Operational feasibility of using whole blood in the rapid HIV testing algorithm of a resource-limited settings like Bangladesh

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Background
Serum-based rapid HIV testing algorithm in Bangladesh constitutes operational challenge to scaleup HIV testing and counselling (HTC) in the country. This study explored the operational feasibility of using whole blood as alternative to serum for rapid HIV testing in Bangladesh.

Methods
Whole blood specimens were collected from two study groups. The groups included HIV-positive patients (n = 200) and HIV-negative individuals (n = 200) presenting at the reference laboratory in Dhaka, Bangladesh. The specimens were subjected to rapid HIV tests using the national algorithm with A1 = Alere Determine (United States), A2 = Uni-Gold (Ireland), and A3 = First Response (India). The sensitivity and specificity of the test results, and the operational cost were compared with current serum-based testing.

Results
The sensitivities [95% of confidence interval (CI)] for A1, A2, and A3 tests using whole blood were 100% (CI: 99.1–100%), 100% (CI: 99.1–100%), and 97% (CI: 96.4–98.2%), respectively, and specificities of all test kits were 100% (CI: 99.1–100%). Significant (P < 0.05) reduction in the cost of establishing HTC centre and consumables by 94 and 61%, respectively, were observed. The cost of administration and external quality assurance reduced by 39 and 43%, respectively. Overall, there was a 36% cost reduction in total operational cost of rapid HIV testing with blood when compared with serum.

Conclusion
Considering the similar sensitivity and specificity of the two specimens, and significant cost reduction, rapid HIV testing with whole blood is feasible. A review of the national HIV rapid testing algorithm with whole blood will contribute toward improving HTC coverage in Bangladesh.

Keywords
Bangladesh, HIV rapid test algorithm, HIV testing and counselling, serum, whole blood specimen

INTRODUCTION
In 2014, there were 36.9 million people living with HIV (PLHIV) and around 15 million of them were on lifesaving antiretroviral therapy [1]. Despite progress in global efforts to control the HIV epidemic, new HIV infection continues to spread relatively unabated in many parts of the world. Bangladesh, for example, is one of nine countries in Asia and the Pacific where HIV infection showed upward trend [2]. HIV prevalence in Bangladesh is low but concentrated among key-affected population (KAP) [3] comprised mainly of people who sell sex, people who inject drugs (PWID), men who have sex with men (MSM), and ‘Hijras’ (male to female transgender people) [4]. The first case of HIV was detected in

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HIV testing with whole blood

Bangladesh in 1989, and as at 2014, there were 3674 reported cases of with HIV in the country [5]. The Joint United Nations Programme on HIV/AIDS (UNAIDS), however, estimated a higher estimate of 11 000 PLHIV in Bangladesh [6] and majority of the PLHIV are unaware of their HIV status.

HIV testing and counselling (HTC) is an entry point to care and support for PLHIV. Client-initiated HTC was initiated in the late 90s in Bangladesh. This was followed by the establishment of voluntary counselling and testing centres and provider-initiated HTC in 2002 and 2006, respectively. The country adopted the WHO guidelines [7] to use three rapid antibody tests for HIV diagnosis in 2005. The national algorithm [8] for rapid HIV testing with serum was validated in Bangladesh in 2005 and the guidelines [9] are in effect as at the time of this study.

There are several limitations associated with the implementation of the national HIV rapid test algorithm with serum in Bangladesh. These limitations include the cost and delicate instrumentation required for serum-based testing, maintenance of cold chain, unstable source of electricity, and difficulties related to external quality assessment (EQA) [10]. These challenges contribute to the low coverage of HTC, especially among KAP in Bangladesh. In 2013, less than one in four KAP know their HIV status [11]. There is therefore, an immediate need to simplify the procedure for HTC to expand HIV diagnosis and linkage to HIV prevention, treatment, and care in Bangladesh.

This study explored the operational feasibility of using whole blood as alternative to serum for rapid HIV testing in Bangladesh. The study was premise upon the WHO recommendation [12] to use whole blood or oral fluid as alternatives to serum for rapid testing of HIV. The authors assumed that using whole blood may reduce cost and ease the procedure of HTC service in Bangladesh.

METHODS

Study design and selection criteria

The observational study was conducted between October and December 2014 and involved two categories of study participants. Overall, there were 400 study participants subdivided into 200 serologically confirmed HIV-positive individuals (group I) and another 200 individuals who were negative for HIV antibody (group II). The sample size is consistent with the recommendation by WHO and UNAIDS [12] to collect approximately 100 HIV antibody-positive specimens and 200 HIV antibody-negative specimens per site for the evaluation of rapid HIV testing kits. All the study participants were selected from the national virology reference laboratory at the Bangabandhu Sheikh Mujib Medical University (BSMMU) in Dhaka, Bangladesh. Blood specimen for study participants in group I were collected alongside laboratory test for measurement of CD4 T lymphocyte from PLHIV presenting at the reference laboratory until the required sample was reached. In Bangladesh, CD4 T-lymphocyte count is a routine laboratory test to measure response to antiretroviral treatment in PLHIV. Specimens from the study participants of group II were collected from HIV-negative individuals who were referred from outpatient clinics to the reference laboratory. The specimens in group II were randomly selected and confirmed through anti-HIV ELISA test (ANI Lab systems, Finland).

Determination of test performance and cost

Whole blood specimens were collected from all the study participants and subjected to three HIV rapid test kits, that is, Alere Determine HIV-1/2 (Alere, United States) as A1, Uni-Gold Recombigen HIV-1/2 (Trinity Biotech, Ireland) as A2 and First Response HIV Test 1–2.0 (PMC Medical, India) as A3. The results were interpreted according to manufacturer’s instructions by a laboratory technologist followed by verification of a clinical virologist. In addition, we estimated the cost of providing rapid HIV test using serum and whole blood, and compared the costs of the two approaches. The cost items were classified into four categories, namely, permanent logistics, consumables, cost for EQA, and administrative cost. The permanent cost calculated in this study includes the cost of purchase of equipment and materials required to establish an HTC service centre in Bangladesh. The salary of HIV counsellor and laboratory technologist was included as administrative cost. The analysis did not include the cost for utilities and maintenance of laboratory equipment.

Statistical analyses

All the data were analysed with SPSS, V-20 (IBM Corp., Armonk, NY 2011). Sensitivity, specificity, negative predictive value, and positive predictive value of the tests were calculated, and the results included 95% confidence interval. A comparison of the cost items were done with paired t-test [13] and $P<0.05$ was considered as significant. The null hypothesis ($H_0$) that there is no difference in the mean of the cost of rapid HIV testing with serum and whole blood and the alternative hypothesis ($H_1$) that there is a difference in the mean of the cost of rapid HIV testing with serum and whole blood were presented as follows: $H_0$: mean (serum–blood) = 0, $H_1$: mean (serum–blood) greater than 0.
Ethical considerations
The study was reviewed and approved by an Institutional Review Board of BSMMU Dhaka, Bangladesh (#BSMMU/2014/10632). Before collection of blood, the purpose of the study was explained to all the participants, and their informed written consent was secured. All the study participants were adults. The specimen was marked for the purpose of classification into the study groups. The names of the participants were, however, not linked to the specimen for confidentiality. In addition, all the documents related to the study were kept under lock and key. Only the principal investigator and coinvestigators had access to all the information.

RESULTS

Patient characteristics
The participants in group I (HIV-reactive samples) were aged between 20 and 65 years with mean age of 36.2 years, and SD of ±8.61 years. Participants in group II (HIV-nonreactive samples) were aged between 21 and 63 years with mean age of 35.9 years and SD of ±6.57 years. The male–female ratio in group I was 49:51 and 50:50 in group II.

Test performance of three rapid tests
The reactivity to HIV rapid test kits for each of the samples in the two groups was recorded as reactive, nonreactive, invalid, or indeterminate. The sensitivity, specificity, negative predictive value, and positive predictive value of the samples in groups I and II are presented in Table 1. In group I, all the samples were reactive to A1 test, 198 (99%) were reactive to A2 test, and 190 (95%) were reactive to A3 test. Invalid responses to A2 and A3 tests were observed in two (1%) and four (2%) samples, respectively, and six (3%) samples were nonreactive to A3 test. All the six nonreactive samples in group I in test A3 were reactive during retest evaluation. Group II samples were nonreactive to A1 test. There were, however, two (1%) invalid samples in group II to both A2 and A3 test kits. All the samples showing invalid test results were excluded from the calculation (Table 1). There were no indeterminate results for all the samples.

Cost analyses
The estimated cost of providing rapid HIV testing with serum and whole blood specimens are presented in Table 2 (in US dollars). The cost for implementing HIV rapid test with whole blood specimen using the three validated rapid HIV test kits [8] in
### Table 2. Breakdown of cost items for rapid HIV test with serum and whole blood

| Cost categories                | Cost items                        | Serum  | Whole blood |
|-------------------------------|-----------------------------------|--------|-------------|
| Estimated permanent logistics | Test tube rack                    | $2.24  | NA          |
|                               | Tourniquets                       | $0.64  | NA          |
|                               | Table top centrifuge              | $153.85| NA          |
|                               | Laboratory coat                   | $3.85  | $3.85       |
|                               | Micropipette [20–200]             | $92.31 | NA          |
|                               | Micropipette rack                 | $3.21  | NA          |
|                               | Long plain forceps                | $1.41  | NA          |
|                               | Timer/stop watch                  | $3.85  | $3.85       |
|                               | Refrigerator 7.5 cft              | $246.15| NA          |
|                               | Dial thermometer                  | $7.69  | NA          |
|                               | Cool box                          | $28.21 | NA          |
|                               | Ice pack                          | $1.03  | NA          |
|                               | Ice gel pack                      | $3.72  | NA          |
|                               | Wash bottle                       | $1.03  | $1.03       |
|                               | Hypochlorite beaker               | $5.77  | $5.77       |
|                               | Plastic bucket with lid           | $0.64  | $0.64       |
|                               | Waste basket (blue)               | $0.64  | $0.64       |
|                               | Autoclave                         | $102.56| NA          |
|                               | Incinerator                       | $25.64 | $25.64      |
|                               | Scissor                           | $0.64  | $0.64       |
|                               | Scandal (plastic)                 | $1.28  | $1.28       |
|                               | Total                             | $686.35| $43.33      |
| Estimated consumables         | Vacutainer                        | $0.21  | NA          |
|                               | Syringe 5 ml and 21G needle       | $0.05  | NA          |
|                               | Hypochlorite solution             | $1.67  | $1.67       |
|                               | Band aid                          | $0.77  | $0.77       |
|                               | Cotton roll                       | $0.64  | $0.64       |
|                               | Gloves (medium)                   | $3.46  | $3.46       |
|                               | Sharp container                   | $0.51  | $0.51       |
|                               | Hexisol (70% alcohol)             | $1.35  | $1.35       |
|                               | Polythene bag                     | $1.28  | $1.28       |
|                               | Serum separation pipette          | $3.85  | NA          |
|                               | Eppendorf tube                    | $8.97  | NA          |
|                               | Cryomarker fine point             | $0.77  | $0.77       |
|                               | Zeep sticker                      | $0.64  | NA          |
|                               | Magic tape with stand             | $1.54  | NA          |
|                               | Masking tape                      | $0.51  | $0.51       |
|                               | Micropipette tips                 | $5.13  | NA          |
|                               | White polythene                   | $1.28  | NA          |
|                               | Sample storage box                | $0.64  | $0.64       |
|                               | Aluminium foil                    | $1.73  | NA          |
|                               | Kitchen tissue roll               | $0.51  | $0.51       |
|                               | Liquid soap                       | $1.09  | $1.09       |
|                               | Cleaning duster                   | $0.13  | $0.13       |
|                               | Towel                             | $0.26  | $0.26       |
|                               | Register note book                | $0.64  | $0.64       |
|                               | Two ring file                     | $0.90  | $0.90       |
Bangladesh was US dollar 24 488, a 36% cost reduction when compared with serum-based testing (Table 3). There were significant reduction in the cost of permanent logistics and consumables by 94% \((P = 0.022)\) and 60% \((P = 0.018)\), respectively, when the rapid HIV test was done with whole blood compared with serum-based testing (Table 4). The cost of EQA and administrative cost reduced by 44 and 39%, respectively, when whole blood was used, though, the difference in the cost were not significant when compared with serum-based rapid HIV testing (Table 4).

**DISCUSSION**

Despite global progress in curbing the HIV epidemics, Bangladesh reported a 25% increase in HIV cases over the past decade [2]. Thus, the need was identify to expand coverage of HIV diagnosis, especially among KAP. Amid of decrease in available global resources [14] and inadequate government contribution [15] to the national HIV response, HTC programme of Bangladesh requires efficient use of the limited resources available for the HIV response. Therefore, there is need to develop an efficient strategy that can maximize the limited resources to expand the availability, access, and uptake of HTC services to meet the increasing need for HIV testing. This study indicates opportunities for cost saving and operational feasibility in rapid HIV testing with whole blood.

In this study, the sensitivity and specificity of rapid HIV testing with whole blood (Table 1) to the first – A1 (Alere Determine) and second – A2 (Uni-Gold) rapid test kits are comparable with serum-based HIV testing validated in the national algorithm [9]. In the national validation, the sensitivity of A1 and A2 were 100%, and the specificity for A1 (98.9%) and A2 (99.7%) were high [8]. This study reported 100% sensitivity and specificity for both A1 and A2 (Table 1). Results from other studies [16,17] that used whole blood specimen reported similar sensitivity and specificity that ranged from 98 to 100% to A1 and A2. A study in Tanzania,

### Table 2 (Continued)

| Cost categories            | Cost items                  | Cost estimates (USD) |
|----------------------------|-----------------------------|----------------------|
|                            | Paper file                  | $0.13                |
|                            | Pen                         | $0.13                |
|                            | Total                       | $38.78               |
|                            | Cost for LIA                | $18 871.79           |
|                            | Cost for ELISA              | $2953.85             |
|                            | Cost for DBS (20)           | NA                   |
|                            | Human resource (seven person) | $14676.92        |
|                            | Total                       | $36502.56            |
| External quality assessment| Cost for counsellor         | $211                 |
|                            | Cost for laboratory technologist | $135                   |
|                            | Total                       | $346                 |
| Administration cost        | Grand total (operational cost) | $38041.03         |

Approximate expenditure are shown, cost can vary as per items number, brands, market prices, etc. DBS, dried blood spot; LIA, line immunoassay; NA, not applicable; USD, US dollar.

### Table 3. Summary cost estimates for rapid HIV test with serum and whole blood

| Expenditure for cost items (in USD) | Cost of HIV rapid test modality (USD) | Cost reduction percentage |
|-------------------------------------|---------------------------------------|---------------------------|
|                                     | Serum       | Whole blood   |                           |
| Permanent logistics                 | $686.35     | $43.33        | 93.7                      |
| Consumables                         | $38.78      | $15.38        | 60.3                      |
| External quality assurance          | $36502.56   | $20688.30     | 43.3                      |
| Administrative cost                 | $346        | $211          | 39.0                      |
| Total (operational) cost            | $37573.69   | $20958.01     | 35.8                      |

USD, US dollar.
however, reported a lower specificity of Alere Determine rapid test kit (A1) to whole blood specimen [18] in one setting. The sensitivity of the Fast Response (A3) was 97% in this study (Table 1). This low performance of Fast Response (A3) when compared with Alere Determine (A1) and Uni-Gold (A2) assays was also observed in the national validation with serum [8] and in another study in Tanzania [16].

The HIV rapid test kits evaluated in this study were based on Immunochromatography. The WHO, however, recommends that HIV test algorithm should include a combination of different test principles and/or antigen systems [12]. The authors are of the view that additional rapid test kits using different methods should be evaluated with whole blood as a backup that could be included in future algorithm to solve discrepancies in test results. There were, however, no samples showing concordantly false positive or false negative results to the three rapid test kits used in this study. In addition, there was no variation related to age and sex in the performance of rapid HIV assays with whole blood in this study. These results indicate comparable quality of whole blood to serum currently used in the national HIV rapid test algorithm of Bangladesh.

The exclusion of permanent logistic items like centrifuge, autoclave, and refrigerator not required for rapid HIV testing with whole blood significantly ($P = 0.022$) reduced the cost of permanent logistics when compared with HIV testing with serum (Table 2). Similarly, microcentrifuge tube, micropipette tips, and other consumables are not required in rapid HIV testing with whole blood, and result in significant reduction ($P = 0.018$) in the cost of consumables. These cost savings are consistent with expectation, as permanent logistics and consumables are critical inputs to establish new HTC centres in Bangladesh, and therefore expand coverage.

The reduction in the cost of EQA, though not significant ($P = 0.244$) was attributed to the use of two ELISAs in place of line immunoassay (LIA) (Table 2). The WHO [19] recommends the use of ELISA in quality assurance of HIV test with whole blood. In line with the WHO guidelines [11], all sera tested for HIV were preserved at $-20^\circ$C (in refrigerator) for EQA. In the reference laboratory, all the sera for EQA are tested by ELISA followed by recheck of all positive samples with LIA. Conversely, whole blood specimen for EQA were spotted on dried blood spot paper, stored at ambient temperature and tested and retested (for positive specimen) with ELISA [20,21]. The EQA approach for blood specimen does not require cold chain and less demanding storage space. The reduction in the administrative costs was also not statistically significant ($P = 0.250$).

The implementation of HTC requires appropriate mix of human resources like counsellors, laboratory technologist, and virologist, irrespective of the specimen used for the test. In this study, the cost elements – human resources for EQA and counsellor in administrative cost – were constant when serum or whole blood specimen were used (Table 2); a further justification for the central place of human resource in HTC. It was, therefore, consistent that the differences in the cost items (EQA and administrative) that include human resources were not significant (Table 4) when different specimens were used for rapid HIV testing.

There are several challenges associated with the implementation of the national HIV rapid testing algorithm using serum. These approach dependents on the use of centrifuge machine, which is expensive and requires electricity for operation. In many occasions, electricity cut constitutes delay in HIV testing in Bangladesh. In addition, the cost of electricity in Bangladesh is expensive. Current approach to rapid HIV testing with serum does not encourage community-based HIV testing because of the cost of setting up multiple HTC centres and the inconvenience of carrying centrifuge machine and refrigerator to the field. On the contrary, HIV testing with whole blood is simple, convenient, and less
procedural. It requires fewer logistics that reduces the cost and operational burden. HIV assays on whole blood can be performed on finger-pricked blood [22], in contrast to plasma/serum assays where venous blood is required. The use of finger-stick blood samples shortens the turnaround time for HTC. Pre and posttest HIV counselling can be offered to clients at the same time HIV testing is done. This simplified approach allows for the expansion of HIV test sites outside of laboratories into community locations. The use of finger-pricked blood is also beneficial in HIV diagnosis among PWID who often present with injection-related injuries including fibroses veins [23]. The highest HIV prevalence of 7.3% was reported among PWID [24] of Bangladesh. This change in blood collection approach will ease the implementation of HTC programme among the PWID.

To implement whole blood strategy for HTC, there are several limitations. To get an appropriate rapid test result with whole blood, there is requirement of a sample application device and one should read the results at the correct time. These require a standard operating procedure (SOP). Lack of adherence to the SOP can easily lead to suboptimal performance with poor sensitivity or specificity [25]. To our knowledge, until now, globally there is lack of standard guidelines and SOPs for HIV testing and EQA using whole blood specimen. Moreover, blood specimen collected through finger sticks is limited to 300 μl, and can only be used for very few laboratory tests. Although a training of limited scope is proposed for nonlaboratory health personnel to attain proficiency in HIV rapid testing with whole blood, and reporting [26], majority of laboratory technologist also require an orientation. The high level of detection accuracy, cost reduction, and operational feasibility reported in this study, however, supports the scale-up of rapid HIV testing with whole blood in resource-limited settings like Bangladesh.

The study established that rapid HIV testing with whole blood is feasible in Bangladesh. The sensitivity and specificity of using whole blood in rapid HIV testing were comparable with the national algorithm for rapid HIV testing with serum. Using finger-pricked blood for HIV testing will contribute more in HTC among PWID and expand coverage to communities in Bangladesh. This study conclude that the current investments in human resources to complement efficient allocation of resources for permanent logistics and consumables required for rapid HIV testing with whole blood will contribute toward scaling up of HTC services in Bangladesh.

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Conflicts of interest
There are no conflicts of interest.

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