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Dried tube specimens: A simple and cost-effective method for preparation of HIV proficiency testing panels and quality control materials for use in resource-limited settings

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HIV testing has rapidly expanded worldwide, but proficiency testing (PT) programs to monitor and improve the quality of testing are often lacking in resource-limited settings (RLS). Traditional PT programs and quality control reagents use serum or plasma specimens requiring stringent conditions for storage and transportation.

A novel, simple and easy to use approach, based on dried tube specimens (DTS), was developed that can help monitor the quality of HIV antibody testing in RLS. DTS were prepared by drying 20 μl of specimen overnight at room temperature. The addition of a green dye (0.1%) made the DTS pellets visible without affecting the test results. Before testing, the DTS were rehydrated with 200 μl of PBS–Tween buffer. A panel of 303 DTS samples (135 HIV positive and 168 HIV negative) was evaluated with two rapid tests. Sensitivity and specificity with the Determine HIV-1/2 test were 99.3% and 99.4%, respectively, and with OraQuick were 98.5% and 100%, respectively. Stability studies showed that HIV-specific antibodies in the DTS specimens were stable at 4 °C and 25 °C for 4 weeks, with only marginal decline at 37 °C and 45 °C over 4 weeks. The DTS-based PT program was piloted successfully in 24 testing sites in Kenya.

Results demonstrate that the DTS is a simple to use, practical method to prepare and distribute PT panels and quality control specimens to monitor HIV testing practices in RLS.

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1. Introduction

The increased commitment and resources from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), the Global Fund for AIDS, Tuberculosis, and Malaria (GFATM) and other major initiatives have resulted in a rapid expansion of testing for human immunodeficiency virus (HIV) in order to meet the prevention, care and treatment goals in many resource-limited countries (Jereni and Muula, 2008; Plate, 2007). HIV testing sites include national and regional laboratories, blood donation centers, antenatal clinics (mother–child health facilities), primary health centers, facility-based and stand-alone counseling and testing centers and mobile facilities. The majority of HIV testing in resource-limited settings is dependent on rapid tests performed by trained health-care professionals or lay counselors working in diverse and often non-laboratory settings, resulting in an expanded need to monitor the quality of testing procedures and ensure the accuracy of the results.

External quality assurance (EQA) is essential to ensure the accuracy of diagnostic testing. Key components of EQA are (a) proficiency testing (PT), (b) periodic retesting of a subset of specimens in a qualified laboratory and (c) site visits/audits by external experts. PT programs have been shown to be very effective in improving the quality of testing for various diseases and analytes (Chalermchan et al., 2007; Goguel, 1991; Hannon et al., 1989; Hofherr et al., 1992; Jackson et al., 1993; Peddecord et al., 1992; Polesky and Hanson, 1990; Reichelderfer and Jackson, 1994; Rickman et al., 1993; Schalla et al., 1990; Schwartz et al., 1988; Schweiger et al., 1997; Yen-Lieberman et al., 1996). However, they are either limited in coverage or lacking altogether in most developing countries,

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where the burden of the diseases is high and laboratory infrastructure is limited.

Several factors account for the limited EQA programs in developing countries. In countries where retesting is the primary means of monitoring the quality of HIV serologic testing, the approach is expensive, time consuming and logistically challenging in decentralized settings. Site visits to thousands of testing sites require a large number of trained personnel and resources as well as subsequent follow-up to ensure corrective actions have been taken. PT programs using liquid serum or plasma specimens are limited to laboratories or sites that are easily accessible and do not include thousands of rapid testing sites. Moreover, liquid specimens require a cold chain, which is expensive and difficult to maintain across the country.

To address the above challenges, a new, cold-chain independent approach is described here for PT program or for preparation of quality control specimens. Serum or plasma specimens, prepared as dried tube specimens (DTS), are stable for at least a month at temperatures encountered in many countries and can be used to prepare panels for PT or as quality control reagents.

2. Materials and methods

2.1. Serum or plasma specimens

Initial evaluation and optimization of DTS and stability studies were performed using a well-characterized panel of 10 specimens (4 HIV-1 antibody positive and 6 HIV negative) routinely used in our laboratory. Following optimization, comparative study of DTS and liquid specimens was performed using a diverse panel of specimens (N = 303, 135 HIV positive and 168 HIV negative). These specimens were obtained from South Africa, Cameroon, Ivory Coast, Kenya and the United States. All specimens were characterized by the US Food and Drug Administration-approved enzyme immunoassay (EIA) (Genetic Systems HIV-1-2-O EIA; Bio-Rad Laboratories, Hercules, CA, USA). Repeatedly EIA-reactive specimens were further confirmed for HIV-1 status by HIV-1 Cambridge Western blot (Maxim Biomedical Corp., Rockville, MD, USA).

2.2. Determination of optimal volume

To determine the optimal volume needed for preparing DTS, various amounts (10 μl, 20 μl, 30 μl, 40 μl and 50 μl) of the 10-panel specimens were dispensed into the bottom of 2 ml Sarsdedt tubes. The tubes were left open and allowed to dry at room temperature for varied durations. Prior to testing, tubes were rehydrated with 0.2 ml of PBS–Tween (0.1 M phosphate-buffered saline, pH 7.4, with 0.1% Tween-20; Sigma Chemical Co, St. Louis, MO, USA), mixed by gentle tapping and left overnight at room temperature to allow solubilization. Tubes were mixed again by gentle tapping and contents were used for HIV testing.

2.3. Preparation of DTS and stability study

DTS specimens were prepared by transferring 20 μl of serum or plasma, premixed with 0.1% (v/v) green dye (food color, Kroger brand), into a 2 ml Sarsdedt tube. The addition of 0.1% green dye did not affect the HIV test results but allowed visualization of the colored pellet at the bottom of the tube. The tubes were left open in a laminar flow hood overnight to dry. They were then capped and stored at 4 °C until rehydrated prior to testing.

For stability studies, multiple sets of DTS specimens were prepared using a "10-member panel" (4 HIV positive and 6 HIV negative) and stored at 4 °C, 25 °C, 37 °C or 45 °C. To evaluate the stability of DTS tubes, one set from each temperature was rehydrated at weeks 1, 2, 3 and 4, and tested for HIV antibodies.

2.4. HIV antibody testing of DTS specimens

PBS–Tween was prepared, filtered through a 0.2 μm filter and aliquoted in 1.5 ml volumes to be used as rehydration buffer (also termed as PT buffer). A day before testing, DTS specimens were rehydrated by adding 200 μl PBS–Tween with a precision pipette, or 7 drops with a plastic disposable transfer pipette (Cat # 13-711-43, Fisher Scientific, Waltham, MA, USA). Seven drops amounted to about 200 μl with this pipette. This resulted in a 1:10 dilution of the original specimen but was treated as undiluted for the purpose of further testing. The specimens were mixed by gentle tapping, without vortexing, to mimic practical conditions encountered at the counseling and testing sites, and were left overnight at room temperature to allow solubilization of dried serum or plasma into the PT buffer. The next day, specimens were mixed again by gentle tapping and used to perform rapid tests or EIA (Fig. 1). Determine HIV-1/2 (Inverness Medical, Waltham, MA, USA), OraQuick Advance HIV-1/2 (OraSure Technologies, Bethlehem, PA, USA), UniGold, and Capillus (both Trinity Biotech, Dublin, Ireland), Bioline (Standard Diagnostics, Korea) and GS HIV1-2-O EIA (GS EIA, Bio-Rad Laboratories, Hercules, CA, USA) were performed according to manufacturers' instructions.

For the stability study, GS enzyme immunoassay (EIA) was performed at 1:1000 dilution, instead of the recommended 3/4 dilution, to observe decline in antibody levels, if any. To determine changes in HIV antibody levels during the stability study at different temperatures, the specimens were also tested by the BED assay, a quantitative HIV antibody assay that measures HIV-IgG as a proportion of total IgG (Parekh et al., 2002).

2.5. DTS-based PT panels and pilots

For a pilot PT program, DTS-based PT panels were prepared using 6 well-characterized specimens (3 positive and 3 negative). The panel members were coded as A1 to A6. Knowing that the PT panels would be tested at counseling and testing sites with no pipetting device or equipment, the instructions were simplified by preparation of a job aid to help guide the process. Each PT panel package included 6 vials of PT panel, 1 vial of PT buffer (PBS–Tween, 1.5 ml), a transfer pipette and a simple job aid, as shown in Fig. 2. Simple reporting forms were developed to capture rapid testing data or EIA data (optical density values), along with test kit information and QC data.

Twenty local laboratory staff members from CDC, Atlanta, participated in a pilot test. Each participant was provided with a PT panel package as described above and instructed to follow the directions on the enclosed handout (Fig. 2). Data on test results were collected and entered into a customized Microsoft Excel spreadsheet developed for this panel. The DTS-PT panels were also piloted in Kenya at 24 counseling and testing sites in collaboration with local laboratory personnel at the National HIV Reference Laboratory (NHRL), Nairobi. These sites were in the vicinity of Nairobi and conveniently located to conduct a pilot. Testing personnel were also asked to provide their comments regarding use of the DTS for PT program.

3. Results

3.1. Optimal volume for DTS preparation

The various amounts (10 μl, 20 μl, 30 μl, 40 μl and 50 μl) of plasma specimens were allowed to dry at room temperature. The tubes with 10 μl and 20 μl dried overnight, but those with 30 μl or greater volume required 2–3 days to dry. Following

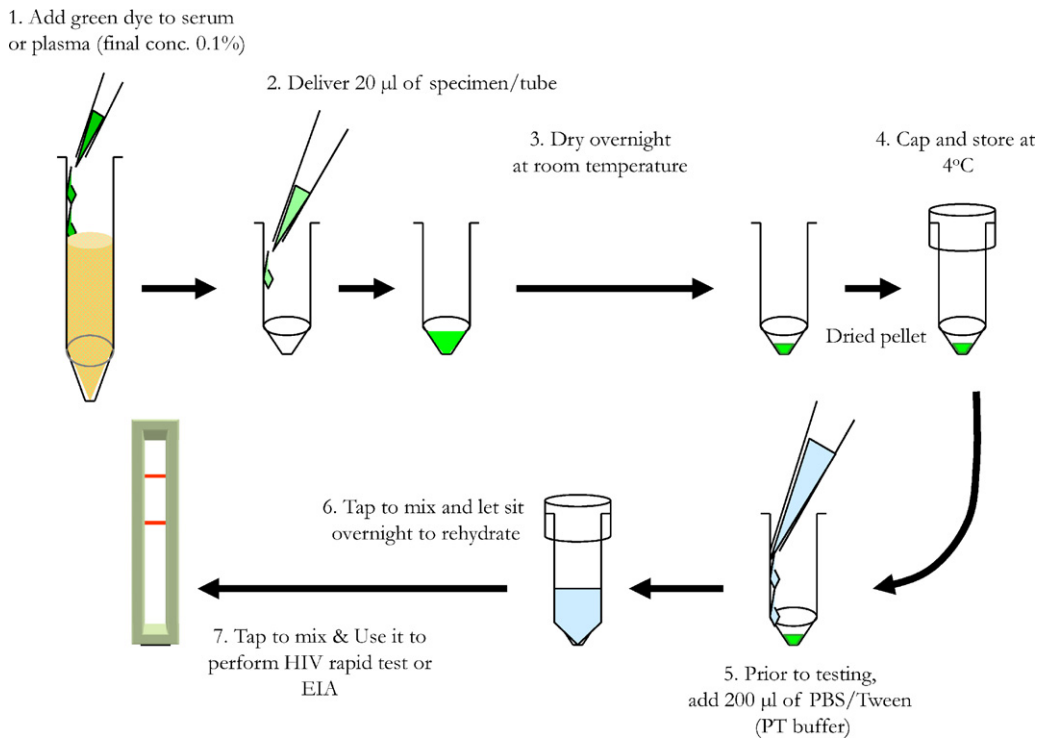

Fig. 1. Schematic of DTS preparation and testing.

rehydration, DTS preparations gave results consistent with expected results for the 10-member panel with Determine and OraQuick rapid tests, irrespective of the volume used. Volume of 20 µl was the preferred, optimal volume for DTS preparation since it dried overnight and had twice the amount of HIV antibodies than the DTS with 10 µl. All subsequent studies were conducted using the DTS prepared with 20 µl volume of serum or plasma.

3.2. Comparison of DTS with liquid specimens

Comparative results were obtained by testing 303 well-characterized specimens, prepared as DTS with two rapid tests, Determine and OraQuick (Table 1A and B). There was excellent agreement (99%, $\kappa = 0.986$) between gold standard reference EIA/Western blot results on liquid specimens and Determine rapid test results on DTS, with sensitivity and specificity of 99.3% and 99.4%, respectively. The only specimen that was missed by Determine had a Western blot profile (gp120/160, gp41 and p24 reactivity) typical of early seroconversion. DTS testing with OraQuick also showed excellent agreement (99%, $\kappa = 0.986$) with reference results and demonstrated a sensitivity of 98.5% and specificity of 100%. Both specimens with EIA/Western blot-positive results but OraQuick-negative results had Western blot profiles suggestive of early seroconversion and included one common specimen that was also missed by the Determine rapid test.

Table 1
Evaluation of rapid testing on DTS specimens compared to reference test results (EIA/WB) of matched serum or plasma specimens. A total of 303 specimens were tested (HIV positive = 135 and HIV negative = 168).

A

Determine DTS	EIA/WB P	EIA/WB N
P	134	1
N	1	167

Sensitivity = 99.3%
Specificity = 99.4%

B

OraQuick DTS	EIA/WB P	EIA/WB N
P	133	0
N	2	168

Sensitivity = 98.5%
Specificity = 100%

3.3. Stability of DTS

Multiple sets of DTS specimens, prepared from the 10-member panel, were stored at 4 °C, 25 °C, 37 °C and 45 °C and tested at 4 weekly intervals. The Determine, OraQuick, Unigold and Capillus test results were consistent with reference EIA/Western blot results for the duration of study (Table 2). For simplicity, only the results of Determine rapid tests at the highest temperature (45 °C) are presented in Table 2A. The results at 4 °C, 25 °C and 37 °C were similar. Additional rapid tests (OraQuick, Unigold and Capillus) were also performed in the same manner (data not shown). Because the DTS specimens gave expected results on rapid tests at all temperatures, including at 45 °C, for 4 weeks, we performed GS HIV-1-2-O EIA at a higher dilution (1:1000) than the recommended 3/4 dilution for this assay to evaluate the potential decline in optical density values over time. All positive specimens gave optical density values of 4.0 (maximum read by the spectrophotometer), even at 1:1000 dilution, while HIV-negative specimens remained negative for the duration of stability study at all temperatures, including 45 °C (Table 2B).

A quantitative HIV antibody assay (the BED-capture enzyme immunoassay) was performed on the same DTS specimens to better observe changes in HIV antibody levels over time at different

Table 2
Stability of 10 DTS specimens at 45 °C over 4 weeks when tested by Determine HIV-1/2 rapid test (A) or Genetic Systems EIA (B). The panel contained 4 HIV positive specimens and 6 negative specimens. The results were similar at 4 °C, 25 °C and 37 °C (not shown). Results of positive specimens are shown in bold.

Specimen ID	Expected results	Day 1	Day 8	Day 15	Day 22	Day 29
(A) Determine						
P06001	**P**	**P**	**P**	**P**	**P**	**P**
P06002	N	N	N	N	N	N
P06003	N	N	N	N	N	N
P06004	N	N	N	N	N	N
P06005	**P**	**P**	**P**	**P**	**P**	**P**
P06006	N	N	N	N	N	N
P06007	**P**	**P**	**P**	**P**	**P**	**P**
P06008	**P**	**P**	**P**	**P**	**P**	**P**
P06009	N	N	N	N	N	N
P06010	N	N	N	N	N	N
(B) GS-EIA (OD-450 nm)						
P06001	**4.000**	**4.000**	**4.000**	**4.000**	**4.000**	**4.000**
P06002	0.064	0.093	0.083	0.106	0.100	0.079
P06003	0.070	0.183	0.150	0.128	0.121	0.180
P06004	0.117	0.077	0.082	0.083	0.122	0.088
P06005	**4.000**	**4.000**	**4.000**	**4.000**	**4.000**	**4.000**
P06006	0.149	0.123	0.112	0.088	0.096	0.125
P06007	**4.000**	**4.000**	**4.000**	**4.000**	**4.000**	**4.000**
P06008	**4.000**	**4.000**	**4.000**	**4.000**	**4.000**	**4.000**
P06009	0.193	0.089	0.076	0.099	0.088	0.106
P06010	0.094	0.110	0.199	0.093	0.103	0.096

temperatures (Fig. 3). HIV antibody levels were stable at 4 °C and 25 °C. Observed changes in HIV-IgG levels were within the variability of the assay, except at 37 °C and 45 °C, when slight declining trends of HIV-IgG were observed for some specimens. It is important to note that none of the HIV positive specimens in the panel became nonreactive on diagnostic rapid tests or EIA over 1 month period.

3.4. Pilots of DTS proficiency testing

Results of our in-house PT pilot with 20 participants from the CDC Atlanta laboratory were in agreement with expected results on all tests performed (Determine, OraQuick and Unigold). In addition, comments and feedback from participants were very positive and they found the job aid useful and simple.

The PT pilot was also conducted at 24 sites in Kenya (Table 3). Of the 144 test results (24 sites × 6 specimens), 143 (99.3%) were in agreement with expected results. One site reported 1 negative specimen as invalid on rapid tests. The same specimen was correctly reported by 23 sites. The comments from testing personnel were positive. All participants found the instructions easy to follow and the general recommendation was that the use of DTS-PT panels should be expanded to all counseling and testing sites. Further details of this and other pilots (from Uganda, Cote d'Ivoire and

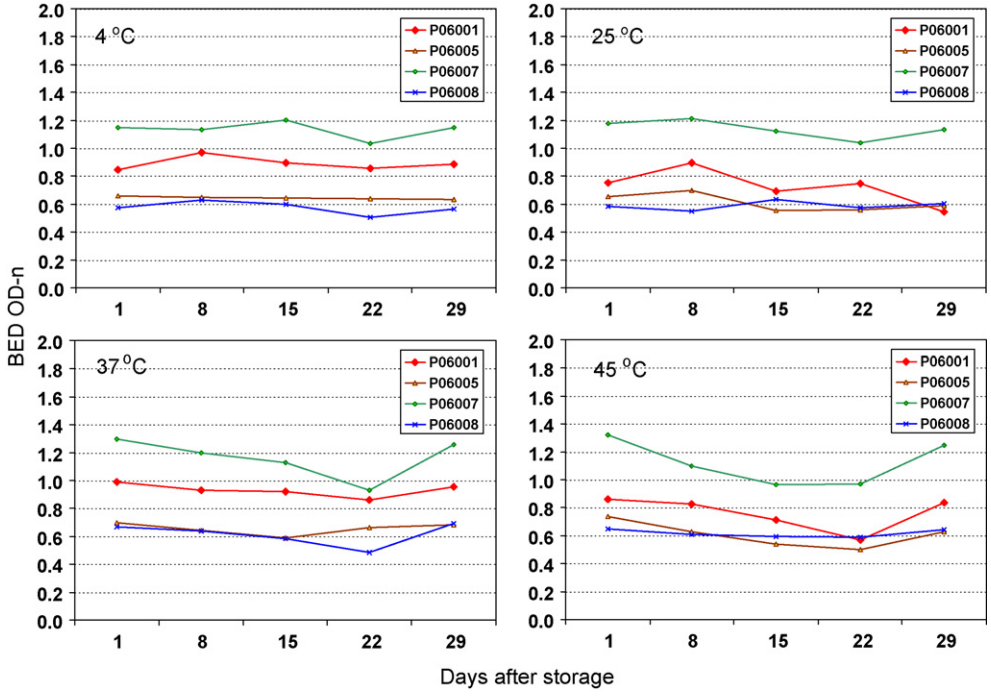

Fig. 2. Stability of 4 HIV positive DTS specimens at 4 °C, 25 °C, 37 °C, and 45 °C over 4 weeks when tested by the BED assay, a quantitative HIV-IgG assay.

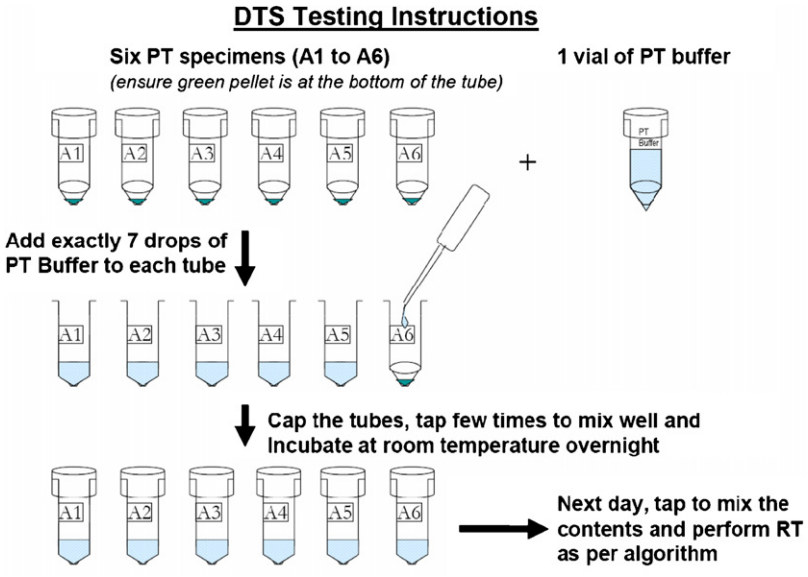

Fig. 3. A schematic job-aid developed to provide simplified instructions on how to use the DTS-based PT panels.

Table 3
Results of a DTS-based PT pilot program in Kenya. Six DTS specimens, coded as A1 to A6, were prepared and delivered to 24 sites. Expected and reported results for each DTS specimen are shown.

Specimen code	Expected results	Reported positive	Reported negative	Reported invalid
A1	Positive	24	0	
A2	Positive	24	0	
A3	Negative	0	24	
A4	Negative	0	24	
A5	Positive	24	0	
A6	Negative	0	23	1

Haiti) and feedback from participants will be shared in a separate report.

4. Discussion

HIV testing is the key entry point for prevention, counseling and service delivery and has expanded rapidly in the last 5 years in resource-limited settings. Access to testing at thousands of counseling and testing sites and task shifting, with testing performed by counselors, nurses and other health-care workers without formal training in the laboratory, provide a challenge to PT and the overall quality management system. In concert with training in rapid testing, assuring the continued quality of testing remains a major challenge. Training of personnel at all levels of testing is essential and should include appropriate external quality assessment practices (Benenson et al., 1989; Francis et al., 1992; Hannon et al., 1989; Hofherr et al., 1992; Peddecord et al., 1992; Polesky and Hanson, 1990; Schalla et al., 1990) and routine use of quality control specimens (Kudlac et al., 1989) for continued quality monitoring and improvement. PT is lacking in many resource-limited settings because of the logistical difficulty of panel preparation and implementation. With the traditional PT program, when it exists, the coverage is mostly limited to national and regional laboratories (Wang et al., 2007). The novel approach described here provides a simple solution for implementing PT programs in most HIV testing sites. The same strategy could also be used to prepare quality control specimens that can be distributed to the HIV testing sites. The DTS, similar to dried blood spot (DBS) specimens, has several advantages. It is safer and less biohazardous than liquid specimens. In addition, the specimens are stable at temperatures expected in many countries, especially during storage and transport, and hence can be transported at room temperature without the need for maintaining an expensive cold chain. Once received at the testing facility, the specimens can be stored at room temperature for few days without negatively affecting the integrity of the specimens. It should be noted that DBS specimens cannot be used for HIV rapid testing because of the hemolysis of red blood cells and potential for interference with rapid test results interpretation.

Overall, the DTS approach has great potential to facilitate expansion of PT programs to include all testing sites. The new approach uses a 10-fold less volume of specimens than most PT programs, which use specimen volumes from 0.2 ml to 0.5 ml. DTS specimens, once rehydrated, can be tested by rapid tests or EIA, and therefore can be used by all laboratories and sites performing HIV testing at different levels. We have also used HIV-2 specimens successfully for DTS preparation (data not shown) which can be included in the PT or QC specimens, if needed. A recent report (Learmonth et al., 2008) described a quality assurance program that includes sending out photographs of rapid test results to assess interpretation proficiency. This approach is not a true proficiency testing program because it does not assess the testing process, i.e. ability to perform rapid tests. Moreover, it cannot be used at sites that do not perform rapid tests.

Our data indicate that there could potentially be some loss of HIV-specific antibodies over 4 weeks when DTS is stored at temperatures at or above 37 °C (Fig. 3). However, this did not affect diagnostic test results by multiple rapid tests or EIA. Because the process includes a further 1:10 dilution and has potential slight loss of antibodies at higher temperature, we do not recommend using borderline, weak positive specimens with low amounts of HIV antibodies for preparing a DTS-based PT panel or QC specimen. However, avoiding extended exposure to higher temperatures can minimize loss of antibodies and may allow use of moderately reactive specimens.

Considerable resources have been invested to train personnel to collect, store and transport DBS specimens in countries (e.g., Kenya, Rwanda) that focus on retesting as part of the EQA program (Chaillet et al., 2009). DBS collection for the purpose of retesting requires an adequate supply of filter paper, drying racks, plastic bags and silica at each site. In addition, considerable resources are needed at the national level to perform the testing, manage the database,

and establish effective communication links with the sites. A DTS-based PT program would simplify the process and enable expansion of the program to most sites. The methodology has been adapted successfully to develop a simplified PT program and was piloted in CDC laboratory in Atlanta as well as in counseling and testing sites in Kenya.

4.1. Conclusion

The DTS-based PT program should be an integral component of external quality assurance strategies for HIV serology in developing countries to monitor and improve the quality and accuracy of testing. The same strategy can also be used for preparation of quality control specimens. This general approach may be extended to preparation of PT panels and QC materials to include HIV-1 group-O, HIV-2 and for other diagnostic tests, especially those that rely on detection of antibodies such as hepatitis, syphilis and measles.

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