A report on preparation, expansion and future outlook of COVID-19 testing in Gambia

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Abstract

**Background:** The outbreak of COVID-19 disease and rapid spread of the virus outside China led to its declaration as a Public Health Emergency of International Concern (PHEIC) in January 2020. Key elements of the early intervention strategy focused on laboratory diagnosis and screening at points of entry and imposition of restrictions in cross-border activities.

**Objective:** We report the role the Medical Research Council Unit, The Gambia (MRCG) played in the early implementation of molecular testing for COVID-19 in The Gambia as part of the national outbreak response.

**Methods:** Laboratory staff members, with experience in molecular biology assays, were identified and trained on COVID-19 testing at the Africa CDC training workshop in Dakar, Senegal. Thereafter risks assessments, drafting of standard operating procedures (SOPs) and in-house training enabled commencement of testing using commercial RT-PCR kits. Subsequently, testing was expanded to the National Public Health Laboratory and also implemented across field sites for rapid response across the country.

**Results:** Capacity for COVID-19 testing at MRCG was developed and can process approximately 350 tests per day, which can be further scaled up as the demand for testing increases.

**Conclusion:** The long presence of the Unit in The Gambia and strong collaborative relationship with the National Health Ministry, allowed for a synergistic approach in mounting an effective response that contributed in delaying the establishment of community transmission in the country.

Keywords: COVID-19 testing, sub-Saharan Africa, SARS-CoV-2.

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INTRODUCTION

COVID-19 disease, caused by the novel human coronavirus strain, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a Public Health Emergency of International Concern (PHEIC) on 31st January 2020 by the World Health Organization (WHO) (1). This followed from a risk assessment of an outbreak of a cluster of pneumonia of unknown etiology in Wuhan, the capital city of Hubei province in China (2). With the rapid spread of the virus outside China, diagnostic testing, isolation and contact tracing rapidly became the major strategy for containment (3).

Similar to other countries in sub-Saharan Africa, The Gambia faced the challenge of rapidly implementing a nucleic acid amplification test (NAAT) for routine diagnosis. The establishment of an African task force for coronavirus preparedness and response (AFT-COR) enabled early identification of the key elements of an intervention strategy focusing on laboratory diagnosis and subtyping, surveillance screening at points of entry and restriction in cross-border activities (4). SARS-CoV-2, which is a positive-sense single-stranded RNA (+ssRNA) virus, is detected by reverse transcriptase real-time PCR (RT-PCR) assay that amplify specific regions in the viral target genome (5). In February 2020, the Africa Centres for Disease Control and Prevention (Africa CDC) in collaboration with the Institute of Pasteur, Dakar, Senegal, organised a training workshop for African scientists on molecular diagnosis of SARS-CoV-2 using RT-PCR techniques (6).

The Medical Research Council Unit, The Gambia (MRCG) played a central role in the early implementation of molecular testing for COVID-19 in The Gambia. Established in the late 1940s, MRCG has a long history of research presence in the Gambia, with an international reputation for ground-breaking research on some of the leading causes of morbidity and mortality in sub-Saharan Africa. The MRCG Unit was initially established as a UK Research Institute for Infectious diseases. In addition to the main campus in Fajara, located in the more urban, densely populated coastal region, the MRCG operates field sites located in the more rural parts of the country (Figure 1). Prior to the COVID-19 outbreak, an ongoing surveillance study in the Unit had established capacity for viral molecular diagnosis (7). MRCG also had a Biosafety level 3 (BSL-3) laboratory dedicated to handling infectious pathogens, which had been upgraded in 2014 during the Ebola outbreak in parts of West Africa. All these enabled the Unit to quickly establish the laboratory diagnostic capacity to support a robust national response to the COVID-19 outbreak. Here, we report the processes undertaken to implement SARS-CoV-2 RT-PCR diagnostic testing and to scale up testing capacity in response to the emerging pandemic.

MATERIALS AND METHODS

Assessment of resources – personnel, equipment and facilities

In the preparatory stages, MRCG worked with guidelines on COVID-19 testing developed by the UK National Health Service (NHS) (8). These guidelines required laboratories to assess how continuous testing at maximum capacity can be provided and to identify potential bottlenecks in the testing pathway that may restrict processing capacity, such as number of staff required, other assays that use the same equipment, logistics and supply chain issues (8). Assessment and monitoring of resource availability and stock levels was done at regular intervals and more frequently following the imposition of lockdown measures and declaration of State of Public Emergency in The Gambia. The challenge of supply chain disruption leading to difficulty in procurement of reagents and consumables for large scale testing in the face of increasing demand was acknowledged and contingency procurement plans were established, including the exchange of reagents with collaborating labora-

Supplementary information The online version of this article (Figures/Tables) contains supplementary material, which is available to authorized users.

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Laboratories in the subregion when shortages occurred.

**Training and Workflow development**

Laboratory staff members with experience in molecular biology assays were identified for training on COVID-19 testing at the Africa CDC training in Dakar, Senegal. Upon return from the Africa CDC workshop in Dakar, Senegal, the team drafted Standard Operating Procedures (SOPs) for the different stages of COVID-19 testing. The SOPs were reviewed and approved through the MRCG Quality Department. Subsequently, trainings were conducted for identified key personnel and their competence assessed and documented before commencement of COVID-19 diagnostic testing.

**Health and safety assessment**

An assessment of the risks associated with handling samples potentially containing SARS-CoV-2 was undertaken prior to the commencement of routine testing. Good laboratory practice, including the use of standard biological safety precautions, appropriate personal protective equipment (PPE), a plan for regular training and re-training of staff involved, as well as the use of standard operating procedures (SOPs), were implemented to minimise potential risks of exposure to the virus (9). Samples for testing, were inactivated and processed inside a biosafety cabinet in a full containment level BSL-3 facility. Compartmentalisation of the workflow and workstations was established to reduce risk of cross-contamination of samples and exposure of team members when community transmission became established.

**Handling COVID-19 sample types - transport, reception and documentation**

Sample types included nasopharyngeal swabs (NPS), throat swabs (TS) or a combination of both (NPS/TS), were collected into universal viral transport media (UTM) that preserves viral integrity for up to 48 hours at 2-25°C. The samples were transported according to WHO specifications in a triple package system – primary, secondary and tertiary (10). Samples and accompanying laboratory request forms were received into the BSL-3 laboratory through a sample transfer hatch. Each layer of packaging was disinfected with 1% bleach solution before unpacking. Details of samples and patient clinical information, date and time of receipt were documented in a log book. The forms were then scanned and saved to a secure network folder with controlled access to maintain confidentiality. Samples were initially inactivated in a biosafety cabinet class IIIC however, due to increasing number of tests as the outbreak progressed, the inactivation step was re-evaluated and subsequently deemed safe to be performed in a Biosafety Class I cabinet which allowed to speed up the testing process.

**Sample inactivation and nucleic acid extraction**

Sample inactivation and downstream nucleic acid extraction was done using pre-made buffers available in commercial kits. Several molecular testing kits such as the IndiSpin pathogen kit (INDICAL BIOSCIENCES GmbH, Germany); QIAamp Viral RNA minikit (Qiagen, Germany); DaAn nucleic acid extraction kit (DaAn Gene, China) have so far been evaluated. All waste from sample processing were collected in secured biohazard bags, autoclaved and incinerated daily for safe disposal.

**Nucleic acid amplification**

The RT-PCR assay used targeted the RNA-dependent RNA polymerase gene (RdRP), the nucleocapsid gene (N) and Envelope protein (E) (5) using the LightMix® Modular SARS-CoV (COVID-19) kits (TIB MolBiol GmbH, Germany), or the open reading frame ORF1ab region, (DaAn Gene, China). The assays were run on the LightCycler 96 Instrument (Roche Diagnostics GmbH, Germany) because of the capability for remote access and emailing of test data files for analysis. The assay was also optimised for other real time PCR machines available at MRCG, such as the Biorad CFX 96 Real time PCR System (Biorad Laboratories, Inc. USA). Though the assay was qualitative, the threshold cycle (Ct) for the fluorescent signal from samples with higher viral load occur earlier resulting in lower Ct values.

**Certification and external quality assessment (EQA) of RT-PCR test results.**

Proficiency and certification for COVID-19 testing was done in March 2020 by the World Health Organisation (WHO) External Quality Assessment Program for the detection of SARS-CoV-2 by RT-PCR
prior to receipt of the index case. In line with Good Clinical Laboratory Practice (GCLP), ISO 15189 standards and WHO recommendations for laboratories undertaking COVID-19 testing programs, selected samples were sent to a WHO designated reference laboratory for external quality assurance and cross validation of results. Discordant samples were investigated further by sequencing using the ARTIC Sars-CoV-2 amplicon tiling protocol (11).

Implementation with first suspected case and expansion of diagnostic capacity to MRCG field sites

The index COVID-19 case in The Gambia was a self reported case to the MRCG Clinical Service Department, who had just returned from the UK with symptoms of the disease (12). The patient presented at the Outpatient Department and was tested intermittently using the RT-PCR assay until a negative result was obtained.

In anticipation of the spread of transmission due to the closely knit lifestyle and culture, the need to expand testing to other parts of the country was assessed. Staff from the MRCG field sites were identified for a two-week intensive training on COVID-19 testing. A risk assessment was carried out at the different field sites to ascertain their suitability for processing COVID-19 samples. Modifications and implementation of appropriate safety precautions were undertaken such as procurement of biosafety cabinet class 1 for sample reception, redesign of sample reception and workflow for reduced personnel contact and use of a different real-time PCR machine – CFX96 Touch real-time PCR detection system (Biorad, USA).

Staffing considerations

Working teams were created in the lab in compliance with COVID-19 social distancing rules, with weekly rota schedules updated to allow team members get some time off and to reduce the workload per staff member. A buddy system was adopted to have two persons working together for each of the processes – from sample reception, nucleic acid extraction and RT-PCR to result reporting. Closed user group (CUG) services for mobile phone networks were used for easy communication within and across team members.

Pooling matrix for scale up of diagnostic testing

Anticipating an increase in the demand for COVID-19 diagnostic testing, a sample pooling approach was explored. We investigated the appropriate pooling strategy that would increase capacity of diagnostic testing for COVID-19 while retaining adequate sensitivity, in the context of large-scale surveillance in The Gambia. Sample pools at dilution factors ranging from 1:5 1:10, and 1:20, consisting of negative samples and positive samples with Ct values identified as low (<22), medium (22–30 and 31–36) and high (>36) were made. Replicates of the pooled samples were tested in a 25μl and 50μl reaction volumes.

RESULTS

Detailed RT-PCR test outcomes of the index case are summarized in Table 1, with viral load decrease extrapolated from the RT-PCR Ct values. All subsequent COVID-19 cases were similarly documented and monitored. A total of 2, 825 tests were carried out in MRCG by August 2020, of which 243 were follow up samples of known positive cases and the rest were suspected new cases. The mean age of individuals tested was 33 years in both males and females (Table 2), with higher positivity rate among male individuals in ages above the mean (P < 0.05). At the end of August 2020, MRCG had increased testing up to 350 individual COVID-19 samples daily, from the initial >50 samples; with five different working groups consisting of six members and a team lead to oversee the entire daily process.

The results of the EQA gave a concordance of 86.7%, with two discordant results out of 15 (10 negative and 5 positive) samples sent for external assessment (Table 3). Sequencing of the discordant samples detected SARS-CoV-2 sequences in one of them. However, both samples had high Ct values (sample GC19-0241 = 38.09 and 39.55; sample GC19-0268 = 37.86 and 37.76 for the N and ORF1ab genes, respectively) in our lab and at the lower range of the assay detection limit.

In the pooling test, all samples with Ct values <32 amplified in all the replicates at the 1:5 sample pool
but samples with Ct values >37 did not produce reproducible results (Table 4). There were differences in the amplification of the N gene and ORF1ab and reproducibility was poor with the 1:10 sample pool, whereas there was no amplification in the 1:20 sample pool for both 25µl and 50µl reaction volumes at Ct values above 37.

**DISCUSSION**

We report the rapid response and scaling up of capacity to provide quality molecular testing in sub-Saharan African laboratory, in the midst of a global disease pandemic. Although prevalence of the disease in The Gambia is relatively low, with 2895 confirmed cases out of 12460 tests by August 27th 2020 (13), the capacity to scale up testing as the need arose was established in the MRCG Unit. The EQA results were within acceptance limits at 86.7% concordance. The additional freeze-thaw cycle at the reference laboratory could have contributed to the discrepancy observed. A pooling strategy that could increase sample processing throughput by up to five times was established and optimised for deployment in community testing and surveillance.

With anticipated increase in COVID-19 cases from establishment of community transmission in many countries across West Africa, the major bottleneck was regular supply of items required such as gloves, surgical masks and appropriate protective gears for frontline staff. MRCG engaged with local entrepreneurs to identify solutions to overcome some of these challenges such as 3D printing of face shields, use of plastic aprons over disposable lab coats, making of cloth masks for non-critical work to spare the surgical masks and respirators needed for high risk laboratory work.

Another potential bottleneck was anticipated when frontline staff members working in close proximity become infected. Therefore, systematic testing of frontline staff have been implemented to identify and isolate infected individuals, and prevent further spread of the disease amongst frontline staff members. Other in-house solutions currently being evaluated to address projected bottlenecks for COVID-19 testing in The Gambia include, the use of in-house prepared buffers and sample collection medium as well as testing of antigen-based diagnostic techniques with shorter turnaround time and less requirement for sophisticated laboratory skills and infrastructure.

**CONCLUSIONS**

From a state of minimal preparedness, the global COVID-19 pandemic has forced The Gambia to face and summort the challenge of implementing NAATs for routine disease diagnosis. The presence of an established International Medical Research Institute with the human resources and laboratory facilities made it possible to respond to the early challenge of detecting imported cases and served to delay the entry of the virus and establishment of local transmission. This gave the public health authorities the window of opportunity to prepare a robust public health response to the pandemic before onset of local transmission. With over 70 years of research presence in The Gambia, MRCG leveraged the good working relationship with the National Health Ministry, to mount an effective response and to train staff of the National Public Health system to independently conduct COVID-19 testing in support of the National outbreak response. The gains achieved by this quick response and increased testing can be further accelerated through automated sample processing and molecular systems, to increase assay throughput and address the bottleneck of the manual steps required for conducting the gold standard RT-PCR test.

**INFORMATION**

**Author contributions**: OS led the safety trainings and organized various aspects of the diagnostic facility and teams; ECO conceptualized and drafted the manuscript; SJ contributed to all Standard Operating Procedures (SOPs) development and implementation as well as training of lab staff and drafting the manuscript; NH and AV contributed to training of laboratory staff, SOP development and implemen-
tation; MN was responsible for result documentation, dissemination and archiving; DN contributed to manuscript concept and provided supervision for the diagnostic group. All authors contributed to review of the manuscript.

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TABLE 1: Summary of results for index COVID-19 case in The Gambia.

| Test | Date       | Ct value of screening gene (E) | Ct value of confirmatory gene (RdRP) | Result |
|------|------------|-------------------------------|-----------------------------------|--------|
| 1    | 17/03/2020 | 30.45                         | 28.60                              | Positive |
| 2    | 20/03/2020 | 29.58                         | 29.06                              | Positive |
| 3    | 22/03/2020 | 30.17                         | 28.73                              | Positive |
| 4    | 26/03/2020 | 34.37                         | 38.31                              | Positive |
| 5    | 30/03/2020 | No Ct                         | No Ct                              | Negative |
| 6    | 31/03/2020 | No Ct                         | No Ct                              | Negative |

TABLE 2: Summary of individuals tested in MRCG by August 2020.

|          | Mean age | Median age | Total tested | New cases | Negative result | Indeterminate | Positive result | Positive cases ³33 years | Positive cases <33 years |
|----------|----------|------------|--------------|-----------|-----------------|---------------|------------------|---------------------------|--------------------------|
| Male     | 33       | 31         | 1825 (64.60%) | 1658 (64.21%) | 1591 (64.36%) | 20 (62.50%) | 47 (60.26%) | 32 (68.09%) | 15 (48.39%) |
| Female   | 33       | 30         | 1000 (35.40%) | 924 (35.79%) | 881 (35.64%) | 12 (37.5%) | 31 (39.74%) | 15 (31.91%) | 16 (51.61%) |
| Total    |          |            | 2825         | 2582      | 2472            | 32            | 78              | 47            | 31          |

TABLE 3: Comparison of Ct values of positive results sent for EQA.

| S/N | Sample ID   | N - gene | ORF1ab Gene | Discrepancy |
|-----|-------------|----------|-------------|-------------|
| 1   | GC19-0077   | 32.63    | 33.80       | 37          | 34.34        | No           |
| 2   | GC19-0214   | 22.46    | 20.78       | 23.2        | 22.46        | No           |
| 3   | GC19-0241   | 38.09    | 0           | 39.55       | 0            | Yes          |
| 4   | GC19-0268   | 37.86    | 0           | 37.76       | 0            | Yes          |
| 5   | GC19-0278   | 38.61    | 31.25       | 39.62       | 36.02        | No           |
| 6   | GC19-0020   | NA       | NA          | NA          | NA           | No           |
| 7   | GC19-0021   | NA       | NA          | NA          | NA           | No           |
| 8   | GC19-0022   | NA       | NA          | NA          | NA           | No           |
| 9   | GC19-0024   | NA       | NA          | NA          | NA           | No           |
| 10  | GC19-0025   | NA       | NA          | NA          | NA           | No           |
| 11  | GC19-0028   | NA       | NA          | NA          | NA           | No           |
| 12  | GC19-0030   | NA       | NA          | NA          | NA           | No           |
| 13  | GC19-0031   | NA       | NA          | NA          | NA           | No           |
| 14  | GC19-0032   | NA       | NA          | NA          | NA           | No           |
| 15  | GC19-0033   | NA       | NA          | NA          | NA           | No           |
FIGURE 1: Map of The Gambia showing location of the Medical Research Council Unit’s study sites (modified from Scott et al. 2014. https://doi.org/10.1371/journal.pone.0107280 under licence CC BY 4.0).
TABLE 4: Comparison of replicate results for sample pooling at the different classification of Ct values.

| S/N | Target gene | Sample ID | Ct prior to dilution | Dilution | 25ul RT-PCR rxn | 50ul RT-PCR rxn |
|-----|-------------|-----------|----------------------|----------|-----------------|-----------------|
|     |             |           |                      |          | Ct_1            | Ct_2            |
| 1   | N- gene     | 1322      | 21.89                | 1:5      | 24.11           | 24.16           |
|     |             |           |                      |          | 24.07           | 24.24           |
| 2   |             |           |                      | 1:10     | 25.03           | 25.19           |
|     |             |           |                      |          | 25.27           | 25.26           |
| 3   |             |           |                      | 1:20     | 26.42           | 26.51           |
|     |             |           |                      |          | 26.62           | 26.61           |
| 4   | 1277        | 29.04     | 1:5                  |          | 25.03           | 25.19           |
|     |             |           |                      |          | 25.27           | 27.23           |
| 5   |             |           |                      | 1:10     | 29.20           | 28.80           |
|     |             |           |                      |          | 29.02           | 29.14           |
| 6   |             |           |                      | 1:20     | 30.88           | 30.83           |
|     |             |           |                      |          | 30.76           | 30.73           |
| 7   | 1197        | 31.46     | 1:5                  |          | 29.50           | 29.41           |
|     |             |           |                      |          | 29.40           | 29.5            |
| 8   |             |           |                      | 1:10     | 32.63           | 32.94           |
|     |             |           |                      |          | 33.17           | 33.11           |
| 9   |             |           |                      | 1:20     | 33.77           | 34.00           |
|     |             |           |                      |          | 34.06           | 34.15           |
| 10  | 1374        | 37.66     | 1:5                  |          | 37.08           | 37.19           |
|     |             |           |                      |          | No CT           | 38.08           |
|     |             |           |                      |          | 39.16           | 39.16           |
| 11  |             |           |                      | 1:10     | No CT           | No CT           |
|     |             |           |                      |          | No CT           | No CT           |
| 12  |             |           |                      | 1:20     | No CT           | No CT           |
|     |             |           |                      |          | No CT           | No CT           |
| 13  | Negative    | No CT     |                      | 1:10     | No CT           | No CT           |
|     |             |           |                      |          | No CT           | No CT           |
| 14  | ORF1ab      | 1322      | 22.62                | 1:5      | 24.56           | 24.74           |
|     |             |           |                      |          | 24.8            | 24.88           |
| 15  |             |           |                      | 1:10     | 26.11           | 26.07           |
|     |             |           |                      |          | 26.00           | 26.09           |
| 16  |             |           |                      | 1:20     | 27.07           | 27.19           |
|     |             |           |                      |          | 27.32           | 27.67           |
| 17  | 1277        | 30.11     | 1:5                  |          | 28.41           | 28.07           |
|     |             |           |                      |          | 28.01           | 28.22           |
| 18  |             |           |                      | 1:10     | 29.48           | 29.48           |
|     |             |           |                      |          | 29.53           | 29.73           |
| 19  |             |           |                      | 1:20     | 31.19           | 31.59           |
|     |             |           |                      |          | 31.07           | 31.57           |
| 20  | 1197        | 32.34     | 1:5                  |          | 30.81           | 30.63           |
|     |             |           |                      |          | 30.40           | 30.81           |
| 21  |             |           |                      | 1:10     | 33.27           | 33.60           |
|     |             |           |                      |          | 33.82           | 33.87           |
| 22  |             |           |                      | 1:20     | 34.74           | 34.51           |
|     |             |           |                      |          | 34.80           | 35.30           |
| 23  | 1374        | 38        | 1:5                  |          | No CT           | No CT           |
|     |             |           |                      |          | No CT           | 38.14           |
| 24  |             |           |                      | 1:10     | 38.34           | No CT           |
|     |             |           |                      |          | No CT           | No CT           |
| 25  |             |           |                      | 1:20     | No CT           | No CT           |
|     |             |           |                      |          | No CT           | No CT           |
| 26  | Negative    | No CT     |                      | 1:10     | No CT           | No CT           |
|     |             |           |                      |          | No CT           | No CT           |