

Performance of an Early Infant Diagnostic Test, AmpliSens DNA-HIV-FRT, Using Dried Blood Spots Collected from Children Born to Human Immunodeficiency Virus-Infected Mothers in Ukraine

Joy Chang,^a Tetyana Tarasova,^b Vedapuri Shanmugam,^a Marianna Azarskova,^c Shon Nguyen,^a Mackenzie Hurlston,^a Jennifer Sabatier,^a Guoqing Zhang,^a Saladin Osmanov,^a Dennis Ellenberger,^a Chunfu Yang,^a Charles Vitek,^c Maria Liulchuk,^d Natalya Nizova^d

Centers for Disease Control and Prevention, Atlanta, Georgia, USA^a; UNICEF, Kiev, Ukraine^b; Centers for Disease Control and Prevention, Kiev, Ukraine^c; Ukrainian Center for Disease Control, Ministry of Health of Ukraine, Kiev, Ukraine^d

An accurate accessible test for early infant diagnosis (EID) is crucial for identifying HIV-infected infants and linking them to treatment. To improve EID services in Ukraine, dried blood spot (DBS) samples obtained from 237 HIV-exposed children (≤ 18 months of age) in six regions in Ukraine in 2012 to 2013 were tested with the AmpliSens DNA-HIV-FRT assay, the Roche COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HIV-1 Qual test, and the Abbott RealTime HIV-1 Qualitative assay. In comparison with the paired whole-blood results generated from AmpliSens testing at the oblast HIV reference laboratories in Ukraine, the sensitivity was 0.99 (95% confidence interval [CI], 0.95 to 1.00) for the AmpliSens and Roche CAP/CTM Qual assays and 0.96 (95% CI, 0.90 to 0.98) for the Abbott Qualitative assay. The specificity was 1.00 (95% CI, 0.97 to 1.00) for the AmpliSens and Abbott Qualitative assays and 0.99 (95% CI, 0.96 to 1.00) for the Roche CAP/CTM Qual assay. McNemar analysis indicated that the proportions of positive results for the tests were not significantly different ($P > 0.05$). Cohen's kappa (0.97 to 0.99) indicated almost perfect agreement among the three tests. These results indicated that the AmpliSens DBS and whole-blood tests performed equally well and were comparable to the two commercially available EID tests. More importantly, the performance characteristics of the AmpliSens DBS test meets the World Health Organization EID test requirements; implementing AmpliSens DBS testing might improve EID services in resource-limited settings.

Ukraine experienced the most severe human immunodeficiency virus (HIV) epidemic in Europe before prevention measures began to slow transmission (1–3). In 2013, approximately 210,000 persons were living with HIV, and the HIV prevalence was 0.8% among adults 15 to 49 years of age and women at childbirth (1–3). HIV infections in Ukraine mainly are the result of injection drug use (4). However, heterosexual HIV transmission in Ukraine is increasing (4). Because of women's biological and social vulnerability (e.g., women engaging in commercial sex work to provide funds for drugs for their male partners), women are more prone to infection (4, 5). Women now represent 45% of all adults living with HIV in Ukraine, and the majority of those women are of reproductive age (4, 5). In 2001, the Ukrainian government implemented a national program for prevention of mother-to-child transmission. Since then, the rate of mother-to-child transmission has decreased from 21% to 3.7% (6), as of 2011. Although the numbers of HIV-infected pregnant women have plateaued, approximately 4,000 HIV-exposed children are born each year (1, 4).

In order for HIV-infected children to receive antiretroviral treatment as early as possible, accurate accessible early infant diagnosis (EID) of HIV is crucial. The World Health Organization (WHO) strongly recommends using virological tests for EID, because infants and children of HIV-positive mothers acquire antibodies transplacentally and the antibodies can persist in the infants for up to 18 months after birth (7). Ukraine has had insufficient capacity for full coverage with EID virological methods. Since 2006, Ukrainian laboratories have been using a DNA-based PCR test, the AmpliSens DNA-HIV-FRT PCR assay (InterLabService, Moscow, Russia), for EID using infants' venous whole

blood (8). Using venous whole blood for EID presents challenges in resource-limited settings and remote areas, because whole-blood samples require cold-chain transportation and the conditions for sample storage and processing are more stringent. Dried blood spot (DBS) samples offer multiple advantages for EID, because the samples can be collected easily by heel prick or finger stick and can be stored at ambient temperature for long periods (9–15). Because of their greater stability at ambient temperature, DBS samples can be transported by postal mail to centralized laboratories for testing and, if packaged correctly, do not raise biosafety concerns regarding the health of postal workers (9–15). Published data indicate that DBS samples are suitable and practical for quality EID and have aided service expansion in sub-Saharan Africa (16–21). In our study, for the first time, the performances of the AmpliSens DNA PCR assay and two commercially

Received 8 September 2015 Returned for modification 24 September 2015
Accepted 5 October 2015

Accepted manuscript posted online 7 October 2015

Citation Chang J, Tarasova T, Shanmugam V, Azarskova M, Nguyen S, Hurlston M, Sabatier J, Zhang G, Osmanov S, Ellenberger D, Yang C, Vitek C, Liulchuk M, Nizova N. 2015. Performance of an early infant diagnostic test, AmpliSens DNA-HIV-FRT, using dried blood spots collected from children born to human immunodeficiency virus-infected mothers in Ukraine. *J Clin Microbiol* 53:3853–3858. doi:10.1128/JCM.02392-15.

Editor: A. M. Caliendo

Address correspondence to Joy Chang, ckc7@cdc.gov, or Tetyana Tarasova, ttarasova@unicef.org.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

available and widely used DBS EID tests, i.e., the Roche COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) Qual test (Roche Diagnostics, Indianapolis, IN, USA) and the Abbott RealTime HIV-1 Qualitative test (Abbott Laboratories, Abbott Park, IL, USA) (22–27), were evaluated by using DBS samples from HIV-exposed infants in Ukraine and were compared with the performance of the EID test in Ukraine, the AmpliSens whole-blood test.

MATERIALS AND METHODS

Samples and testing. From February 2012 to April 2013, DBS samples were derived from venous blood collected in EDTA-containing tubes, which was left over from testing of HIV-exposed infants ≤ 18 months of age at six oblast (equal to state or province) AIDS centers. In Ukraine, infant HIV status is determined by two PCR results using the AmpliSens whole-blood test. All specimens routinely collected for EID testing were eligible for inclusion in this study. The residual blood was transported to the Ukraine National AIDS Center for DBS preparation. The blood was shipped under cold conditions, ≤ 24 h after collection. During the study period, more than 2,600 infants born to HIV-infected mothers, from six oblast AIDS centers in Ukraine, were tested. Among the infants, 100 tested positive for HIV. Along with the 100 HIV-positive samples, 137 HIV-negative samples were randomly selected from the six oblast AIDS centers and were included in the study. Among the 237 samples, 29 were from the Crimean oblast, 41 from the Dnipropetrovsk oblast, 54 from the Donetsk oblast, 35 from the Mykolaiv oblast, 35 from the Kiev oblast, and 43 from the Odessa oblast. At the National AIDS Center, the DBS samples were prepared by spotting 70 μ l of whole blood onto Whatman 903 filter paper for each spot, by using a measuring pipette. For each patient, two DBS cards were obtained, with each card containing 5 DBSs. The DBS cards were dried overnight at room temperature and then covered with folded leaflets. The DBS cards were then packed in low-gas-permeable plastic bags with desiccants and were stored at -20°C until they were ready to be shipped to the Centers for Disease Control and Prevention (CDC) (Atlanta, GA, USA) for testing. All DBS samples were tested blindly with the AmpliSens, Roche CAP/CTM Qual, and Abbott Qualitative assays.

DIA-DNA-HIV-FRT whole-blood DNA extraction protocol. The DIA-DNA-HIV-FRT whole-blood DNA extraction procedure (DiaProph Med, Kiev, Ukraine) includes three steps, i.e., (i) hemolysis and leukocyte pelleting, (ii) nucleic acid (NA) extraction, and (iii) NA washing and pelleting. In the first step, 250 μ l of EDTA-treated whole blood from each patient was hemolyzed twice. First a 6-min hemolysis procedure was conducted in 1 ml of hemolytic solution, followed by centrifugation (Biofuge Stratos Heraeus, Kendro, Germany) at 8,000 rpm for 2 min to obtain the leukocyte pellet, and then a 3-min hemolysis procedure was conducted in 0.5 ml of hemolytic solution, followed by centrifugation at 8,000 rpm for 2 min to obtain the leukocyte pellet. In the second step, the leukocyte pellet was incubated with 300 μ l of lysis solution and 25 μ l of universal sorbent solution at room temperature for 10 min, to lyse the leukocytes and to precipitate the NA. In this step, the extraction controls, i.e., a negative control (5 μ l of Tris-EDTA [TE] buffer) and a positive control (5 μ l of HIV-positive DNA) provided with the kit, were processed along with the patients' samples to monitor the NA extraction process. After centrifugation of the lysed leukocytes at 5,000 rpm for 30 s, the NA pellets were obtained by removing the supernatant from each sample tube. In the third step, the NA pellets were first washed with 300 μ l of washing solution 1, followed by centrifugation at 5,000 rpm for 30 s. After centrifugation, the supernatant was removed, and the NA pellets were washed again with 500 μ l of washing solution 2, followed by centrifugation at 10,000 rpm for 30 s. At the end of the centrifugation, the supernatant was removed from each tube, and the NA pellet was dried at 65°C for 10 min. The dried NA pellet was then dissolved in 50 μ l of TE buffer, followed by centrifugation at 12,000 rpm for 1 min. The purified NA solution was then ready for DNA amplification or was stored at -70°C until testing.

AmpliSens DBS DNA extraction protocol. The DBS samples were processed by following the instruction manual (Federal Budget Institute

of Science, Central Research Institute for Epidemiology, Moscow, Russia) instruction manual (28). One 12-mm DBS was processed per patient, to compare the same amount of DNA input as with the Roche CAP/CTM Qual and Abbott Qualitative EID tests. The AmpliSens DBS DNA extraction procedure included the lysis, NA extraction and pelleting, and NA pellet washing steps. At the end of the procedure, 50 μ l of DNA was obtained from each patient sample.

AmpliSens DNA amplification and result interpretation. The AmpliSens DNA amplification was performed by using two real-time PCR instruments, i.e., the Rotor-Gene 6000 (Qiagen, Germantown, MD, USA) and Agilent-Stratagene Mx3000P (Agilent Technologies, Santa Clara, CA, USA) systems, both of which had been validated and recommended for the AmpliSens test kit (29). The Rotor-Gene 6000 system was used at the oblast AIDS centers in Ukraine for amplification of whole-blood samples, and the Agilent-Stratagene Mx3000P system was used at the CDC for amplification of DBS samples. The real-time PCR results were interpreted by following the result interpretation matrix provided in the kit (28). Briefly, when the extraction and amplification controls meet the criteria in the matrix, any sample with a cycle threshold (C_T) value of <40 for the target and a positive result for the internal control (IC) is considered to demonstrate a positive result. A negative result would have no C_T value for the target but a positive result for the IC.

Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Qual test. The Roche CAP/CTM Qual test was performed by following the manufacturer's instructions (30).

Abbott RealTime HIV-1 Qualitative assay. The Abbott Qualitative assay was performed by following the manufacturer's instructions (31) except for the specimen input volume; instead of two 12-mm DBSs, one 12-mm DBS was processed per patient. We chose to compare the same input volume of whole blood (DNA) as for the Roche CAP/CTM Qual and AmpliSens tests.

Statistical analysis. The sensitivity and specificity of each DBS test were calculated by using the AmpliSens whole-blood test as the reference group. The Wilson score method was used to calculate 95% confidence intervals (CIs) for binomial proportions. The McNemar test was used to assess whether the proportions of positive results for each DBS test differed from each other. If the P value was >0.05 , then the proportions of positive results for the DBS tests did not differ significantly from each other. Cohen's kappa was used to assess the agreement between the AmpliSens whole-blood test and the DBS tests in HIV diagnosis. Kappa values of 0.81 to 1.0 indicated almost perfect agreement. All statistical analyses were performed by using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

Ethics review. The study was reviewed and approved by the State Service of Ukraine on HIV/AIDS and Other Socially Dangerous Diseases. DBS samples were delinked from identifying patient information before being sent to the CDC for testing. The anonymous testing at the CDC was determined to be nonhuman subjects research by the Associate Director for Science at the Center for Global Health, CDC (Atlanta, GA, USA).

RESULTS

Patient ages. The median age of the 237 children was 5 months. Among the 237 children, 9% (21/237 patients) were <3 months of age, 82% (195/237 patients) were 3 to <12 months of age, and 9% (21/237 patients) were 12 to <18 months of age.

Performance of AmpliSens DBS test versus AmpliSens whole-blood test. A total of 237 DBS samples, including 100 HIV-1-positive samples and 137 HIV-1-negative samples, were tested with the AmpliSens assay (Table 1). Two HIV-negative DBS samples demonstrated no DNA results because their endogenous controls (β -globin) were not detected by the AmpliSens test. These two samples were excluded from the data analysis for specificity. Results indicated that AmpliSens testing using DBS samples could detect all of the positive samples that were detected by AmpliSens

TABLE 1 Performance of Abbott Qualitative, AmpliSens DBS, and Roche CAP/CTM Qual tests versus AmpliSens whole-blood test for early infant diagnosis

DBS test platform and result ^a	No. with AmpliSens whole-blood test result of:		Total no.	Sensitivity (95% CI)	Specificity (95% CI)	McNemar <i>P</i>	Kappa
	Positive	Negative					
Abbott ^b				0.96 (0.90–0.98)	1 (0.97–1.00)	0.13	0.97
Positive	96	0	96				
Negative	4	137	141				
Total	100	137	237				
AmpliSens ^c				0.99 (0.95–1.00)	1 (0.97–1.00)	1	0.99
Positive	99	0	99				
Negative	1	135 ^d	136				
Total	100	135	235				
Roche ^e				0.99 (0.95–1.00)	0.99 (0.96–1.00)	1	0.98
Positive	99	1	100				
Negative	1	136	137				
Total	100	137	237				

^a DBS, dried blood spot; CI, confidence interval.

^b Abbott Laboratories (Abbott Molecular, Inc., Des Plaines, IL, USA).

^c Federal Budget Institute of Science, Central Research Institute for Epidemiology (Moscow, Russia).

^d Two DBS samples with no DNA results were excluded.

^e Roche Molecular Systems, Inc. (Branchburg, NJ, USA).

testing using whole-blood samples, except for one sample that tested positive by whole-blood testing but negative by DBS testing (Table 1). For the 135 samples that tested negative with the AmpliSens whole-blood test, the DBS test results matched exactly (Table 1). The sensitivity and specificity of the AmpliSens DBS test were 0.99 (95% CI, 0.95 to 1.00) and 1.00 (95% CI, 0.97 to 1.00), respectively. The McNemar *P* value for the AmpliSens DBS test was 1.0, indicating that the proportion of positive results obtained with the AmpliSens DBS test was not significantly different from the proportion obtained with the AmpliSens whole-blood test. The Cohen's kappa value for the AmpliSens DBS test was 0.99, indicating almost perfect agreement between the AmpliSens DBS test and the AmpliSens whole-blood test (Table 1).

Performance of Abbott Qualitative and Roche CAP/CTM Qual DBS tests versus AmpliSens whole-blood test. A total of 237 DBS samples were tested with both the Abbott Qualitative and Roche CAP/CTM Qual tests. Among the 100 whole-blood samples that tested positive with the AmpliSens test, four of the paired DBS samples were not detected by the Abbott Qualitative test (Table 1). For all 137 whole-blood samples that tested negative with the AmpliSens blood test, the Abbott Qualitative assay yielded the same results with DBS samples (Table 1). Compared with the AmpliSens whole-blood test, the sensitivity and specificity of the Abbott Qualitative test were 0.96 (95% CI, 0.90 to 0.98) and 1.00 (95% CI, 0.97 to 1.00), respectively (Table 1). The McNemar *P* value for the Abbott Qualitative test was 0.13, indicating that the proportion of positive results obtained with the Abbott Qualitative assay was not significantly different from that obtained with the AmpliSens whole-blood test. The Cohen's kappa value for the Abbott Qualitative assay was 0.97, indicating almost perfect agreement between the Abbott Qualitative assay and the AmpliSens whole-blood test (Table 1).

The Roche CAP/CTM Qual test results indicated that, among the 100 whole-blood samples that tested positive with the Am-

pliSens test, one of the paired DBS samples was not detected by the Roche CAP/CTM Qual assay (Table 1). For all 137 whole-blood samples that tested negative with the AmpliSens assay, the Roche CAP/CTM Qual assay demonstrated the same results with DBS samples, except for one DBS sample with a weak positive result (Table 1). Compared with the AmpliSens whole-blood test, the sensitivity and specificity of the Roche CAP/CTM Qual assay were 0.99 (95% CI, 0.95 to 1.00) and 0.99 (95% CI, 0.96 to 1.00) (Table 1), respectively. The McNemar *P* value for the Roche CAP/CTM Qual assay was 1.0, indicating that the proportion of positive results obtained with the Roche CAP/CTM Qual assay was not significantly different from that obtained with the AmpliSens whole-blood test. The Cohen's kappa value for the Roche CAP/CTM Qual assay was 0.98, indicating almost perfect agreement between the Roche CAP/CTM Qual assay and the AmpliSens whole-blood test (Table 1).

Discordant results between DBS tests and AmpliSens whole-blood test. Among the three DBS tests, six DBS samples yielded discordant results, compared with the results from the AmpliSens assay using whole blood (Table 2). Human errors and potential cross-contamination were ruled out for the six discordant samples by repeating the tests with another DBS sample from the same patient. The HIV status of the six discordant samples was confirmed at the Ukraine oblast AIDS centers. The negative result from AmpliSens whole-blood testing among the six discordant results was confirmed with an HIV enzyme-linked immunosorbent assay (ELISA), and the five positive results from AmpliSens whole-blood testing were confirmed with an HIV load test (Table 2). The results presented in Table 2 demonstrate that the discordant results were not age or oblast specific. The available C_T values from the AmpliSens and Roche CAP/CTM Qual DBS tests were considered high and indicated weak positive results.

TABLE 2 Discordant DBS test results

Sample	Patient age (mo)	Oblast	AmpliSens whole-blood test result	DBS test result ^a		
				Abbott Qualitative ^b	AmpliSens ^c	Roche CAP/CTM ^d
1	7	Crimean	Negative ^e	Negative	Negative	Positive, C_T of >34
2	5	Kiev	Positive ^f	Negative	Positive, C_T of 35	Positive, C_T of >34
3	3	Dnipropetrovsk	Positive ^f	Negative	Positive, C_T of >35	Negative
4	14	Dnipropetrovsk	Positive ^f	Negative	Positive, C_T of 33	Positive, C_T of >34
5	4	Donetsk	Positive ^f	Positive ^g	Negative	Positive, C_T of >34
6	6	Odessa	Positive ^f	Negative	Positive, C_T of 34	Positive, C_T of >34

^a DBS, dried blood spot; C_T , cycle threshold.

^b Abbott Laboratories (Abbott Molecular, Inc., Des Plaines, IL, USA).

^c Federal Budget Institute of Science, Central Research Institute for Epidemiology (Moscow, Russia).

^d Roche Molecular Systems, Inc. (Branchburg, NJ, USA).

^e Confirmed by an ELISA (Ani LabSystems Ltd., Finland) and the Genscreen Ultra HIV Ag-Ab test (Bio-Rad, France).

^f Confirmed by the Abbott HIV Viral Load test.

^g The C_T value was not provided by the Abbott test software, but the amplification curve indicated a weak positive result.

DISCUSSION

DBS samples serve as suitable practical samples for quality EID in multiple countries (16–21). To help Ukraine expand its EID services and to increase infants' and children's access to EID in resource-limited settings or remote areas, the performance of Ukraine's EID test, i.e., the AmpliSens assay, as well as the Roche CAP/CTM Qual and Abbott Qualitative assays, was evaluated by using DBS samples from HIV-exposed Ukrainian infants or children; those results were compared with the performance of Ukrainian EID testing using venous whole-blood samples.

The results of our study demonstrated that the performance of the AmpliSens assay using DBS samples was as good as that of the AmpliSens assay using whole-blood samples and was comparable to the performance of the Roche CAP/CTM Qual and Abbott Qualitative EID tests. The sensitivity of 0.99 (95% CI, 0.95 to 1.00) and the specificity of 1.00 (95% CI, 0.97 to 1.00) for the AmpliSens DBS test exceeded the WHO 2010 recommendations for virological tests, i.e., ≥ 0.95 for sensitivity and 0.98 for specificity at different HIV prevalence levels (7).

The AmpliSens DBS test had one discordant result, compared with the AmpliSens whole-blood test. This discordant sample tested positive with the AmpliSens whole-blood test but negative with the DBS test (Table 2). This particular sample tested positive with the Roche CAP/CTM Qual assay but had a high C_T value of >34, indicating a weak positive result. The Abbott Qualitative assay also yielded a weak positive result for that sample, as determined by reviewing the amplification curve. However, the Abbott software did not provide a specific C_T value for the qualitative test. The discrepant results between the AmpliSens DBS test and the AmpliSens whole-blood test might be caused by different sample input volumes. The AmpliSens whole-blood test required 250 μ l of whole blood per test, whereas the AmpliSens DBS test used only one DBS per test, containing only 70 μ l of blood. The Abbott Qualitative and Roche CAP/CTM Qual assays also used one DBS per test, with 70 μ l of blood, which might explain why the Abbott Qualitative assay missed four samples and the Roche CAP/CTM Qual assay missed one sample that tested positive with the AmpliSens whole-blood test (Table 2). Other factors (e.g., amplification cycle numbers and targeted regions for the tests) also might contribute to the discordant results among the three DBS tests. The total amplification cycle numbers were 37 for the Abbott

Qualitative assay, 40 for the Roche CAP/CTM Qual assay, and 42, with 5 additional cycles of non-real-time PCR amplification, for the AmpliSens whole-blood and DBS tests. The targeted regions for the tests also were different. The Roche CAP/CTM Qual assay targets the *gag* region and the Abbott Qualitative assay targets the *pol* region of the HIV-1 virus (30, 31). Which region the AmpliSens test targets is not known, because that information is not provided in the package insert. The efficiency of NA extraction for each test and the HIV subtypes of the samples also might contribute to the discordant results, as discussed for an EID test evaluation study in Kenya (22). HIV subtype information was not available in this study, but the subtypes for clinical samples in Ukraine are primarily subtypes A and B (32–34).

Although all three DBS tests missed detection of one or a few positive samples detected by the AmpliSens whole-blood test, a sample that tested negative with the AmpliSens whole-blood test was detected by the Roche CAP/CTM Qual assay with a weak positive result, with a C_T value of 34 (Table 2). Because this sample was confirmed as negative by an ELISA with a follow-up sample in Ukraine (Table 2), this weak positive result indicated a false-positive result from the Roche CAP/CTM Qual assay. The potential false-positive results from the Roche CAP/CTM Qual assay were reported and discussed in an evaluation by Maritz et al. (23) of the Roche CAP/CTM Qual assay versus the Roche COBAS Amplicor DNA test, version 1.5 (Roche Molecular Systems Inc., Pleasanton, CA, USA), for EID. Maritz et al. (23) demonstrated that a false-positive sample identified by the Roche CAP/CTM Qual assay tended to have a flat amplification slope and a high C_T value, which together indicate a nonspecific signal. Our findings are consistent with those of Maritz et al. (23). To rule out potential false-positive results from the Roche CAP/CTM Qual assay, Maritz et al. recommended that Roche CAP/CTM Qual assay users review the C_T value and fluorescence intensity of the amplification curve for each sample, as reported by the AMPLILINK software (Roche Molecular Systems Inc., Pleasanton, CA, USA) (23). If Roche CAP/CTM Qual assay users observe a sample with an absolute fluorescence intensity of <5 and a C_T value of >32, then they should request a follow-up sample from the patient and repeat the testing for confirmation (23).

The interpretation of the results of the AmpliSens test should be noted. According to the AmpliSens kit instructions, when the

extraction and amplification controls meet the requirements indicated in the package insert, any sample result with a C_T value of <40 for the target and a positive signal for the IC should be considered an HIV-positive result. In our study, however, only a few clinically positive DBS results had C_T values of >35. Given our experience regarding potential false-positive results with high C_T values, we recommend that, when a sample with a C_T value of 35 to 40 is observed, AmpliSens test users may consider requesting a follow-up sample from the patient and repeating the test to confirm the patient's HIV status.

The technical procedure for the AmpliSens assay is labor-intensive and requires highly trained technicians, because it is challenging to work with a small NA pellet from each sample in a multistep procedure. Additionally, close attention needs to be paid during the sample processing and amplification set-up steps, to avoid potential sample cross-contamination, with sample tubes being opened multiple times and processed next to each other. When a laboratory chooses to use the AmpliSens test for EID, the laboratory should have comprehensive quality assurance and quality control procedures in place, and those procedures should be followed closely. The format of the AmpliSens test allows users to process 96 samples per day. However, processing 96 samples in a day when working with such a long procedure is challenging and may be impractical for most laboratories. This test may be best suited to a laboratory with low or medium EID sample input (from a few to several dozen samples per week). If a laboratory chooses to run a full 96-sample run for the amplification, then the laboratory can combine extracted DNA samples from different days or runs. However, this approach should be contingent on the return of results to patients not being delayed.

In conclusion, our study demonstrates that the AmpliSens DBS test performs as well as testing with whole-blood samples and matches the expected outcomes of the two widely used commercial EID tests. Furthermore, the AmpliSens assay meets or exceeds the WHO EID test qualification requirements, and using DBS samples might improve EID services in resource-limited settings. The findings in this study also may benefit countries that are using the AmpliSens test with venous whole-blood samples for EID.

ACKNOWLEDGMENTS

This research was supported by the President's Emergency Plan for AIDS Relief through the Centers for Disease Control and Prevention.

Special thanks go to Ruslan Malyuta at the UNICEF Regional Office for Central and Eastern Europe and the Commonwealth of Independent States for his introduction of the idea of using DBS testing for EID services in Ukraine. The study was made possible because of his work to facilitate the connections and dialogue among the partners who were involved in the study.

This study also was made possible by the reagents donated by AmpliSens, Federal Budget Institute of Science, Central Research Institute for Epidemiology (Moscow, Russia). AmpliSens did not take part in the data collection, data analysis, or writing of the manuscript.

The use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Department of Health and Human Services or the Centers for Disease Control and Prevention. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

REFERENCES

- Vitek CR, Cakalo JI, Kruglov YV, Dumchev KV, Salyuk TO, Božičević I, Baughman AL, Spindler HH, Martsynovska VA, Kobyscha YV, Abdul-Quader AS, Rutherford GW. 2014. Slowing of the HIV epidemic in Ukraine: evidence from case reporting and key population surveys, 2005–2012. *PLoS One* 9:e103657. <http://dx.doi.org/10.1371/journal.pone.0103657>.

- UNAIDS. 2013. HIV and AIDS estimates: Ukraine. <http://www.unaids.org/en/regionscountries/countries/ukraine>. Accessed 20 March 2014.
- Ministry of Health of Ukraine. 2012. Ukraine harmonized AIDS response progress report: reporting period: January 2010–December 2011. Ministry of Health of Ukraine, Kiev, Ukraine. http://www.unaids.org/sites/default/files/en/dataanalysis/knownyourresponse/countryprogressreports/2012countries/ce-UA_Narrative_Report%5B1%5D.pdf.
- UNICEF. 2013. Ukraine: HIV/AIDS, children and youth programme. http://www.unicef.org/ukraine/activities_11384.html. Accessed 21 October 2014.
- UNICEF. 2011. Ukraine country programme document 2012–2016. UNICEF, New York, NY. http://www.unicef.org/about/execboard/files/Ukraine_final_approved_2012-2016_20_Oct_2011.pdf.
- Ministry of Health of Ukraine. 2014. Ukraine harmonized AIDS response progress report, reporting period: January 2012–December 2013. Ministry of Health of Ukraine, Kiev, Ukraine. http://www.unaids.org/sites/default/files/country/documents/UKR_narrative_report_2014.pdf.
- World Health Organization. 2010. WHO recommendations on the diagnosis of HIV infection in infants and children. World Health Organization, Geneva, Switzerland.
- Mahdavi S, Malyuta R, Semenenko I, Pilipenko T, Thorne C, Ukraine European Collaborative Study Group. 2010. Treatment and disease progression in a birth cohort of vertically HIV-1 infected children in Ukraine. *BMC Pediatr* 10:85.
- Leelawiwat W, Young NL, Chaowanachan T, Ou CY, Culnane M, Vanprapa N, Waranawat N, Wasinrapee P, Mock PA, Tappero J, McNicholl JM. 2009. Dried blood spots for the diagnosis and quantitation of HIV-1: stability studies and evaluation of sensitivity and specificity for the diagnosis of infant HIV-1 infection in Thailand. *J Virol Methods* 155: 109–117. <http://dx.doi.org/10.1016/j.jviromet.2008.09.022>.
- van Deursen P, Oosterlaken T, Andre P, Verhoeven A, Bertens L, Traub MA, Ligeon V, de Jong J. 2010. Measuring human immunodeficiency virus type 1 RNA loads in dried blood spot specimens using NucliSENS EasyQ HIV-1 v2.0. *J Clin Virol* 47:120–125. <http://dx.doi.org/10.1016/j.jcv.2009.11.021>.
- Nyambi PN, Franssen K, De Beenhouwer H, Chomba EN, Temmerman M, Ndinya-Achola JO, Piot P, van der Groen G. 1994. Detection of human immunodeficiency virus type 1 (HIV-1) in heel prick blood on filter paper from children born to HIV-1-seropositive mothers. *J Clin Microbiol* 32:2858–2860.
- Cassol S, Salas T, Gill MJ, Montpetit M, Rudnik J, Sy CT, O'Shaughnessy MV. 1992. Stability of dried blood spot specimens for detection of human immunodeficiency virus DNA by polymerase chain reaction. *J Clin Microbiol* 30:3039–3042.
- Cassol SA, Lapointe N, Salas T, Hankins C, Arella M, Fauvel M, Delage G, Boucher M, Samson J, Charest J. 1992. Diagnosis of vertical HIV-1 transmission using the polymerase chain reaction and dried blood spot specimens. *J Acquir Immune Defic Syndr* 5:113–119.
- Biggar RJ, Miley W, Miotti P, Taha TE, Butcher A, Spadaro J, Waters D. 1997. Blood collection on filter paper: a practical approach to sample collection for studies of perinatal HIV transmission. *J Acquir Immune Defic Syndr Hum Retrovirol* 14:368–373. <http://dx.doi.org/10.1097/00042560-199704010-00010>.
- Comeau AM, Pitt J, Hillyer GV, Landesman S, Bremer J, Chang BH, Lew J, Moye J, Grady GF, McIntosh K. 1996. Early detection of human immunodeficiency virus on dried blood spot specimens: sensitivity across serial specimens. *J Pediatr* 129:111–118. [http://dx.doi.org/10.1016/S0022-3476\(96\)70197-X](http://dx.doi.org/10.1016/S0022-3476(96)70197-X).
- Sherman G, Stevens G, Jones S, Horsfield P, Stevens W. 2005. Dried blood spots improve access to HIV diagnosis and care for infants in low-resource settings. *J Acquir Immune Defic Syndr* 38:615–617. <http://dx.doi.org/10.1097/01.qai.0000143604.71857.5d>.
- Mehta N, Trzmielina S, Nonyane BA, Eliot MN, Lin R, Foulkes AS, McNeal K, Ammann A, Eulalievyyolo V, Sullivan JL, Luzuriaga K, Somasundaran M. 2009. Low-cost HIV-1 diagnosis and quantification in dried blood spots by real time PCR. *PLoS One* 4:e5819. <http://dx.doi.org/10.1371/journal.pone.0005819>.
- Chohan BH, Emery S, Wamalwa D, John-Stewart G, Majiwa M, Ng'ayo M, Froggett S, Overbaugh J. 2011. Evaluation of a single round polymer-

- ase chain reaction assay using dried blood spots for diagnosis of HIV-1 infection in infants in an African setting. *BMC Pediatr* 11:18. <http://dx.doi.org/10.1186/1471-2431-11-18>.
19. Rollins NC, Dedicoat M, Danaviah S, Page T, Bishop K, Kleinschmidt I, Coovadia HM, Cassol SA. 2002. Prevalence, incidence, and mother-to-child transmission of HIV-1 in rural South Africa. *Lancet* 360:389. [http://dx.doi.org/10.1016/S0140-6736\(02\)09599-5](http://dx.doi.org/10.1016/S0140-6736(02)09599-5).
 20. Lilian RR, Kalk E, Bhowan K, Berrie L, Carmona S, Technau K, Sherman GG. 2012. Early diagnosis of in utero and intrapartum HIV infection in infants prior to 6 weeks of age. *J Clin Microbiol* 50:2373–2377. <http://dx.doi.org/10.1128/JCM.00431-12>.
 21. Lilian RR, Kalk E, Technau KG, Sherman GG. 2013. Birth diagnosis of HIV infection in infants to reduce infant mortality and monitor for elimination of mother-to-child transmission. *Pediatr Infect Dis J* 32:1080–1085. <http://dx.doi.org/10.1097/INF.0b013e318290622e>.
 22. Chang J, Omuomo K, Anyango E, Kingwara L, Basiye F, Morwabe A, Shanmugam V, Nguyen S, Sabatier J, Zeh C, Ellenberger D. 2014. Field evaluation of Abbott Real Time HIV-1 qualitative test for early infant diagnosis using dried blood spots samples in comparison to Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Qual test in Kenya. *J Virol Methods* 204:25–30. <http://dx.doi.org/10.1016/j.jviromet.2014.03.010>.
 23. Maritz J, Preiser W, Zyl GU. 2012. Establishing diagnostic cut-off criteria for the COBAS AmpliPrep/COBAS TaqMan HIV-1 qualitative test through validation against the Amplicor DNA test v1.5 for infant diagnosis using dried blood spots. *J Clin Virol* 53:106–109. <http://dx.doi.org/10.1016/j.jcv.2011.12.002>.
 24. Schumacher W, Frick E, Kauselmann M, Maier-Hoyle V, van der Vliet R, Babiak R. 2007. Fully automated quantification of human immunodeficiency virus (HIV) type 1 RNA in human plasma by the COBAS® AmpliPrep/COBAS® TaqMan® system. *J Clin Virol* 38:304–312. <http://dx.doi.org/10.1016/j.jcv.2006.12.022>.
 25. Stevens W, Erasmus L, Moloi M, Taleng T, Sarang S. 2008. Performance of a novel human immunodeficiency virus (HIV) type 1 total nucleic acid-based real-time PCR assay using whole blood and dried blood spots for diagnosis of HIV in infants. *J Clin Microbiol* 46:3941–3945. <http://dx.doi.org/10.1128/JCM.00754-08>.
 26. Jani IV, Sabatier J, Vubil A, Subbarao S, Bila D, Sousa A, Mabunda N, Garcia A, Skaggs S, Ellenberger D, Ramos A. 2012. Evaluation of a high-throughput diagnostic system for detection of HIV-1 in dried blood spot samples from infants in Mozambique. *J Clin Microbiol* 50:1458–1460. <http://dx.doi.org/10.1128/JCM.00107-12>.
 27. Kageha S, Okoth V, Kadima S, Vihenda S, Okapesi E, Nyambura E, Maiyo A, Ndung'u N, Khamadi S, Mwau M. 2012. Discrepant test findings in early infant diagnosis of HIV in a national reference laboratory in Kenya: challenges and opportunities for programs. *J Trop Pediatr* 58:247–252. <http://dx.doi.org/10.1093/tropej/fmr076>.
 28. Federal Budget Institute of Science, Central Research Institute for Epidemiology. 2011. AmpliSens® DNA-HIV-FRT PCR kit instruction manual. Federal Budget Institute of Science, Central Research Institute for Epidemiology, Moscow, Russia. <http://www.pcrdiagnostics.eu/files/documents/products/pdf/20140310/DNA-HIV-FRT%20141112.pdf>.
 29. AmpliSens Biotechnologies. 2009. HIV infection. Federal Budget Institute of Science, Central Research Institute for Epidemiology, Moscow, Russia. <http://www.pcrdiagnostics.eu/files/documents/brochures/hiv.pdf>.
 30. Roche Molecular Systems, Inc. 2010. COBAS AmpliPrep/COBAS TaqMan HIV-1 Qual test package insert. Roche Molecular Systems, Inc., Branchburg, NJ.
 31. Abbott Molecular, Inc. 2010. Abbott RealTime HIV-1 Qualitative assay package insert. Abbott Molecular, Inc., Des Plaines, IL.
 32. Novitsky VA, Montano MA, Essex M. 1998. Molecular epidemiology of an HIV-1 subtype A subcluster among injection drug users in the southern Ukraine. *AIDS Res Hum Retroviruses* 14:1079–1085. <http://dx.doi.org/10.1089/aid.1998.14.1079>.
 33. Nabatov AA, Kravchenko ON, Lyulchuk MG, Shcherbinskaya AM, Lukashov VV. 2002. Simultaneous introduction of HIV type 1 subtype A and B viruses into injecting drug users in southern Ukraine at the beginning of the epidemic in the former Soviet Union. *AIDS Res Hum Retroviruses* 18:891–895. <http://dx.doi.org/10.1089/08892220260190380>.
 34. Saad MD, Shcherbinskaya AM, Nadai Y, Kruglov YV, Antonenko SV, Lyulchuk MG, Kravchenko ON, Earhart KC, Sanchez JL, Bix DL, Carr JK. 2006. Molecular epidemiology of HIV type 1 in Ukraine: birthplace of an epidemic. *AIDS Res Hum Retroviruses* 22:709–714. <http://dx.doi.org/10.1089/aid.2006.22.709>.