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Dried blood spots are a useful tool for quality assurance of rapid HIV testing in Kigali, Rwanda

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Summary A study was conducted in two primary health facilities in Kigali, Rwanda, to determine whether dried blood spots (DBS) used for quality control of HIV testing would give comparable results with serum after being stored for a period of 14 days and 30 days at ambient temperature. DBS and serum specimens were collected from patients undergoing HIV testing. ELISA performed on serum at baseline (gold standard) was compared with DBS results. The study included a total of 491 patients, comprising 92 (19%) males and 399 (81%) females with a median age of 27 years. A total of 148 individuals (30%) were HIV-positive. The average ambient temperature under which DBS specimens were stored at the health facilities was 23 °C (range 18—25 °C). The κ statistic at 14 days and 30 days was 0.99 (99.4% agreement) and 0.98 (99.2% agreement), respectively, signifying almost 'perfect agreement ($P < 0.001$)' with the gold standard. In a resource-limited sub-Saharan African country embarking on scaling-up of HIV testing, DBS stored at ambient conditions for up to 1 month were found to be a useful and robust tool to perform quality control of rapid HIV testing at the health centre level.

1. Introduction

Scaling-up of voluntary counselling and HIV testing (VCT), which is the entry point to all preventive and care-related interventions including antiretroviral treatment (ART), is a public health priority in sub-Saharan Africa. The use of point-of-care (on-site) rapid whole-blood tests for performing HIV testing at VCT sites has several advantages over conventional laboratory-based tests, and the WHO has thus recommended their routine use in resource-limited settings.[1] The main operational advantages of using rapid whole-blood tests[2—5] are: they are relatively simple to perform; finger-prick is used to obtain blood instead of venous puncture, facilitating its use by lower-level health cadres (task shifting); results are readily available on site and

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within minutes, which is valuable from a client perspective; and, since whole blood is used, no additional laboratory resources are needed. The overall testing costs are also considerably lower than laboratory-based tests.

However, one of the principal disadvantages of the recommended rapid whole-blood testing strategy is that since the testing strategy involves a finger-prick procedure for obtaining blood, it does not involve any routine storage of blood specimens and external independent quality assessment of HIV testing is currently not possible.

In Rwanda, the Ministry of Health (MoH) currently uses rapid tests at all VCT sites in the country. Owing to the understandable concern of running external quality control, the MoH decided that all VCT sites would withdraw 'venous blood' instead of using a finger-prick and the HIV testing procedure would be done on serum/plasma[6] instead of whole blood. Specimens are thus centrifuged on-site and stored in a deep freezer for transport and external quality control at the central laboratory. This approach negates many of the core advantages of using a simple finger-prick strategy as it is human resource intensive, complicates the HIV testing procedure and is more time consuming for patients and staff. The current strategy thus poses a potential barrier to scaling-up of HIV testing to decentralised sites where there are human, infrastructural and financial limitations. The use of a dried blood spot (DBS) (a spot of whole blood placed on a simple filter paper and allowed to dry) for HIV diagnosis using ELISA might allow VCT sites to use whole blood for external quality control instead of serum.[7–12] If feasible and robust, DBS would be of particular interest in Rwanda and in other sub-Saharan African countries as they would permit whole-blood HIV testing to be done using a finger-prick strategy and at the same time not compromise quality control.

The aim of this study was to ascertain whether the DBS used for quality control would give comparable results with serum after being stored for a period of 2 weeks to 1 month at ambient temperature conditions in Kigali, Rwanda.

2. Methods

2.1. Study setting and population

The study was conducted in two primary health centres (Kimironko and Kinyinya) that offer VCT services in Kigali, the capital of Rwanda. The study population included a consecutive cohort of individuals undergoing HIV testing. Children under the age of 2 years were excluded from the study owing to the presence of maternal antibodies that affect the reliability of rapid HIV testing. The study was conducted during a 6-week period in August–September 2005.

2.2. Samples and laboratory procedures

Patients accepting an HIV test had 5 ml of venous blood withdrawn into a Vacutainer and 3 ml withdrawn into an EDTA tube (with anticoagulant). The 5 ml sample was centrifuged and the serum was used (as is routine) for on-site HIV testing using a combination of Determine HIV-1/HIV-2 (Abbott Laboratories, Wiesbaden-Delkenheim, Germany) and Uni-Gold HIV-1/HIV-2 (Trinity Biotech, Bray, Co. Wicklow, Ireland), in line with the WHO strategy III for HIV antibody testing.[1] The remaining part of this serum sample was stored in a deep-freezer compartment (−20 °C) and transported (under cold chain) on a scheduled basis to the reference laboratory in Kigali where HIV testing was performed using ELISA. Serum results drawn through venipuncture are currently the accepted procedure for quality assurance using ELISA in Rwanda and this was set as the gold standard.

From the 3 ml EDTA tube, 50 μl of blood was spotted onto circles of Guthrie card filter paper (Schleicher & Schuell, Keene, NH, USA). Samples were left to dry at room temperature for a minimum of 3 h and stored in a zipped polythene bag containing a desiccant. These constituted the DBS samples. A total of three such samples were made for each patient; one served as a backup and the other two served for HIV testing using ELISA at 14 days and 30 days of specimen collection. DBS samples were kept at room (ambient) temperature. Ambient temperature was recorded twice daily.

ELISA testing for HIV status was performed on DBS samples (after undergoing blood elution[13]) and on serum using standard international ELISA testing procedures. ELISA test kits used included Vironostika (HIV Uni-Form ll Ag/Ab; bioMérieux, Craponne, France) and Murex (HIV-1.2.0; Abbott Laboratories). A confirmed HIV-positive result with ELISA meant a positive HIV outcome with both Vironostika and Murex.

2.3. Data collection, sample size and statistical analysis

Data collection sheets at the VCT sites were used to gather basic sociodemographic information and information related to samples. Since this was a validation study, the minimum sample size required to estimate sensitivity and specificity was used. This meant inclusion of a minimum of 100 HIV-positive and HIV-negative samples. Based on an estimated HIV prevalence rate of 25% among those undergoing HIV testing at the two sites, a minimum of 450 individuals were required for the study.

The sensitivity and specificity of the ELISA assays done on DBS samples stored for 14 days and 30 days were compared with serum results. The measure of agreement between DBS testing and baseline serum testing was determined using the κ statistic graded as follows: 0.81–1.0, almost perfect; 0.61–0.80, substantial; 0.41–0.60, moderate; 0.21–0.40, fair; 0.01–0.20, slight; and <0.001, poor. The level of significance was set at $P \leq 0.05$ and 95% CIs were used throughout. Data were entered into Epi Info 6.04 (CDC, Atlanta, GA, USA) and analysed using STATA 10 (Stata Corp., College Station, TX, USA).

3. Results

Specimens were drawn from a total of 493 patients. One patient was excluded from the analysis owing to a missing serum Vironostika test result and one sample was incomplete. Among the 491 patients for whom data were complete, there were 92 (19%) males and 399 (81%) females with a median age of 27 years (interquartile range 22–33 years). Among the individuals, 311 (63%) were married, 92 (19%) were single (unmarried), 66 (13%) were widowed or

Table 1 Validity of ELISA HIV test results using dried blood spots (DBS) exposed to ambient temperature for 14 days and 30 days compared with serum samples (gold standard) ($n = 491$)

DBS	Sensitivity	Specificity	PPV	NPV	κ	Measure of agreement
14 days	156/156 (100%)	332/335 (99.1%)	156/159 (98.1%)	332/332 (100%)	0.99	99.4% ($P < 0.001$)
30 days	155/156 (99.4%)	332/335 (99.1%)	155/158 (98.1%)	332/333 (99.7%)	0.98	99.2% ($P < 0.001$)

PPV: positive predictive value; NPV: negative predictive value.

divorced and 22 (4%) were children above 2 years of age. Farmers, street vendors and housewives were the most common occupations, comprising 67% of all patients. A total of 148 individuals (30%) were HIV-positive. The average ambient temperature during the study period was 23 °C (range 18—25 °C).

Table 1 shows the validity (sensitivity, specificity, and positive and negative predictive values) of ELISA HIV test results from DBS samples stored for 14 days and 30 days at ambient temperature compared with serum at baseline (the gold standard). The κ statistic at 14 days and 30 days was 0.99 (99.4% agreement) and 0.98 (99.2% agreement), respectively, signifying almost 'perfect agreement ($P < 0.001$)' with the gold standard.

Rapid HIV test results compared with the gold standard had a sensitivity and specificity of 91.7% and 98.5%, respectively. The κ statistic was 0.91 and the measure of agreement was 96.3%.

4. Discussion

This study shows that DBS samples left at ambient temperature for up to 1 month at health centre level and then used for quality control of rapid HIV testing compare well with serum. The measure of agreement using DBS was >99% after 14 days and 30 days of specimen collection and the κ statistic was almost perfect.

The findings of this study are encouraging for a number of reasons. First, although many sub-Saharan African countries such as Rwanda are scaling-up rapid whole-blood HIV testing as an entry point to prevention and care,[14] there are no systematic measures in place for quality assurance of HIV testing. The use of DBS introduces the possibility of quality control and is of particular operational advantage within the scaling-up process; DBS specimens can be stored at ambient conditions for periods of 2 weeks to 1 month, implying that no cold chain is required at the health facility level for either storage or specimen transport; DBS can be transported for quality control to the reference laboratory on a monthly basis during supervision visits, thus facilitating the related logistics; and finally DBS can be stored in envelopes, which puts less demand on the often limited space available at peripheral health facilities.

Second, the use of ELISA techniques is robust, assay procedures are standardised, the equipment is often available at district or provincial laboratories in many resource-limited countries, and laboratory staff are conversant with its use. Eluting DBS samples and running quality control testing for HIV using the ELISA technique is thus feasible without additional equipment.

Third, some countries such as Rwanda have been hesitant to use a finger-prick strategy and 'whole blood' for rapid HIV testing owing to the understandable concern of running external quality control. As such, VCT sites have been obliged to resort to venipuncture and the use of serum.[6] The possibility of running external quality control using DBS allows the use of the finger-prick strategy (and whole blood) for rapid HIV testing without compromising on quality control with its related advantages, namely: (a) task shifting of HIV testing from laboratory technicians to lay counsellors would be possible and this would favour decentralisation to sites where there are shortages of qualified staff; (b) the HIV testing procedure would be less human resource intensive and time consuming as centrifugation would be unnecessary; (c) the need for syringes, needles and blood tubes associated with venipuncture would be avoided; and (d) cold chain facilities to store serum for quality control would no longer be required.

Despite these advantages, an issue that needs to be resolved in order to reap the potential benefits of DBS would be to set out standardised guidelines on sampling for quality control at different sites. This will have to be tapered according to HIV prevalence and throughput. This could involve testing, for example, a percentage (e.g. 10%) of specimens selected on a random basis (e.g. every 2 months) or consecutive specimens at a given time of the day during each week.[15]

Possible limitations of this study include the fact that serum was used for rapid HIV tests instead of whole blood, which is the general practice in most other countries, and DBS relied on controlled application of EDTA blood on filter paper instead of whole blood collected directly from finger-prick in a real-world situation.

Further development of specific DBS protocols is necessary in order to assist with the expansion of quality control of rapid HIV testing, especially at remote sites.

In a resource-limited sub-Saharan African country embarking on scaling-up of HIV-testing, DBS were found to be a useful and robust tool to perform quality control of rapid HIV testing services.

Authors' contributions: PC, RZ and ER were involved with the study conception and study design; PC and ER were involved with the field implementation and laboratory testing; PC, RZ, KH and ADH were involved with data analysis and interpretation; RZ, KH and PC drafted the first version of the manuscript; ADH improved the intellectual content. All authors read and approved the final manuscript. RZ is guarantor of the paper.

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