

Scaling Up HIV Rapid Testing in Developing Countries

Comprehensive Approach for Implementing Quality Assurance

Bharat S. Parekh, PhD,¹ Mireille B. Kalou, MD, MPH,¹ George Alemnji, PhD,² Chin-Yih Ou, PhD,³ Guy-Michel Gershy-Damet, PhD,⁴ and John N. Nkengasong, PhD¹

Key Words: HIV diagnosis; Rapid testing; Quality assurance; Developing countries

DOI: 10.1309/AJCPTDIMFR00IKYX

Abstract

In the last few years, the use of HIV rapid testing has expanded worldwide in response to the call for universal access to prevention, care, and treatment by UNAIDS and the World Health Organization. HIV rapid testing is performed by people with varied skills in laboratory and nonlaboratory settings. Accurate HIV diagnostic testing is the first step to identifying infected persons for follow-up referral and care. However, there are several challenges related to test kit quality, test selection, testing algorithms, training, quality assurance (QA), quality of new lots, and postmarket performance. We highlight various issues that impact the quality of HIV rapid testing and provide solutions to monitor and improve test accuracy, especially in resource-limited settings. These include the use of validated kits, training with emphasis on QA, use of a standardized log book, dried-tube specimen-based proficiency testing, new kit lot verification, and postmarket surveillance. Systematic implementation of these tools should greatly enhance the quality of HIV rapid testing.

Since 2003, there has been a surge in funding from the Global Fund to Fight AIDS, Tuberculosis and Malaria, the US President's Emergency Plan for AIDS Relief (PEPFAR), the World Bank, and other bilateral donors to combat HIV/AIDS in developing countries in the areas of prevention, antiretroviral treatment, and care.¹ As a result, HIV testing has expanded rapidly. The World Health Organization (WHO)/UNAIDS estimates that more than 5 million persons are presently receiving antiretroviral therapy,² and in 2009 alone, more than 29 million people were tested for HIV infection in PEPFAR-supported countries at thousands of HIV testing and counseling sites. This represented a doubling of the number tested in 2007. HIV testing is the first step to identify HIV-infected persons so that appropriate care and treatment services can be provided in a timely manner. To broaden the testing coverage of at-risk populations, many countries are adopting opt-out testing.

To meet the continuously increasing need for testing in resource-limited countries, the primary method has shifted from enzyme linked immunosorbent assays (ELISAs) to rapid tests (RTs). Most HIV RTs are simple to perform and do not require cold chain for transportation and storage, making them suitable for nonlaboratory settings and to meet the demands of large testing volume.³ For example, there are several thousand lay persons performing RTs in more than 5,500 sites in Kenya (personal communication, Jane Mwangi, MD). The rapid expansion of RTs has not kept pace with quality assurance (QA) programs to monitor performance, raising potential concerns about accuracy.⁴ Various testing strategies have been implemented to reduce testing errors and to shorten turnaround time. The concerns about the accuracy of RTs are further compounded by the large number of different HIV RT kits in the market. The need for accurate HIV rapid

testing cannot be overemphasized as it has direct implications for people and for prevention, treatment, and transmission. Aghokeng et al⁵ evaluated 4 RT kits in Cameroon and found the test sensitivities varied from 94.1% to 100% and specificities from 88% to 98.8%. Findings in other studies have been mixed, with some studies showing good performance and others showing poor performance.⁶⁻⁹ However, with a high volume of testing, even a small error can result in a large number of misdiagnosed cases. For example, a 1% error rate in testing 1 million people could result in the erroneous diagnosis of 10,000 cases (false-positives and false-negatives), emphasizing the need for high QA standards.

This report addresses challenges of implementing traditional QA programs and describes practical approaches that should be considered to improve the accuracy of RT results. The accuracy of HIV diagnosis is affected by at least 4 main factors: (1) the quality of the HIV RT kits as manufactured, (2) the quality of the product at the time of use in the field, (3) test selection and the testing algorithm used at the testing site, and (4) the quality of the testing performed in the field. These steps are further discussed in more details in subsequent sections.

Quality of HIV RT Kits as Manufactured

The HIV diagnostic market has expanded enormously, with a large number of different RT kits on the market. Not all test kits are manufactured with the highest standards. Some kits are produced by the same manufacturers but sold under different names by different companies, and, occasionally, the same test kit or its components are manufactured in multiple facilities, some of which may lack appropriate inspection or approval by regulatory agencies.

Figure 1 summarizes steps that should be followed to assess the quality of test kits at various levels of the evaluation process. Several measures have been put in place to ensure the quality of RT kits. WHO implemented a prequalification program performed at its Collaborating Center Laboratory in Antwerp, Belgium, since 2001.¹⁰ The US Agency for International Development (USAID), in collaboration with the US Centers for Disease Control and Prevention (CDC), also instituted a vigorous test kit validation program in 2006 so that users in PEPFAR-supported countries could have access to RT kits that are not yet Food and Drug Administration (FDA)-approved but meet the performance criteria.¹¹ This evaluation is performed at the CDC using a well-characterized global panel of serum and plasma specimens obtained from several countries with diverse HIV-1 subtypes and HIV-2. Criteria for acceptable performance are a sensitivity of 99% or more and a specificity of 98% or more, consistent with FDA requirements and the WHO prequalification program. Validation also includes the comparison of 3 kit lots to assess lot-to-lot consistency. Test kits are

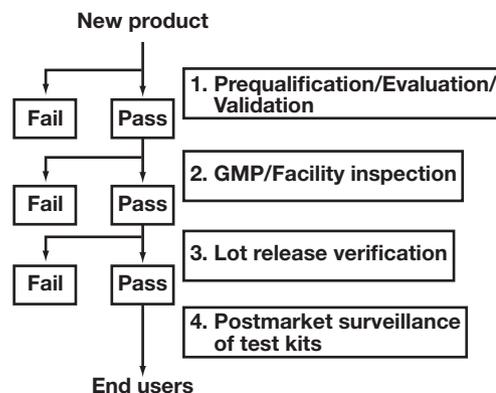


Figure 1 Critical steps to assess the quality of HIV rapid test kits at various stages of the evaluation process. GMP, good manufacturing practices.

rated for other characteristics, including provision of all testing components and devices used in the field, recommended storage temperature, shelf life, training needs, and ease of use and interpretation. The list of USAID-approved test kits is available online and updated regularly (http://www.usaid.gov/our_work/global_health/aids/TechAreas/treatment/scms.html).

There were 41 RT kits listed on the USAID waiver list as of February 2010.¹¹ These kits are manufactured in developed and developing countries including Canada, France, India, Ireland, Israel, Japan, Nigeria, Norway, Spain, Thailand, the United Kingdom, and the United States. Of these kits, 35 are not US FDA approved but have been approved by an equivalent regulatory agency in Europe or Canada or have been validated in the CDC laboratory. Three RT kits use oral fluid, and 9 require low-temperature storage.

The current CDC evaluation does not include manufacturing facility inspection to determine if the manufacturing process is compliant with good manufacturing practices. The ability to manufacture consistent kit lots is taken as a proxy for good manufacturing practices. Lot release verification and postmarket surveillance of test kits (Figure 1) are important steps to further ensure quality of RTs.

Selection of RT Kits and Testing Algorithms

Selected test kits should be suitable for the field sites and for test providers. Most RTs can be completed in less than 30 minutes, but tests that give results in 10 to 15 minutes are preferred because they can facilitate counseling and testing during the same visit. In addition, the kits should be stable at ambient temperature, requiring no refrigeration. Kits that need storage at 4°C to 8°C should be avoided unless there is a good refrigeration facility and constant power supply. In addition to the required sensitivity and specificity, test kits should be selected based on additional criteria such as ease

of use and interpretation, storage requirements, and amount of waste generated. Some countries have annual or biannual bidding processes for the procurement of HIV test kits. Cost of the kits is an important factor affecting procurement, but the sensitivity and specificity of the test kit should be the most important deciding factors.

Several countries use venous blood to perform RTs,¹² which requires separation of plasma or serum. Most RTs can also be used with whole blood specimens. More countries are currently shifting toward the use of whole blood directly from finger sticks to circumvent the need for plasma or serum preparation.¹³ Finger sticks can also be used for collection of dried blood spot (DBS) specimens, if needed, for the purpose of external quality assessment.^{12,14,15} The use of finger-stick blood samples shortens the turnaround time, and, thus, post-test counseling can be offered to patients during the same visit to reduce the chance of loss of patients to follow-up. It also allows the expansion of testing to sites without laboratory equipment or skills for blood drawing. Whole blood testing should be performed according to the standard operating procedure (SOP) for each RT, which includes use of an appropriate sample application device (eg, capillary or loop) and reading the results at the correct time. Lack of adherence to the SOP can easily lead to suboptimal performance with poor sensitivity or specificity.¹⁵

New oral fluid-based RT kits (OF RT) have begun to enter the market,¹⁶ and, currently, 3 OF RTs are on the USAID waiver list. A field evaluation of 2 OF RT tests in Mozambique recently demonstrated outstanding performance of these test kits, meeting or exceeding whole blood-based RT.¹⁷ Moreover, most clients preferred OF RT compared with whole blood RT. The use of OF RT will further simplify testing, facilitating expansion while reducing biohazards associated with the disposal of lancets and needles.

Testing algorithms should include a more sensitive assay as the first test followed by a more specific assay as the second test to confirm initially reactive results. The WHO and USAID lists include several tests that meet these criteria. Cost should not dictate the order in which the tests are used. Initially, many countries adopted a uniform national testing algorithm, but this turned out not to be the best choice because of a lack of flexibility.¹⁸ We recommend that countries choose at least 2 algorithms involving different test kits with similar performance; they can be used as alternative algorithms in case of stock outs or unavailability and will permit flexibility of HIV testing in various settings.

The choice to use serial or parallel testing algorithms is important and depends on several factors. The WHO recommends the use of a serial algorithm. Serial algorithms require fewer tests and provide cost savings. In contrast, parallel algorithms using 2 different RT kits simultaneously require less time to perform but increase the reagent costs. In some testing

circumstances such as testing pregnant women at delivery, at sites with very high client loads, in operating rooms, and in blood transfusion facilities, parallel testing can provide quick results for timely diagnosis and clinical decision making. This may also be more practical in situations in which the prevalence rate is high and personnel costs compete with costs of testing.

If assays are selected properly, the positive predictive value (PPV) of 2 sequential reactive tests (specificity $\geq 99\%$) is more than 90%, even in low-prevalence populations (0.1%), and more than 99% in populations with a prevalence of 1% or higher. If the tests have a specificity of 98%, the PPV is more than 90% in populations with a prevalence of 0.5% or higher. A third RT kit is used in some countries as a tie-breaker when the 2 RT results are discordant (eg, a first reactive test followed by a second nonreactive test in a serial algorithm or discordant result in a parallel algorithm). However, the use of a tie-breaker may not always resolve the HIV status with accuracy. The outcome depends on selection of the test kit used as the tie-breaker. Moreover, it is difficult and often impractical to procure and store RT kits with a limited shelf life for occasional tie-breaker use. In some countries, a blood specimen is collected from the persons with discordant results and forwarded to a facility capable of performing additional testing to verify the infection status. Often, clients are retested after 3 or 4 weeks to resolve the infection status instead of using the tie-breaker test. This approach is more likely to provide a correct diagnosis, although clients may not return for follow-up testing. An appropriate and strong counseling message at this stage may help ensure a return visit.

Along with HIV-1, HIV-2 is present in West Africa and is occasionally found in other areas of the world. Several RT kits claim to discriminate HIV-1 and HIV-2 infections, but dual reactivity is common. Misclassification can occur due to significant antibody cross-reactivity with viral antigens between the 2 HIV types, and, thus, singly infected persons could be misdiagnosed as having dual infection.^{19,20} A careful selection of test kits that can discriminate accurately between HIV-1 and HIV-2 is important, and additional more specific testing using peptide-based or polymerase chain reaction assays to truly differentiate these infections is warranted if true discrimination is necessary.²¹

The new fourth-generation HIV RT (Determine HIV 1/2 Ag/Ab Combo, Inverness BioMedical, Waltham, MA) evaluated recently by the CDC/USAID demonstrated detection of the HIV antigen about 10 days (mean time) before antibody detection when using seroconversion panels. It could be a useful tool in screening most at-risk populations but can also be used in blood banks for transfusion safety when more sophisticated testing is not available. Training, good record keeping practices, and proper counseling messages will be critical in ensuring the quality of this new technique because the test strips contain 3 lines representing antigen, antibody, and control.

Standardized Log Book as a QA Tool

The US CDC developed a simple but practical QA tool to monitor and improve the quality of testing while reducing operational cost and human resource needs. To simplify the monitoring process, a standardized log book was developed for use at all testing sites within a country, irrespective of the implementing partner or testing venue. Sites often maintain inadequate testing information. Test registers include hand-drawn columns in a blank register that may vary from site to site or even at the same site. Information about individual tests used is not consistently recorded in the log book. Most often only the final result, instead of 2 or 3 individual test results, is recorded. The lack of test kit names, lot numbers, and expiration dates makes it impossible to identify or troubleshoot problems. It is also critically important to train first-line test performers in the field to recognize abnormalities themselves. To this end, a simple paper-based standardized log book (Figure 2) was designed to capture specific information, including kit name, lot number, expiration date, and individual test results. Test result options are preprinted, thus

allowing test performers to simply circle the correct result. Page totals at the bottom of each page allow test performers to monitor the testing in real time by determining the concordance (agreement) between tests 1 and 2 and also between final reported HIV status and results of the external quality assessment (EQA; ie, retesting), if performed. This log book can be customized for specific use as long as critical information about individual tests and page totals are retained.

Supervisors overseeing testing sites should assess site performance through the data collected in the log book at regular intervals. These data can be collected by short text messages from mobile phones or verbally by phone. Monitoring frequency depends on the volume of testing; weekly monitoring is recommended for high-volume sites, while monthly monitoring would be sufficient for low-volume sites. Concordance between the 2 tests from log book page totals can be used to identify testing problems at a site if the site is monitored over time (Figure 3A), to compare the performance of different operators and identify operators needing more training (Figure 3B) and to compare the performance

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Serial No.	Client or Specimen ID	Age (Yrs)	Sex	Date Tested	TEST 1	TEST 2	TEST 3	FINAL RESULTS	Operator Name / Initials	Sent for Further QA Testing	Date Sent	Final QA Results	Date Received	Comments
					Kit Name: <u>Determine</u> Lot No. <u>X1180</u> Exp. Date <u>Sept 2009</u>	Kit Name: <u>Unigold</u> Lot No. <u>U-22111</u> Exp. Date <u>June 2009</u>	Kit Name: _____ Lot No. _____ Exp. Date _____							
1		M	F		R NR I	R NR I	R NR I	P N I		<input checked="" type="checkbox"/>		P N I		
2		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
3		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
4		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
5		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
6		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
7		M	F		R NR I	R NR I	R NR I	P N I		<input checked="" type="checkbox"/>	3/3/08	P N I	10/3/08	Neg on 2 EIAs
8		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
9		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
10		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
11		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
12		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
13		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
14		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
15		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
16		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
17		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
18		M	F		R NR I	R NR I	R NR I	P N I		<input checked="" type="checkbox"/>		P N I		
19		M	F		R NR I	R NR I	R NR I	P N I		<input type="checkbox"/>		P N I		
20		M	F		R NR I	R NR I	R NR I	P N I		<input checked="" type="checkbox"/>		P N I		

PAGE TOTAL					
Total Tested	20	20	20		
Total Reactive/Positive	4	3	3	3	3
Total Non-Reactive/Negative	16	17	16		1
Total Invalid (or Indeterminate)	0		1	1	

R = Reactive, NR = Nonreactive, and I = Invalid P = Positive, N = Negative and I = Indeterminate

Figure 2 An example of a page from standardized log book for recording and monitoring HIV testing. Individual test information is recorded, and page totals from each page (bottom section) are compiled for ongoing concordance between tests. EIA, enzyme immunoassay; QA, quality assurance.

of different sites and identify sites requiring more attention **Figure 3C**. Finally, ongoing concordance between tests 1 and 2 can be used to assess validity of the testing algorithm. If there is a high level of discordance at all sites **Figure 3D**, the problem is likely related to test kit quality or an inappropriate testing algorithm. This approach highlights the use of existing data at each site and analysis for ongoing quality assurance.

External Quality Assessment

EQA is an integral component of QA and is usually practiced through a combination of the following 3 approaches: (1) participation in external proficiency testing (PT) programs, (2) supervisory site visits by external experts, and (3) retesting a subset of specimens in another competent laboratory or site at a higher level.

PT programs are generally quite effective as an EQA tool in identifying poorly performing sites.²² Traditional PT programs and quality control (QC) reagents use serum and plasma specimens requiring stringent conditions for storage and transportation. This is expensive and difficult to implement in resource-limited settings. To overcome the cold-chain transportation and PT panel delivery problems, we developed a dried tube specimen (DTS)-based²³ PT **Figure 4**. A small volume (20 μ L per tube) of specimen is allowed to dry in the tube overnight. Once dried, DTSs are stable at room temperature for at least 1 month and can be rehydrated at the testing site for PT purposes. The DTS, similar to 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

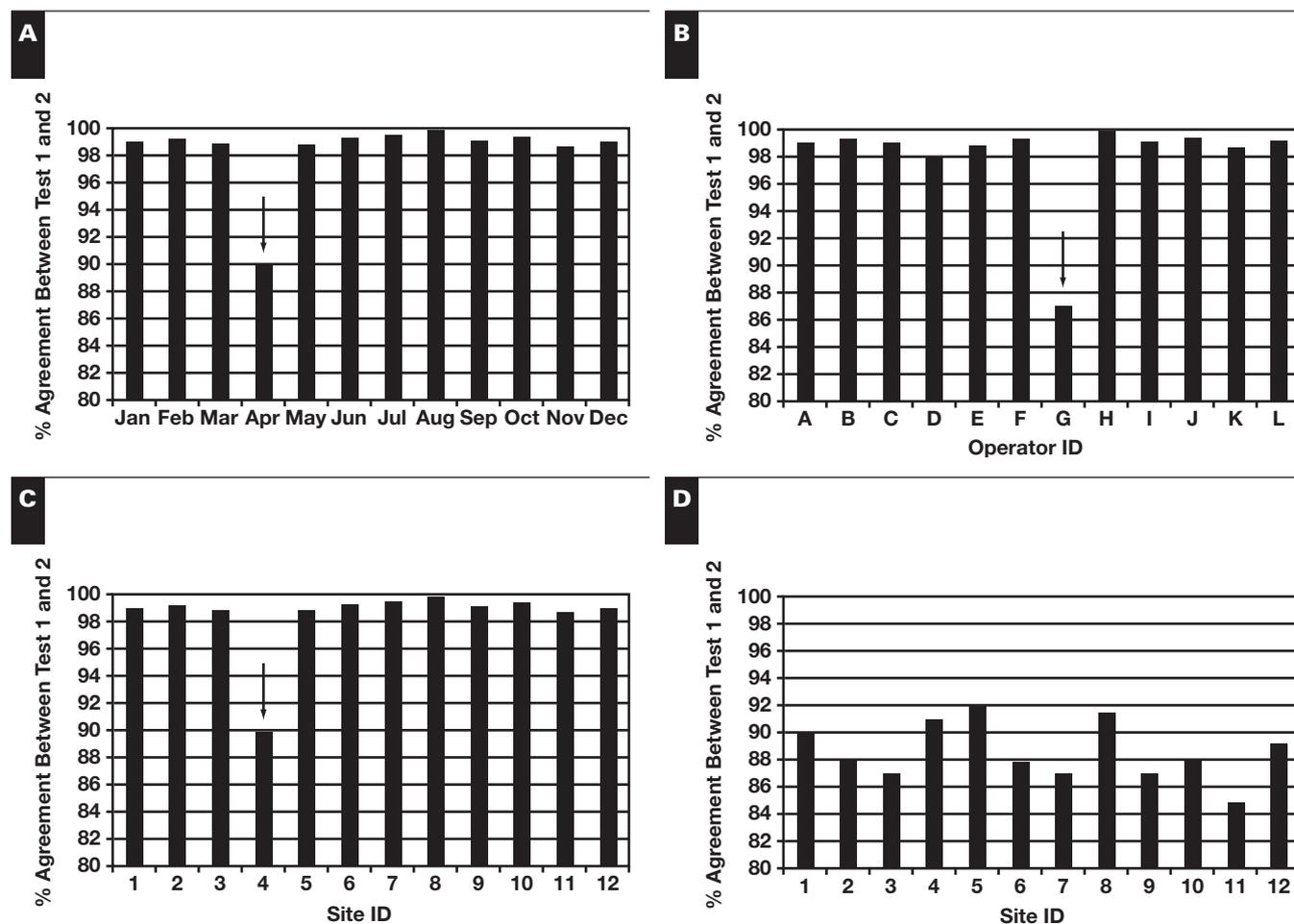


Figure 3 Use of page total data to assess concordance between test 1 and test 2 to monitor quality of testing. **A**, Monitoring concordance over time at a single site to identify problems. **B**, Comparing concordance among different operators to detect operators with poor performance. **C**, Comparing concordance among different sites to identify sites with problems. **D**, Poor concordance at multiple sites may indicate poor test performance or an inappropriate testing algorithm. Arrows indicate problems requiring further investigation.

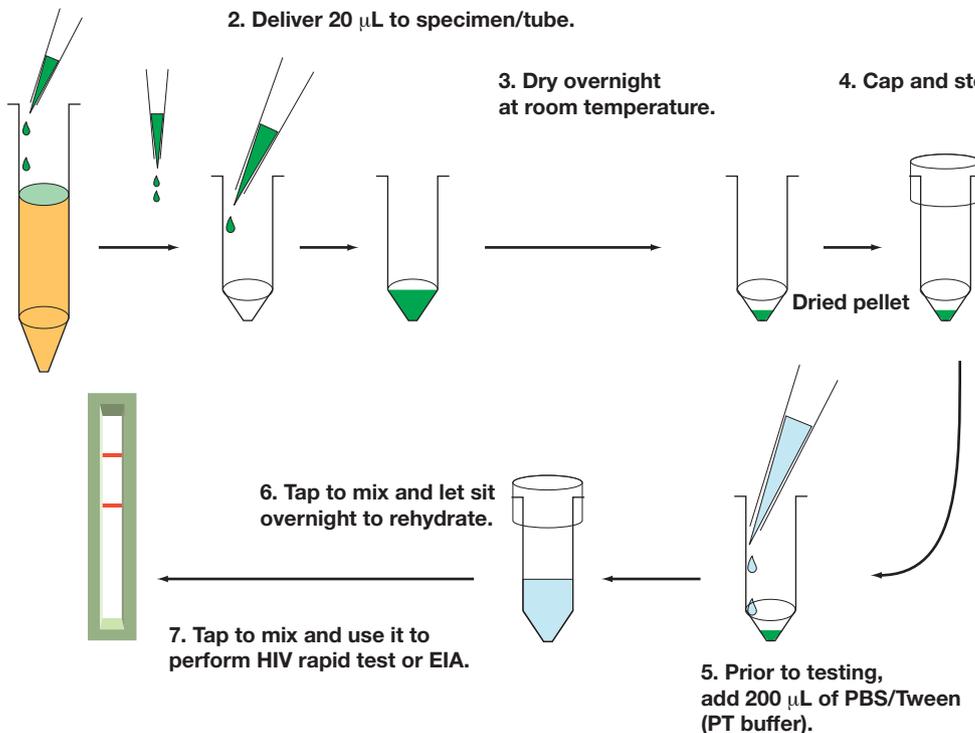
A

1. Add green dye to serum or plasma (final concentration of 0.1%).

2. Deliver 20 μ L to specimen/tube.

3. Dry overnight at room temperature.

4. Cap and store at 4°C.



B

Six PT Specimens (A1 to A6)
(ensure green pellet is at the bottom of the tube)

1 vial of PT buffer

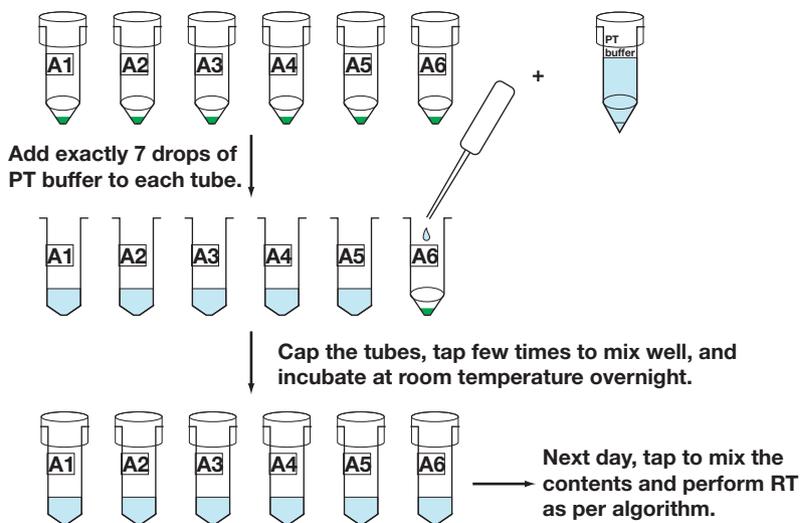


Figure 4 **A**, Schematic to illustrate preparation of dried tube specimens (DTS) and testing for HIV antibodies. **B**, Job aid to assist with proficiency testing of DTS panels. EIA, enzyme immunoassay; PBS, phosphate-buffered saline; PT, proficiency testing; RT, rapid testing.

expensive cold chain. Once received at the testing facility, the specimens can be stored at room temperature for a few days without negatively affecting the integrity of the specimens. This general approach may also be extended to preparation of QC materials. Several countries are in the expansion phase of implementing DTS-based PT programs.

Some countries have embarked on retesting strategy as a component of EQA in which every 10th specimen is collected as serum or DBS and sent to a reference laboratory for retesting to determine the extent of field site accuracy. Although useful in some settings, retesting has proved not to be very practical in most countries owing to the large volume of testing. This is neither practical nor achievable without enormous human and financial resources. Moreover, countries conducting even limited retesting have not been able to complete the exercise in a timely manner to provide feedback and take corrective action to improve performance, thus not meeting the purpose of EQA. Retesting, when performed, should always be combined with other EQA practices and should be limited, targeting only new sites or sites with problems. If the retesting data show high agreement (>99%) with site data, retesting may be discontinued or limited. Moreover, retesting should be quick and timely to be effective, with plans for corrective action and follow-up.

Periodic supervisory site visits are another means to perform EQA and require a large pool of trained professionals to visit and conduct assessments. To make this effort efficient and sustainable, a thorough and careful top-down tiered inspection scheme or network needs to be created. There are additional challenges in training personnel and standardizing assessments. Because this exercise can be very costly and time-consuming, it should be used in conjunction with other approaches to target poorly performing sites.

New Kit Lot Verification

The quality of new kit lots can vary; however, current practices do not include assessment and qualification of new kit lots when they are procured in the country. Therefore, it is important to have a standardized mechanism in place to verify the quality of new lots before bulk procurement. Dilution panels, prepared by 5-fold dilution of reactive specimens, can be used to determine the detection limits of new lots. A validated kit lot with acceptable sensitivity and specificity is used to generate reference data (Table 1) (only 2 panels are shown for simplicity). Five to ten such panels should be used for

Table 1
Examples of New Kit Lot Verification for Consistency Using Panels of Serially Diluted (5-fold) HIV+ Specimens*

Dilution	Specimen ID	Verified New Lots			Failed New Lots		
		Reference Lot	New Lots		Reference Lot	New Lots	
		Lot A	Lot B	Lot C	Lot A	Lot D	Lot E
Dilution panel A							
0	RTV-DT 21	P	P	P	P	P	P
5	RTV-DT 22	P	P	P	P	P	P
25	RTV-DT 23	P	P	P	P	P	P
125	RTV-DT 24	P	P	P	P	P	P
625	RTV-DT 25	P	P	P	P	P	P
3,125	RTV-DT 26	P	P	P	P	P	P
15,625	RTV-DT 27	P	P	P	P	P	P
78,125	RTV-DT 28	P	P	P	P	P	N [†]
390,625	RTV-DT 29	P	P	P	P	N [†]	N [†]
1,953,125	RTV-DT 30	N	N	N	N	N	N
Agreement (%)		—	100	100	—	90	80
Dilution panel B							
0	RTV-DT 41	P	P	P	P	P	P
5	RTV-DT 42	P	P	P	P	P	P
25	RTV-DT 43	P	P	P	P	P	P
125	RTV-DT 44	P	P	P	P	P	P
625	RTV-DT 45	P	P	P	P	P	N [†]
3,125	RTV-DT 46	P	P	P	P	N [†]	N [†]
15,625	RTV-DT 47	P	P	N [†]	P	N [†]	N [†]
78,125	RTV-DT 48	N	N	N	N	N	N
390,625	RTV-DT 49	N	N	N	N	N	N
1,953,125	RTV-DT 50	N	N	N	N	N	N
Agreement (%)		—	100	90	—	80	70
Overall agreement (%)		—	100	95	—	85	75

N, negative; P, positive.

* Kit lots B and C pass the requirements with overall agreement of 95% with the reference lot. Kit lots D and E fail the requirements because overall agreement is <90% with the reference lot.

† New lot results that do not match with reference data (lot A).

detection sensitivity. An acceptable kit lot should have similar end-point titers that are identical or do not differ by more than 1 dilution per panel when compared with the reference data, with an overall agreement of 90% or more. A kit lot that differs by more than 10% when compared with the reference lot is likely to have issues with sensitivity or specificity and should be further investigated. In addition, it is critical that ministries of health, in collaboration with key stakeholders including the WHO, develop policy and establish or strengthen the existing national regulatory authority to empower national authorities to take appropriate actions, including rejecting procurement of the lot, if necessary.

Postmarket Surveillance of RT Kits

Postmarket surveillance relates to monitoring the quality of test kits after procurement. Although most RT kits are stable at room temperature, this recommended temperature usually ranges from 15°C to 30°C. The test kit quality may be impacted adversely by suboptimal transport, storage temperature, humidity, or other environmental factors. Ambient temperature can vary significantly in many countries and can be as high as 45°C to 50°C in storage or during transport. Therefore, additional measures may be needed to ascertain the quality of kits after procurement. Stored kits may be tested at regular intervals or randomly picked from field sites for postmarketing surveillance. Dilution panels (as described for new kit lots) can be used to validate performance of the test kits. Any major deviation in end-point sensitivity may be indicative of suboptimal performance of the assay. Such results should lead to further investigation about storage or transportation conditions and other issues. Again, appropriate policies need to be in place for a course of action in case compromised test kits are detected.

Training and Certification of Personnel and Sites

All individual test operators should go through comprehensive hands-on training. Experience has shown that improved results are obtained when test operators undergo hands-on training. The WHO/CDC HIV rapid testing training package provides excellent guidance to proper training.²⁴ Training should focus on the principles of rapid testing, various formats of RTs, and the importance of following the manufacturer's performance procedures, including QC, thorough and accurate results recording, and results interpretation. The manufacturer's kit insert also contains important instructions regarding sample volume, wait time, storage requirements, shelf life, and troubleshooting. It is critically

important to educate testing personnel to take ownership, ensure the accuracy of RT results, and recognize unusual results in a timely manner. QA should be integrated into practical training, including the value of standardized record keeping. Trainees should be informed of the importance of EQA and ways this can be accomplished.

It is important that countries develop a plan that outlines minimum competency requirements for the test site facility and testing personnel. These requirements should be linked to a formal certification scheme operated and recognized by the government. New test performers should be officially certified after completing the training and successfully testing 50 specimens that include HIV+ specimens under the supervision of the experienced certified testers. An official certificate should be displayed at the testing site to assure clients seeking testing. A process should be instituted to periodically renew certification to maintain workforce competencies and to update skills. Considering the huge scope of HIV testing and testing personnel in each country, certification requirements should be part of the national policy of each country. Certificates should be provided by a national reference laboratory (NRL) or equivalent institution that oversees training and competencies.

Similarly, we recommend that testing sites have minimum certification requirements, which would include proper organization of the traffic flow and counseling/testing area, adequate storage and inventory management, appropriate disposal of biohazard waste, disinfection procedures, and biosafety measures. A standardized SOP describing the general layout of the test site and detailed testing procedures, checklists, and testing algorithms should be developed to assist new field sites to become operational in a timely manner. A supervisory checklist should be used for periodic announced or unannounced supervisory visits as part of EQA.

Step-Wise Approach to Introducing RTs in the Country

The standardized and thorough evaluations conducted by the WHO and USAID/CDC provide a solid, scientific, and evidence-based kit performance data set for resource-constrained countries to make procurement decisions without embarking on new rounds of in-country evaluation processes. However, if it is determined necessary by the country ministry of health, the government should appoint an official body with proper authority to coordinate this effort and use well-standardized evaluation criteria to conduct an evaluation in an established laboratory facility with qualified personnel. This process should be conducted in a way that the assessment is safeguarded from direct and indirect influence exerted by manufacturers. It also requires specimen evaluation panels

with adequate samples and proper QA measures. Similar to the specimen evaluation panel used in the USAID/CDC program, the specimens present in the country evaluation panel should contain viral subtypes representative for the country and the region. This evaluation panel will be constituted by anonymous, unlinked discarded blood units obtained from blood banks and transfusion centers. This panel should include a high proportion of HIV+ samples, with a range of antibody titers, to ensure that the information gain of a test, defined as the difference between the pretest and posttest probability of a disease, is greatest when the pretest probability is close to 40% to 60%. Specimens should be well characterized by using a standard diagnostic algorithm and additional testing, if available, to determine the diversity of subtypes and stage of infection (recent infection).

Countries are advised to train a core group of testing staff, including laboratory and nonlaboratory personnel. This core group will provide training to new testing personnel in the field. The initial implementation of RTs should be piloted at a few sites and the results compared with an existing testing algorithm, including ELISA, whole blood, or other RTs to identify potential operational challenges. Systematic collection and analysis of data using a standardized log book (Figures 2 and 3) from these initial sites for the first 2 or 3 months should serve the purpose of in-country validation and confidence building. This process will speed up the implementation and expansion of RTs in the country, which otherwise may be a time-consuming effort to have the evaluation specimen panel assembled and appropriately used to determine RT kit quality. Therefore, it is strongly recommended that resource-limited countries use the validated tests evaluated by the WHO and USAID/CDC and not embark on expensive and time-consuming evaluation of their own unless it is necessary.

A significant departure from the aforementioned situation is in China, which has its own well-developed biologic production capacity and already has produced more than 10 domestic HIV ELISA kits and approximately 10 RT kits. The production of these kits is regulated by China's FDA, and their postmarket evaluation is performed by the National AIDS Reference Laboratory and by some provincial HIV central laboratories. Evaluation results are accessible through Web sites. Most provinces with low HIV prevalence use the National AIDS Reference Laboratory evaluation information to make their procurement decisions. Despite the presence of these governmental premarketing and postmarketing evaluations, occasional instances of inadequate performance were found in some kit lots in domestic RT kits. In addition to the enforcement of production consistency and compliance with good manufacturing practices, the provincial laboratory should institute a lot-to-lot inspection using strategies described in this article.

QA Strategy in Low-Prevalence Populations

Approaches to QA may have to be modified in areas with a very low prevalence of HIV. Often testing sites may not observe a positive result for several weeks. DTS can be used to prepare positive and negative QC samples, which should be used with higher frequency in low-prevalence populations. The QC results should be recorded in the log book, just like client results, compiled, and reviewed regularly to ensure that the results of QC specimens are consistent and match expected results. Because of the low PPV in low-prevalence populations, a retesting of 5% to 10% of negative specimens and all or a large fraction of positive specimens is appropriate in these settings. Testing sites should be provided timely feedback about retesting results. In addition, mandatory participation in PT programs is important to ensure that site personnel can correctly diagnose HIV-1 infection when encountered.

As indicated earlier, the PPV of 2 reactive tests is more than 90% in populations with a prevalence of 0.1% and individual test specificities are 99%. A third test may be used to increase the PPV to 99%. However, if the second test has a specificity of 100%, use of the third test may not be necessary, even in low-prevalence populations. Continued monitoring of the field data comparing agreement values between test 1 and test 2 vs all 3 tests would provide actual information about the usefulness of including a third test in the algorithm. Data collected in a standardized manner using a log book (Figures 2 and 3) would greatly assist in the data review and recommendations for a final algorithm.

Decentralized Approaches to EQA: the Role of an NRL and Partnership With Local Organizations or Institutions

EQA is designed to identify sites and testers needing assistance. An effective EQA exercise has to be timely with integrated corrective actions. The EQA is not meaningful if data are not collected and analyzed in a timely manner and sites are not provided feedback. Recognizing the large scope of work in most countries with thousands of testing sites, it may not be feasible for one entity (eg, the NRL) to implement various EQA strategies and successfully monitor outcomes effectively nationwide. Therefore, it is appropriate to use a combination of a decentralized approach and indigenous nongovernmental organizations or other entities that can be trained to run the EQA program and provide coverage in specific geographic regions, tiered levels, or programs. Regional and provincial reference laboratories can be set up to provide oversight and compile regional and provincial data for overall analysis and coordination by the NRL or a central reference laboratory (Table 2). More critically,

Table 2
Proposed Roles and Responsibilities of Ministry of Health/National AIDS Control Program, National Reference Laboratory, and Others Involved in Monitoring and Improving the Quality of HIV Testing

Who	What
Ministry of health or national AIDS control program	Stakeholders' meetings: Introduction of program Progress report Designate national reference laboratory or laboratories Communicate information Select and engage testing sites/labs Funding Design and distribute standardized logbook Site supervision and corrective actions Use external quality assessment data to guide decision making for HIV rapid testing
National reference laboratory	Develop implementation strategy Coordinate trainings Prepare and distribute proficiency testing panels Determine distribution schedule Analyze results Generate reports, provide feedback and corrective actions Share feedback report
Local partners or implementing partners	Collaborate with national reference laboratory Provide technical support Determine level of coverage Collect site data
Testing sites/laboratories	Enrollment/participation Ensure all staffs properly trained Site supervisors review proficiency testing data and logbook Laboratory focal person to review monthly report Report issues

countries should establish partnerships with indigenous nongovernmental organizations and local experts to develop local capacity to produce and distribute the DTS PT samples and log books and collect information, therefore ensuring adequate coverage and sustainability with lower cost.

Programs must allocate resources at the national and regional levels to implement EQA approaches. This should include the hiring of dedicated staff members who can expand programs over time as they become more integrated in the health system. In addition, NRL and supporting staff must develop plans for supervisory site visits and corrective action when necessary. Corrective actions may include close examination of testing practices, record keeping, and refresher training, as appropriate. Limited retesting of specimens from sites with persistent problems is a good complementary strategy. The overall goal of any EQA activity is to measure the current level of competency while striving for improvement in quality of testing over time.

Conclusions

With the scale-up of HIV rapid testing and the expanded use RTs, ensuring the quality of testing has become challenging. Traditional approaches that served well in laboratory settings may not translate well into field settings. However, significant progress has been made by devising new approaches

to QA and following a multistep approach **Figure 5**. New RT kits are routinely evaluated by the WHO and/or CDC to ensure satisfactory performance. The kits on the approved list could be used immediately at the country level, but continued monitoring is always a good practice.

Additional QA should be conducted for new lot verification and postmarket surveillance. Accuracy of HIV testing and testing algorithms can be monitored by analysis of collected data at each site. This is facilitated by the use of a standardized register or log book to produce a real-time test summary that is reviewable by field site personnel and readily available to higher tier supervisors. Ongoing QA tools may be further expanded to accommodate data for other testing such as malaria and tuberculosis as testing becomes more integrated. Novel DTS-based PT programs and QC specimens can also help with monitoring and improving the quality of HIV rapid testing.

A good training program lays the foundation of quality HIV testing and should incorporate various QA elements. Adoption of strategies outlined herein should promote continuous quality improvement of rapid testing and accuracy of HIV diagnosis in HIV testing and counseling settings.

From the ¹Division of Global HIV/AIDS, Centers for Disease Control and Prevention, Atlanta, GA; ²CDC-Caribbean Regional Office, Bridgetown, Barbados; ³CDC-China Office, Beijing; and ⁴World Health Organization Regional Office for Africa, Ouagadougou, Burkina Faso.

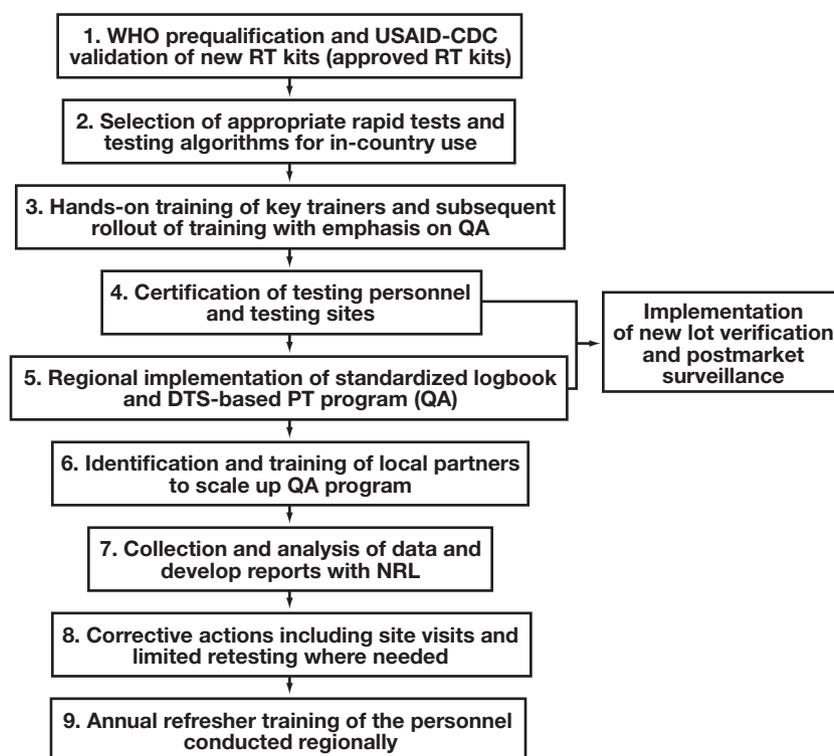


Figure 5 Multistep approach to assure the quality of HIV testing. CDC, Centers for Disease Control and Prevention; DTS, dried tube specimens; NRL, national reference laboratory; QA, quality assurance; RT, rapid testing; WHO, World Health Organization.

Address reprint requests to Dr Parekh: Serology/Incidence and Diagnostics Team, GAP International Laboratory Branch, 1600 Clifton Rd, MS-G19, Bldg 15/2611, Centers for Disease Control and Prevention, Atlanta, GA 30333.

Acknowledgments: We thank Elliot Cowan, PhD, and Marc Bulterys, MD, for critical review and helpful suggestions and Christine Korhonen, MPH, for editorial assistance.

References

- Kates J, Lief E, Avila C. *Financing the Response to AIDS in Low- and Middle-Income Countries: International Assistance From the G8, European Commission and Other Donor Governments in 2008*. Geneva, Switzerland: UNAIDS; and Menlo Park, CA: Kaiser Family Foundation; 2009.
- http://www.who.int/mediacentre/news/releases/2010/hiv_treatment_20100719/en/index.html. Accessed August 16, 2010.
- World Health Organization. *Task Shifting: Global Recommendations and Guidelines*. Geneva, Switzerland: World Health Organization; 2008.
- World Health Organization, US Department of Health and Human Services, Centers for Disease Control and Prevention. *Guidelines for Assuring the Accuracy and Reliability of HIV Rapid Testing: Applying a Quality System Approach*. Geneva, Switzerland: World Health Organization; 2005.
- Aghokeng AF, Ewane L, Awazi B, et al. Evaluation of four simple/rapid assays and two fourth-generation ELISAs for the identification of HIV infection on a serum panel representing the HIV-1 group M genetic diversity in Cameroon. *J Acquir Immune Defic Syndr*. 2004;37:1632-1640.
- Gray JJ, Wreghitt TG, McKee TA, et al. Internal quality assurance in a clinical virology laboratory, II: internal quality control. *J Clin Pathol*. 1995;48:198-202.
- Plate DK. Evaluation and implementation of rapid HIV tests: the experience in 11 African countries. *AIDS Res Hum Retroviruses*. 2007;23:1491-1498.
- Tegbaru B, Wolday D, Messele T, et al. Assessment of the implementation of HIV-rapid test kits at different levels of health institutions in Ethiopia. *Ethiopian Med J*. 2007;45:293-299.
- van den Berk GE, Frissen PH, Regez RM, et al. Evaluation of the rapid immunoassay Determine HIV 1/2 for detection of antibodies to human immunodeficiency virus types 1 and 2. *J Clin Microbiol*. 2003;41:3868-3869.
- World Health Organization. *HIV Assays. Operational Characteristics (Phase I): Report 14: Simple/Rapid Tests*. Geneva, Switzerland: World Health Organization; 2004.
- USAID Bureau for Global Health. http://www.usaid.gov/our_work/global_health/aids/TechAreas/treatment/testkit_explain.pdf. Accessed August 9, 2010.
- Chaillet P, Zachariah R, Harries K, et al. Dried blood spots are a useful tool for quality assurance of rapid testing in Kigali, Rwanda. *Trans R Soc Trop Med Hyg*. 2009;103:634-637.

13. Liu A, Kilmarx PH, Supawitkul S, et al. Rapid whole-blood finger-stick test for HIV antibody: performance and acceptability among women in northern Thailand. *J Acquir Immune Defic Syndr*. 2003;33:194-198.
14. Granade T, Parekh BS, Tih PM, et al. Evaluation of rapid prenatal human immunodeficiency virus testing in rural Cameroon. *Clin Diagn Lab Immunol*. 2005;12:855-860.
15. Mashauri FM, Siza JE, Temu MM, et al. Assessment of quality assurance in HIV testing in health facilities in Lake Victoria zone, Tanzania. *Tanzania Health Res Bull*. 2007;9:110-114.
16. Pai N, Joshi R, Dogra S, et al. Evaluation of diagnostic accuracy, feasibility and client preference for rapid oral fluid-based diagnosis of HIV infection in rural India. *PLoS One*. 2007;2:e367. doi:10.1371/journal.pone.000036.
17. Sema C, Raposo C, Correia D, et al. Prospective evaluation of two oral fluid rapid tests in Mozambique. Presented at the International AIDS Conference; July 18-23, 2010; Vienna, Austria.
18. Wright R, Stringer JS. Rapid testing strategies for HIV-1 serodiagnosis in high-prevalence African settings. *Am J Prev Med*. 2004;27:42-48.
19. Maofeng Q, Liu X, Jiang Y, et al. Current HIV-2 diagnostic strategy overestimates HIV-2 prevalence in China. *J Med Virol*. 2009;81:790-797.
20. Alcaro M, Peroni E, Rovero P, et al. Synthetic peptides in the diagnosis of HIV infection. *Curr Protein Peptide Sci*. 2003;4:285-290.
21. *Clinical Evaluation of National HIV Antibody Diagnostic Reagents by China Centers for Disease Control*. Beijing, China: China-CDC; 2009:30-33.
22. Peddecord KM, Benenson AS, Hofherr LK, et al. Analytic results of HIV-1 testing using blind proficiency testing. *Clin Lab Sci*. 1992;5:165-171.
23. Parekh B, Anyanwu J, Patel H, et al. 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 [published online ahead of print October 28, 2009]. *J Virol Methods*. 2010;163:295-300. doi:10.1016/j.jviromet.2009.10.013.
24. World Health Organization, Centers for Disease Control and Prevention, UNAIDS. HIV Rapid Test Training Package. Geneva, Switzerland: World Health Organization; 2006. http://www.who.int/diagnostics_laboratory/documents/guidance/hivrtraining_overview/en/. Accessed August 9, 2010.