Samore, 2020).

Like in the Ghanaian experience, whether we use the one-time pooling or repeated pooling, some studies have shown great potential in testing pooled samples in order to detect the SARS-CoV-2 virus (Warasi *et al.*, 2016; Abdalhamid *et al.*, 2020; Zhu *et al.*, 2020). This approach will conserve huge resources during COVID-19 mass testing and can help effectively curb local COVID-19 outbreaks in early stages, particular in low-income settings, and in containing second wave outbreaks (Abdalhamid *et al.*, 2020). However, these results should only be cautiously used for clinical decision when a specific site validation of the procedure has not been carried out.

Increasing testing in areas with low SARS COV-2 positivity rate and when estimating viral load is not the focus, pooled samples could be used for screening (Prakash *et al.*, 2020). Pooling of samples should not be adopted for patient testing and management (Noriega and Samore, 2020; Prakash *et al.*, 2020). Principles for successful application of group testing involve knowledge of the limit-of-detection, sensitivity, and specificity of the assay, and the prevalence of disease in the population (Hitt *et al.*, 2019; Abdalhamid *et al.*, 2020).

Challenges in using Group Testing ("pooling")

Group testing of pooled specimens also requires the use of highly sensitive assays to avoid missing low positive samples (Hitt *et al.*, 2019; Abdalhamid *et al.*, 2020). Therefore, strategies must be employed to closely monitor the use of pooling as the positive rate of test specimens increases in an outbreak of disease (Hitt *et al.*, 2019; Abdalhamid *et al.*, 2020; Zhu *et al.*, 2020). Additionally, the impact of different extraction methods on the recovery of RNA and overall test sensitivity need to be evaluated (Abdalhamid *et al.*, 2020) especially when different reagents have been made by manufacturers and are been donated or procured per their availability. All quality indicators including recovery, and Lot-to-Lot bridging of reagents performance need to be established for patient safety and test quality.

Some of the challenges we expect involve in particular how pooling multiple samples dilutes the genetic material and may increase false positives (possibly from contamination and when the limit of detection (LOD) is low) and negatives (Zhu *et al.*, 2020). Although one might expect that an analytically sensitive assay should more readily identify those persons, the ability to measure a very small quantity of a substance does not always translate into high diagnostic sensitivity (Saah and Hoover, 1997).

Due to the different matrix effect, more genetic material in some cases, and less in others cases might be sampled (Zhu *et al.*, 2020). Appropriately, genetic material will have to be measured before samples are pooled to ensure the same concentration of genetic material is loaded for each sample (Zhu *et al.*, 2020). The best option for pooling would have been to tag individual samples prior to pooling but this rather adds to cost.

When a positive sample is pooled amidst negative

https://doi.org/10.51374/annalsmls.2022.2.2.0062

samples, the individual samples would have to be tested to ascertain which of the samples is positive and may delay the release of results for prompt patient management. If five samples were pooled together, and one of them was positive, then one would end up running six samples (the pooled sample and running each of the pooled five samples individually).

WAPCMLS Recommendations on COVID-19 diagnosis.

Countries in the West Africa Region are encouraged to use the real-time reverse transcription-polymerase chain reaction (RT-qPCR) diagnostic panel for the detection of SARS COV-2 (World Health Organization, 2020).

Sample pooling dilutes samples which can lead to false negatives.

Sample pooling can be used in areas where the prevalence of COVID-19 is low to avoid wasting of resources to un-pool samples when pooled samples test positive.

Sample pooling for PCR screening may be considered in a community survey for surveillance among asymptomatic individuals but one must operate with best laboratory practices for safety and reporting for patients.

Only mass screening exercises appear ethically suited for mass sampling. Routine surveillance and enhanced contacts tracing cases, with their high pre-test positivity rates, on the other hand, are best not confirmed through pooled sampling.

Pooling is not acceptable for patient management due to its limitations and could delay release of results for patient care. It is also not ideal to estimate viral load.

Sample pooling introduces potential error and stray DNA could amplify because of contamination during sample preparation.

Possible loss of the clients (death) due to anxiety while waiting for the result. Instances of patients dying before the outcome of their COVID-19 test results were released by few isolation centres could be observed. It is noteworthy that occurrence of underlying health challenges such as hypertension, diabetes etc contribute to susceptibility to and severity of SARS CoV-2 infection especially in persons 50 years and above exist.

CONCLUSION

Positioning the College towards producing some COVID-19 related diagnostic materials and consumables to avert the issues of scarcity as realized in the beginning of the COVID-19 pandemic in Africa. There was exhaustion and skyrocketing of the cost of procuring the following commodities: Alcohol based hand sanitizers, personal protective equipment (PPEs) (nose mask, face shield, gowns, boots, etc), Viral Transport Medium (VTM), Specialized Swab sticks, Primers for the RT-qPCR, Viral extraction kits, and other consumables. Members of the College are encouraged to team up for local mass production of the above listed commodities and even export them to other countries within and outside the West Africa Region. In this respect, the College will contribute to self-sustenance and possibly, self-sufficiency in the use of the commodities and would conserve foreign reserves of our respective countries within the region. The need to identify with regional or global corporate bodies: WAPCMLS needs to identify with corporate bodies such as (Coalition for Epidemic Preparedness and Innovation (CEPI) and others. This would possibly attract funds to the College for innovative and collaborative efforts at producing vaccines and other materials needed for diagnosis and surveillance of COVID-19 in West Africa region.

COMPETING INTEREST

Authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

The committee was established by President and Registrar of the Postgraduate College; NA and GO respectively. With TM as chairperson, DAG as

https://doi.org/10.51374/annalsmls.2022.2.2.0062

secretary and the other members; BN, TYR and SU, an approach to deliver the advisory position paper was formulated and task assigned. All inputs were put together, reviewed and accepted by the committee before final submission to the Postgraduate College. All authors contributed to the development of the manuscript, read and approved the final version.

FUNDING

West Africa Postgraduate College of Medical Laboratory Science funded the work of the committee of experts.

ACKNOWLEDGEMENTS

The West Africa Postgraduate College of Medical Laboratory Science and the Diagnostic Experts Committee express their gratitude to all medical laboratory professionals who contributed by sharing information and their work for review. The next gratitude goes to the agencies and professionals that would utilize the recommendations for their practice. and professionals.

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