Research Square

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Evaluation of a Sample Pooling Strategy for Sars-cov-2 Using Real Time PCR

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Abstract

Back ground: Corona Virus Disease 2019 (COVID 19) in Uganda was first reported in a male traveler from Dubai on 21st March, 2020 shortly after WHO had announced the condition as a global pandemic. Timely laboratory diagnosis of COVID -19 for all samples from both symptomatic and asymptomatic patients was observed as key in containing the pandemic and breaking the chain of transmission. However, there was a challenge of limited resources required for testing SARS-COV-2 in low and middle income countries. To mitigate this, a study was conducted to evaluate a sample pooling strategy for COVI-19 using real time PCR. The cost implication and the turn around time of pooled sample testing versus individual sample testing were also compared.

Methods*:* In this study, 1260 randomly selected samples submitted to Uganda Virus Research Institute for analysis were batched in pools of 5, 10, and 15. The pools were then extracted using a Qiagen kit. Both individual and pooled RNA were screened for the SARS-COV-2 E gene using a Berlin kit.

Results*:* Out of 1260 samples tested, 21 pools were positive in pools of 5 samples, 16 were positive in pools of 10 and 14 were positive in pools of 15 samples. The study also revealed that the pooling strategy helps to save a lot on resources, time and expands diagnostic capabilities without affecting the sensitivity of the test in areas with low SARS-COV-2 prevalence.

Conclusion: This study demonstrated that the pooling strategy for COVID-19 reduced on the turnaround time and there was a substantial increase in the overall testing capacity with limited resources as compared to individual testing.

Background

On the 31st of December 2019 in the city of Wuhan, Hubei province of China, SARS-CoV-2 the causative agent for Coronavirus disease (COVID-19) was reported as a new epidemic that causes life threatening respiratory symptoms (Rothan & Byrareddy, 2020). The world Health Organization (WHO) on 12th March, 2020 declared COVID-19 a global pandemic meaning that the disease had spread across the international borders. As of 23rd March 2010, there were 334,981 confirmed cases, including 14,652 deaths putting the case fatality at 4.017% globally. COVID 19 is progressing very rapidly; new cases and fatalities are being reported in many countries across the world (Sohrabi et al., 2020). As of 26/10/2020, 43,376,552 cases of COVID 19 have been reported globally including 1,159534 deaths (4.01%). By 25th October, 2020, 47 countries were affected in Africa with a total of 1281981 cases and 2464 deaths that have been reported (Culp, 2020). The first case in Uganda was reported from a male Ugandan aged 36 years who had returned from Dubai after a business. In a months' time, the number COVID-19 cases had risen to 30 stagnating at 50 cases in May. However, there was a spike in cases at the end of May and June mainly from truck drivers. By mid-July, Uganda had reached 1000 cases. The country has confirmed 12743cases and 112 deaths as of 26th October 2020 (Culp, 2020).

Detecting victims of the virus is fundamental to the epidemic response efforts. It ensures case detection, quarantine of COVID 19 patients to prevent local spread and treatment of the victims and more broadly informs national response measures by providing data to compute the epidemic speed and quantum hence suppressing transmission however the ongoing Coronavirus disease 2019 pandemic is a substantial challenge for health care systems and their infrastructure. Testing in most countries is mostly focused on ill patients of COVID 19 while asymptomatic patients who are potentially infectious remain undiagnosed this is mainly due to shortage of testing kits and factories are struggling to keep up with the demands, it is therefore important to come up with ways to conserve reagents used in diagnosis of SARS-COV-2 Pooling of COVID 19 samples for testing using RT PCR can provide a cheap and effective way of gathering information and identifying prevalence of COVID 19 and identifying new cases in countries where it is hard to carry out a high number of tests since COVID 19 testing is costly in terms of consumables, trained human resource and testing kits and also time consuming.

Methods

Study design

This was a cross sectional study that was carried out between 10th August 2020 to 11th September 2020 at the Uganda Virus Reaserch Institute, Entebbe, Uganda. The study population included all samples from both males and female, children and adults. Samples submitted to the laboratory during the study period with good quality and appropriate volumes were considered for inclusion and were coded with unique identifiers. Samples with inadquate volumes and patients who did not consent were excluded from the study. All methods followed in this study were performed in accordance with the relevant guidelines and regulations.

Sample size and pool size determination

Sample size Calculation of detection of disease by EPI tools method was used to determine the appropriate size of pools (Cowling et al., 1999) Considering a prevalence of 0.02 in Uganda as of July 2020 (CDC, 2020) with 95 percent confidence of detection the following were the calculated minimum sample sizes for the pool sizes used in the study (Table 1).

Table 1
Sample sizes used in the study

Pool size	Minimum sample size	Actual sample size used
5	34	252
10	17	126
15	12	85

Data collection

Samples were collected from patients in the field using nasopharyngeal swabs and or oral swabs then put in sample containers containing 1ml of universal transport medium or Normal saline. Samples were then transported to the COVID 19 testing laboratory at Uganda Virus Research Institute. Case investigation forms for each patient with information such as age of the patient, name, residence were sent with the samples and cold chain was maintained.

Laboratory analysis

Samples were batched in pools of 5, 10 and 15. A total volume of 200 microliters was then aliquoted from each pool in a vial; 40 microliters per sample for the pool containing 5 samples was put in the same vial to make a total volume of 200 microliters and for the pool of 10 samples, 20 microliters per sample was aliquoted making 200 microliters as a total volume and that containing 15 samples, 13.3 microliters was aliquoted. The pools were vortexed vigorously at a high speed for one minute to ensure homogeneity then RNA was extracted.

RNA was purified using the Qiagen RNA extraction kit. The procedure is outlined below;

140 microliters of each pool was added to 560 microliters of lysis buffer, vortexed and pulse spun then 560 microliters of ethanol was added, the mixture was then vortexed and spun at 800RMP for one minute. The solution was then transferred to labelled spin columns and spun at 8000RPM for one minute. Then 500 microliters of AWI wash buffer was added and spun at 8000RPM for one minute, 500 microliters of AW2 wash buffer was then added and the tubes spun at 14000RPM for three minutes and then RNA was eluted in 70 microliters of elution buffer. Positive and a negative control were also included in the extraction process.

The eluted purified RNA was amplified using a Berlin kit that targets the E-gene on SARS-COV-2 A1-step master mix containing reverse transcriptase and DNA polymerase enzymes with 3 primers targeting specific regions of the viral genome. Primers targeting a human gene, such as RNase P were also included as a positive control for swab collection, RNA extraction and amplification.

Amplified products were detected using TaqMan probe fluorescence or DNA-intercalating dyes and a threshold cycle of amplification was set to distinguish positive from negative results. A test result was typically considered positive if amplification was observed for two or more viral targets, while it was considered negative if amplification was observed for the control RNA but for none of the viral targets. (CDC, 2020).

Data analysis

Real-time PCR results were interpreted as recommended in the WHO protocol. A pool or sample was considered positive for COVID-19 if the threshold cycle (C_T) value was less than or equal to 40 (Cornman et al., 2020)

A two by two table was used to compute the sensitivity, specificity and predictive values for each pool strategy. For every actual true pool outcome, a computation of money and time saved was done per pool. Pooled prevalence for fixed pool size and tests with uncertain sensitivity and specificity was used to calculate the estimated pool prevalences in EPI tools as described by Cowling et al., 1999.

Results

Demographic characteristic of SARS-COV-2 suspects

In the present study, 1260 COVID 19 suspected patients were involved and out of these 850 were male and 387 female, 13 (1.8%) males and 9 (2.3%) females were SARS-COV-2 positive (Table 2).

Table 2
Frequencies of demographic characteristics of SARS-COV-2 suspects

Variable	RT-PCR result		X^2	P-Value
	Negative (%)	**Positive (%)**		
Sex Male	850 (2.3)	13 (1.8)	0.929	0.335
Female	387(97.7)	9 (2.3)		
Age group		0(0)	1.343	0.511
0–15	44(3.5)			
16–49	1087(98.2)	20(1.8)		
≥50	106(97.2)	3(2.8)		

Prevalence of samples tested

Out of 1260 samples tested, 252 were pools of 5 samples, 126 pools of 10 samples and 84 pools of 15 samples. Among 252 pools of 5, 21 pools were positive, 16 pools were positive out of the 126 pools of 10 samples and out of 84 pools of 15 samples, 14 pools were positive. All positive pools of 5 samples were retested individually and only 23 samples out of 21 pools were positive as indicated in Table 3.

Table 3
Number of pools and estimated prevalence of pools

Pool size	No. of pools	Positive pools	Positive samples	Number of samples retested	Estimated prevalence (%)
5	252	21	23	105	1.83
10	126	16	20	160	1.59
15	84	14	17	210	1.3

Test performance of pooled samples compared to individual samples

The sensitivity of Individual samples was 100 percent, this was compared to the sensitivity of pools of 5, 10 and 15 samples and it was revealed that sensitivity of the pools was 100, 97 and 92 respectively (Table 4).

Table 4
Comparison of pooled and individual sample testing

No. of specimens in a pool	Amplification of E gene, %	No amplifications	Sensitivity of pools,90% confidence interval	Cumulative sensitivity
1	100	0	100(96–100)	100
5	100	0	100(96–100)	100
10	97	3	97(92–99)	97
15	92	6	92 (90–98)	90

Comparison of CT values of pools and individual samples

The Threshold Cycle values (CT) of pools versus individual samples were also compared, individual samples with a CT value of less than 38 remained positive in pools of 5,10 and 15, those that had a CT value greater than 38, were positive in pools of 5 and negative in pools of 10 and 15. Some samples when tested in a pool had a lower CT value than when tested individually as indicated in Fig. 1.

Cost implication of pooled sample strategy

The cost implications of pooling samples were compared with that of individual sample testing and in this study testing 1260 samples required only 126 tests in pools of 10 samples to detect 23 positive individuals compared to 1260 tests that would be required for individual sample testing. This implies that

pool sample testing reduced on the number of tests required and thus saved on resources which included consumables, extraction and testing kits to almost 70 percent. Considering the Ministry of Health cost of 185,000 Uganda shillings for an individual SARS-COV-2 test, pooling strategy resulted in saving of 209,790,000 Uganda shillings.

Turn around time

It was also observed that the turnaround time of samples run individually compared to those run in a pool was higher and this was due to an increase in the number of samples testing in a pool as compared to those tested individually. It took a minimum of 3 hours to process and report a result for one individual sample implying that it required up to 3780 hours to analyze and report for 1260 samples. Testing one pool of ten samples required only 3 hours to obtain and report results, implying that approximately 378 hours were required to analyze and report results for 1260 samples tested.

Discussion

The detection of COVID 19 using RT-PCR with the pooling of samples greatly reduces on the workload and costs especially when disease prevalence is low (Khai et al., 2020). In this study it was observed that specimen pooling almost certainly reduced on the cost of a test by about 70 percent since the prevalence was as low as 2 % and this in agreement with a study that was performed by Jaya et al that reported that pool testing of 10 samples saves about 60 percent of resources as compared with individual testing. Another study by Abdalhamid et al also indicated that pool testing is an effective way of saving on the resources required in COVID 19 testing in populations with a prevalence that is as low as 10 percent. Also Supaporn et al, 2020 indicated that in areas with a prevalence ≤ 1 percent or low risk populations can drastically decrease the resource burden on laboratory operations by up to 80 percent. However, if the prevalence of COVID 19 is higher than 10 percent then specimen pooling may save very little since most of the pools might turn out positive and thus a second test required therefore, pooling samples for COVID 19 testing is required in areas were the SARS-COV-2 prevalence is low or when carrying out a surveillance in areas with low infection rates.

This study also demonstrated that a pool of 5 samples had 100% sensitivity, 97% sensitivity in pools of ten specimens and 93% in pools of 15 samples this shows that as the number of samples in a pool increase, there is a reduction in sensitivity of a test and this is in agreement with a study that was performed in South Korea in 2020 by So Yeon Kim that indicated that pools with fewer samples had a higher sensitivity as compared to larger pools, they also recommended pooling < 6 specimens in clinical practice in order to prevent false-negative results that would come as a result of testing larger pools. Another study that was performed in India by Garg et al recommended testing pools containing ten samples in areas where the prevalence is less than 10 percent since it was observed that this strategy increased test capacity with existing equipment and test kits and positive samples were detected with sufficient diagnostic accuracy.

It was also observed that all samples that had their CT values less than 38 were all concordant in pools of 5, 10 and 15 as indicated in Table 3 above, however, those CT values higher than 38 turned out negative as the pool sizes increased and this is in agreement with a study by Supaporn et al that indicated how pool testing does not affect the sensitivity of a test if the viral load of patients being tested is greater than 35. Some samples when tested individually had a very low CT value however when tested in a pool, their CT values increased and this was due to having more than one positive sample in a pool. A study that was performed at the Kenyan coast by Agoti et al also compared the CT values of some pools and the individual samples and noted that there was on average a 1.59 Ct value increase for the samples during pooled testing versus the same samples tested singly.

No significant relationship between demographic factors and COVID 19 prevalence were noted in this study. However, other studies reported that males were more affected by COVID 19 than females especially after ages of 50 and above and these differences are attributed to three characteristics: differences in immune function associated with the X chromosome, the effects of sex hormones and gender-related behavior (Lakbar et al., 2020)

Conclusion

Due to the increasing demand of testing COVID 19 alongside global shortage of resources, laboratory professionals need to come up with strategies that reduce on the turnaround time and increase testing of samples using limited resources. This study demonstrated that the pooling strategy for COVID-19 reduced on the turnaround time and there was a substantial increase in the overall testing capacity with limited resources as compared to individual testing.

Sensitivity of PCR was inversely proportional to the pool size, implying that pool sizes should be maintained as small as possible to prevent false negative results. Pooling should not be performed in areas where the prevalence of COVID 19 is high.

Abbreviations

COVID-19: Coronavirus disease 2019SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; RT-PCR: Reverse transcription polymerase chain reaction test.

Declarations

Ethical approval

Ethical approval was sought from the ethical review board of College of Veterinary Medicine, Animal Resources and Biosecurity to carry out the study.

Permission was also sought from the UVRI management to allow the use COVID 19 samples for this study. Furthermore, confidentiality was observed by giving samples identification codes rather than the

use of names. All participants and parent/legally authorized representative of minor participants provided informed consent for the study.

Consent to publish

Not applicable in this study.

Availability of data and materials

The datasets used and/or analysed during the current study is available from the corresponding author on reasonable request.

Authors' contributions

AMN: Conception and design of study, collection, analysis and interpretation of data; drafting and critical review of manuscript, gave final approval for submission of manuscript.

LN: Conception and design of study, collection, analysis and interpretation of data; drafting and critical review of manuscript, gave final approval for submission of manuscript.

SB: Conception and design of study, collection of data; drafting and critical review of manuscript, gave final approval for submission of manuscript

JK: Conception and design of study, collection, analysis and interpretation of data; drafting and critical review of manuscript, gave final approval for submission of manuscript.

JL: Conception and design of study, drafting and critical review of manuscript, gave final approval for submission of manuscript.

AT: Conception and design of study, analysis and interpretation of data, drafting and critical review of manuscript, gave final approval for submission of manuscript.

JMK: Conception and design of study, collection, analysis and interpretation of data; drafting and critical review of manuscript, gave final approval for submission of manuscript.

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Conflict of interest

Authors declare that there is no conflict of interest.

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Figures

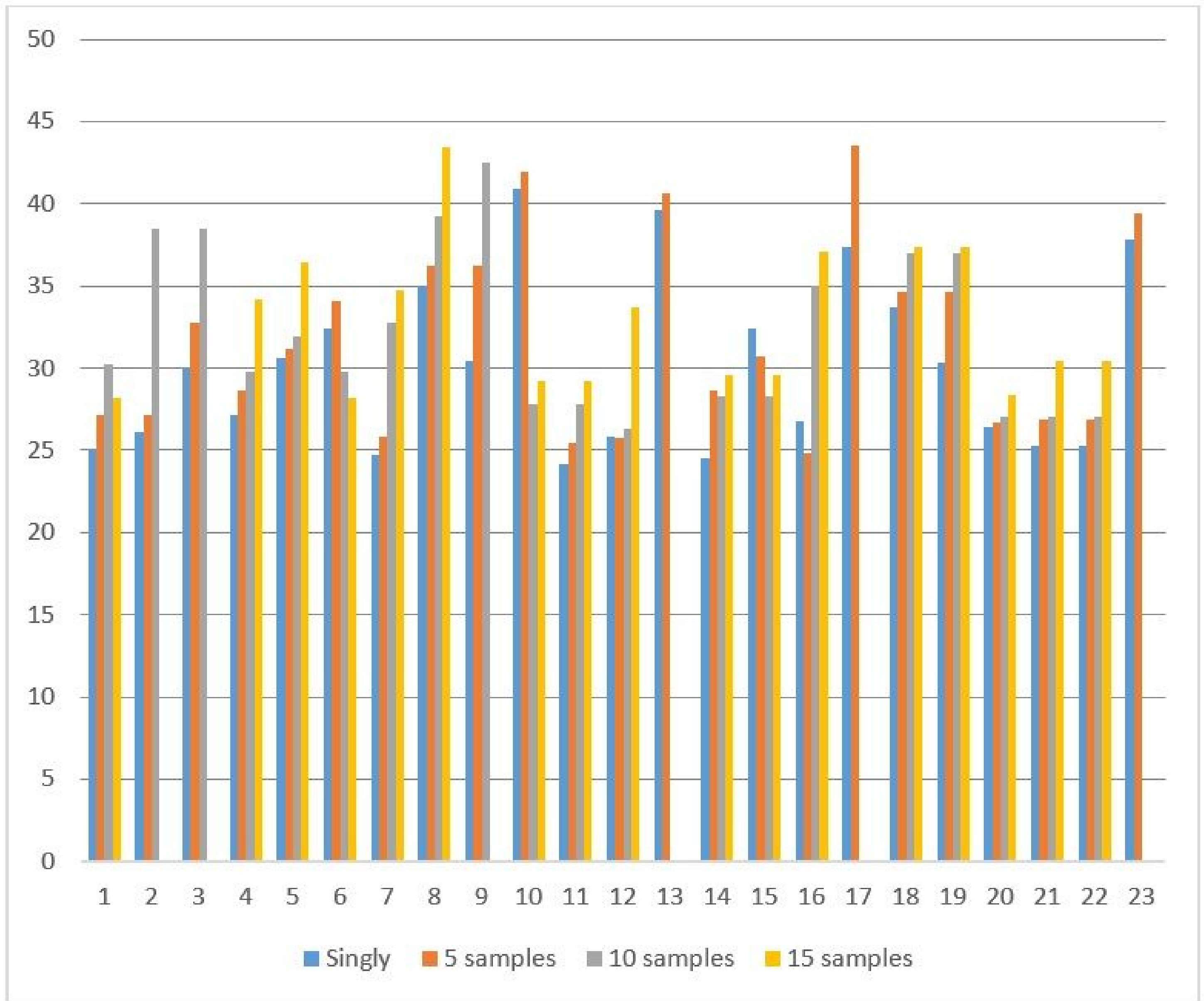

Figure 1

CT values of positive pools and individual samples.