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Evaluation of the Prototype Roche DNA Amplification Kit Incorporating the New SSK145 and SKCC1B Primers in Detection of Human Immunodeficiency Virus Type 1 DNA in Zimbabwe

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We assessed the sensitivity and specificity of a newly developed DNA PCR kit (Roche Diagnostic Corporation, Indianapolis, Ind.) that incorporates primers for all the group M viruses for the detection of human immunodeficiency virus (HIV) type 1 (HIV-1) infection in Zimbabwe. A total of 202 whole-blood samples from adults whose HIV status was known were studied. This included 100 HIV-1-positive and 102 HIV-1-negative samples selected on the basis of concordant results obtained with two enzyme-linked immunosorbent assay kits. The prototype Roche DNA PCR assay had a 100% sensitivity for the detection of HIV-1 DNA and a specificity of 100%. We conclude that the new Roche DNA PCR kit is accurate for the detection of HIV DNA in Zimbabwean samples, in which HIV-1 subtype C dominates.

The use of PCR for the diagnosis of human immunodeficiency virus (HIV) infection has been hampered by a lack of suitable primers for non-subtype A or non-subtype B viruses (1, 2, 5, 6, 8, 11, 13, 16). Since HIV type 1 (HIV-1) is a highly variable virus, its detection by PCR is greatly dependent on the ability of the chosen primers to detect all strains (9). The global variation in the reported rates of mother-to-child transmission of HIV-1 (3, 17) and the timing of the infection have been partially attributed to the lack of a standardized PCR protocol for the detection of all HIV strains in different regions of the world (4). The development and commercialization of a standardized PCR assay with universal primers for the detection of all HIV-1 strains will be useful for investigation of the timing and rates of mother-to-child transmission of HIV-1, assessment of therapeutic interventions aimed at reducing this transmission, and, in general, monitoring of the course and pathophysiology of HIV-1 infection.

We previously used an in-house PCR assay to diagnose HIV infection in infants under the age of 2 years using primers based on the consensus subtype C *gag* gene sequence (18). This PCR system, while sensitive and specific for the dominant subtype C virus in Zimbabwe (7, 14), suffers the disadvantage of being a manual procedure with the inherent problems of labor intensity and relatively high chances of obtaining false-positive and -negative results which may be attributable to several manual manipulations of the samples. Thus, this method would not be suitable for a large clinical trial that generates thousands of samples.

Roche Molecular Systems (Roche Diagnostic Corporation, Indianapolis, Ind.) recently introduced a modified PCR kit for the detection of HIV-1 DNA in peripheral blood mononuclear cells. The modified kit uses a new prototype primer pair system that incorporates all the group M viruses. The main objective of the present study was to investigate the sensitivity and specificity of the new kit with whole blood from asymptomatic HIV-1-seropositive and HIV-seronegative mothers immediately postpartum.

MATERIALS AND METHODS

Whole blood in EDTA was obtained immediately postpartum from women enrolled in an ongoing clinical trial which seeks to assess the effect of vitamin A supplementation on the transmission of HIV. The study, called Zimbabwe Vitamin A for Mothers and Their Babies (ZVITAMBO), plans to recruit 14,000 mother-baby pairs. The main objectives of this study are to test the efficacy of maternal-neonatal vitamin A supplementation in the immediate postpartum period on (i) infant mortality, (ii) mother-to-child transmission of HIV during breast-feeding, and (iii) incidence of HIV infection during the first postpartum year in women not infected at the time of delivery. All women gave informed consent for HIV testing under a protocol approved by the Medical Research Council of Zimbabwe.

The HIV status of the cohort was assessed with the Murex (which detects HIV antibodies to recombinant proteins containing HIV-1 and HIV-2 core and envelope antigens and which is manufactured by Murex Diagnostics, Johannesburg, South Africa) and the GeneScreen (which detects HIV-1 or HIV-2 antibodies to purified HIV-1 recombinant antigens [glycoprotein 160 and p25] and a peptide that mimics the immunodominant epitope of the HIV-2 envelope protein, respectively, and which is manufactured by Sanofi Diagnostics Pasteur PRx, Johannesburg, South Africa) enzyme-linked immunosorbent assay (ELISA) kits by following the manufacturers' instructions. Only samples from women who had concordant enzyme-linked immunosorbent assay results by the two ELISAs were selected for use in the evaluation of the prototype Roche DNA PCR kit. The use of two concordant ELISA results as the standard for diagnosis of HIV infection in adults is in accordance with World Health Organization recommendations, whereby only discordant results with two independent ELISA kits would require retesting by the Western blot assay as the "gold standard" to resolve the discordant ELISA results (12, 15).

The evaluation study comprised a total of 202 subjects; 100 of these women were HIV-1 positive, while 102 were HIV-1 negative. DNA was extracted with the Amplicor Whole Blood Specimen Preparation Kit (Roche Diagnostic Corporation) according to the manufacturer's instructions. Briefly, 500 μl of whole blood in EDTA was added to 1 ml of Specimen Wash Solution in a 1.5-ml Sarstedt screw-cap tube, and the contents were mixed by inversion of the tube; this was followed by a 5-min incubation at room temperature, inversion of the

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TABLE 1. OD_{450}s for HIV-1-positive and -negative samples as determined by PCR

OD_{450}	No. of samples with HIV status	
	Negative	Positive
0.055–0.064	9	
0.065–0.074	26	
0.075–0.084	41	
0.085–0.134	26	
3.028–3.373		3
3.534–3.555		2
3.662–3.999		23
>3.999		72
Total	102	100

tube, and a further 5 min of incubation at room temperature. The tubes were centrifuged at 12,500 × *g* at room temperature for 3 min. The resultant cell pellet was washed three times with Specimen Wash Solution, and the DNA was immediately extracted from the dry pellet obtained after the final wash, and the extract was subsequently amplified and detected. The cell pellets can be stored at −70°C indefinitely until future DNA extraction, amplification, and detection (Roche Diagnostic Corporation). DNA was extracted from the cell pellet by adding 200 μl of Working Extraction reagent, which contained 600 copies of internal control DNA per ml, and the tubes were then incubated for 30 min at 60°C, followed by a further 30-min incubation at 100°C. The samples were mixed briefly by vortexing and were then centrifuged at 12,500 × *g* for 3 s. Amplification and detection were performed with the Amplicor HIV-1 Amplification and Detection kits (Roche Diagnostic Corporation) by following the manufacturer's instructions. Briefly, amplification was performed in 100-μl reaction mixtures containing 50 μl of DNA and 50 μl of a master mixture consisting of dATP, dCTP, dGTP, dUTP, Amperase, AmpliTaq, salts, and biotinylated primers SSK145 (5′-AGTGGGGGGACATCAAGCAGCCATGCAAAT-3′) and SKCC1B (5′-TACTAGTAGTTCCTGCTATGTCACTTCC-3′) (Amplicor HIV-1 Amplification Kit; Roche Diagnostic Corporation). Amplifications were performed in a Perkin-Elmer TC9600 thermal cycler by using the following profile: 50°C for 2 min and then 5 cycles at 95°C for 10 s, 55°C for 10 s, and 72°C for 10 s and 35 cycles at 90°C for 10 s, 55°C for 10 s, and 72°C for 10 s. Finally, the sample was held at 72°C for 15 min. The samples were immediately denatured by adding an equal volume of denaturation solution. The denatured amplicons were then analyzed by adding 25 μl of each reaction mixture to 100 μl of hybridization solution contained in individual wells precoated with amplicon detection probes SK102 and CTG for the test sample and the DNA internal control (IC), respectively. After incubation at 37°C for 60 min, the plates were washed with an automated microwell plate washer (Bio-Tek Instruments, Montreal, Quebec, Canada) and were reincubated with 100 μl of an avidin-horseradish peroxidase conjugate per well. Following a further washing to remove unbound conjugate, 100 μl of chromogenic substrate (tetramethylbenzidine) was added to each well, and the plates were placed in the dark for 10 min at room temperature. The color reaction was stopped by the addition of 100 μl of stop reagent, and the plate was read on a microwell plate reader (Bio-Tek Instruments) at a wavelength of 450 nm. Samples with optical densities (ODs) greater than 0.8 were considered positive for HIV-1. Samples were considered negative for HIV-1 if the OD at 450 nm (OD_{450}) was less than 0.2 and that of the DNA IC was greater than 0.2. For specimens with OD_{450} values in the gray zone (equal to or greater than 0.2 and less than 0.8), the manufacturer recommends duplicate repeat testing of the processed specimen regardless of the IC OD_{450} result. For final interpretation of repeat test results, (i) a sample is considered positive for HIV-1 if at least one of the repeat tests has an OD_{450} equal to or greater than 0.2, but the IC OD_{450} result can be any value; (ii) a sample is presumed to be negative for HIV-1 if the results of both repeat tests for HIV-1 have OD_{450} values less than 0.2 and the results of both repeat tests for IC have OD_{450} values greater than 0.2; and (iii) if the results of both repeat tests for HIV-1 have OD_{450} values less than 0.2 and at least one repeat test result for IC has an OD_{450} less than 0.2, the sample is considered indeterminate for HIV-1 (Roche Diagnostic Corporation). Appropriate positive and negative controls provided by the manufacturer were included in all assays.

RESULTS

As shown in Table 1, all 100 samples confirmed to be HIV-1 positive by ELISA had very strong positive results by PCR. Seventy two of 100 (72%) of the samples had OD_{450} values above the detection limit of the microwell plate reader, while only 5 of 100 (5%) of the samples had OD_{450} values between 3.0 and 3.6; the remaining 23 of 100 (23%) samples had OD_{450} values that ranged from 3.662 to 3.999. Similarly, all samples negative for HIV by ELISA were PCR negative. All of the ELISA-confirmed HIV-1-negative samples had OD_{450}s below 0.134 (Table 1). Thus, the sensitivity and specificity of this assay for the 202 samples tested were both 100%, with the positive predictive value and the negative predictive value both being 100%.

DISCUSSION

We and others, using the heteroduplex mobility assay, have previously shown that subtype C is the dominant HIV-1 virus in Zimbabwe (7, 14).

The prototype Roche HIV DNA kit showed 100% sensitivity and 100% specificity (compared to a gold standard of two concordant ELISA results) when it was used to assess its suitability for detection of HIV infection in samples from Zimbabwe, where subtype C virus dominates (7, 14).

In summary, the new Roche microwell assay (version 1.1) for the detection of HIV-1 DNA is straightforward, is easy to follow, and involves very few manipulations. Not only is it semiautomated, thereby reducing the chances of false-positive results, but it also incorporates the enzyme uracil-*N*-glycosylase, which catalyzes the destruction of amplicons from previous runs, further reducing the chances of false-positive results arising from carryover contamination. The kit is useful for studies involving large numbers of samples, such as in clinical trials in which thousands of samples must be analyzed for the presence of HIV-1 DNA. The major constraint of the assay when used in such studies is its expense. Whole-blood samples need to be washed within 4 days of collection, as there is a loss of signal if the samples are stored as whole blood at 4°C for more than 4 days, particularly those samples that may be initially weakly PCR positive (10a). The ongoing clinical trial of vitamin A is intended to recruit 14,000 mother-baby pairs. At the current 30% prevalence of HIV infection among recruited mothers, approximately 4,700 babies will be at risk of vertical transmission of HIV. PCR of archived samples collected at birth, at 6 weeks of age, and thereafter at 3-month intervals will be used retrospectively to determine timing of infection among babies who test ELISA positive at age 2 years. Although this approach reduces the number of samples to be analyzed by PCR, all whole-blood samples will be washed within 4 days of collection, which in itself is an expensive and labor-intensive exercise.

Since we found the Roche kit to be very sensitive and specific for the detection of subtype C virus in Zimbabwe (7, 15), there is a need to conduct large-scale global evaluations to assess its performance with the rest of the group M viruses. Its ability to detect HIV-1 infection globally will prove useful in comparisons of rates of mother-to-child transmission of the virus as well as the timing of this transmission. Recently, Respess and colleagues (10) evaluated the prototype Roche DNA PCR assay in comparison with the standard Roche Amplicor HIV-1 test and a modified version of the latter test using a panel of 136 genetically diverse group M and O virus isolates from adults from outside the United States and Europe. Interestingly, they reported 100% sensitivity for detection of subtype A, B, C, D, E, and G viruses and 92% sensitivity for detection of subtype F viruses with the prototype kit. However, those investigators had only one subtype C virus-infected sample from South America.

In conclusion, the prototype Roche DNA PCR kit was accurate in the detection of HIV DNA in samples from adults from Zimbabwe, where subtype C virus dominates (14). It

remains to be established if this assay will be equally sensitive in the early detection of HIV infection in infants with low numbers of viral particles.

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REFERENCES

1. **Borkowsky, W., K. Krasinski, H. Pollack, W. Hoover, A. Kaul, and T. Ilmet-Moore.** 1992. Early diagnosis of human immunodeficiency virus infection in children <6 months of age: comparison of polymerase chain reaction, culture, and plasma antigen capture techniques. J. Infect. Dis. **166:**616–619.
2. **Chadwick, E. G., R. Yogev, S. Kwok, J. J. Snisky, D. E. Kellogg, and S. M. Wolinsky.** 1989. Enzymatic amplification of the human immunodeficiency virus in peripheral blood mononuclear cells from pediatric patients. J. Infect. Dis. **160:**954–959.
3. **Dabis, F., P. Msellati, D. Dunn, P. Lepage, M.-L. Newell, C. Peckham, P. Van de Perre, and the Working Group on Mother-to-Child Transmission of HIV.** 1992. Estimating the rate of mother to child transmission of HIV. Report of a workshop on methodological issues in Ghent (Belgium), 17–20 February. AIDS **7:**1139–1148.
4. **Dunn, D., M. L. Newell, A. E. Ades, and C. S. Peckam.** 1992. Risk of human immunodeficiency virus type I transmission through breastfeeding. Lancet **340:**585–588.
5. **Edwards, J. R., P. P. Ulrich, P. S. Weintrub, M. J. Cowan, J. A. Levy, D. W. Wara, and G. N. Vyas.** 1989. Polymerase chain reaction compared with concurrent viral cultures for rapid identification of human immunodeficiency virus infection among high-risk infants and children. J. Pediatr. **115:**200–203.
6. **Esaich, S., M. Wallon, I. Bagniski, J. Ritter, N. Philippe, Y. Bertrand, O. Claris, D. Raudrant, M. Sepetjan, and C. Trepo.** 1991. Comparison of HIV detection by virus isolation in lymphocyte cultures and molecular amplification of HIV DNA and RNA by PCR in offspring of seropositive mothers. J. Acquir. Immune Defic. Syndr. **4:**130–135.
7. **Hu, D. J., T. J. Dondero, M. A, Rayfield, J. R. George, G. Schochetman, H. W. Jaffe, C. Luo, M. L. Kalish, B. G. Weniger, C. P. Pau, C. A. Schable, and J. W. Curran.** 1996. The emerging genetic diversity of HIV. JAMA **275:**210–216.
8. **Krivine, A., A. Yakudima, M. LeMay, V. Pena-Cruz, A. S. Huang, and K. McIntosh.** 1990. A comparative study of virus isolation, polymerase chain reaction, and antigen detection in children of mothers infected with human immunodeficiency virus. J. Pediatr. **116:**372–376.
9. **McCutchan, F. E., E. Sanders-Buell, C. W. Oster, R. R. Redfield, S. K. Hira, P. L. Perine, B. L. Ungar, and D. S. Burke.** 1991. Genetic comparison of human immunodeficiency virus (HIV) isolates by polymerase chain reaction. J. Acquir. Immune Defic. Syndr. **4:**1241–1250.
10. **Respess, R. A., A. Butcher, H. Wang, T. Chaowanachan, N. Young, N. Shafer, T. D. Mastro, B. Biryahwaho, R. Downing, A. Tanuri, M. Schechter, R. Pascu, L. Zekeng, L. Kaptue, L. Gurtler, J. Eberle, D. Ellenberger, C. Fridlund, M. Rayfield, and S. Kwok.** 1997. Detection of genetically diverse human immunodeficiency virus type 1 group M and O isolates by PCR. J. Clin. Microbiol. **35:**1284–1286.

10a. **Roche Diagnostics.** Personal communication.

11. **Rogers, M. F., C.-Y. Ou, M. Rayfield, P. A. Thomas, E. E. Schoenbaum, E. Abrams, K. Krasinski, P. A. Selwyn, J. Moore, A. Kaul, K. T. Grimm, M. Bamji, and G. Schochetman.** 1989. Use of polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. N. Engl. J. Med. **320:**1649–1654.
12. **Sato, P. A., W. J. Maskill, H. Tamashiro, and D. L. Heymann.** 1994. Strategies for laboratory HIV testing: an examination of alternative approaches not requiring Western blot. Bull. W.H.O. **72:**129–134.
13. **Scarlatti, G., V. Lombard, A. Plebani, N. Principi, C. Vegin, G. Ferraris, A. Bucceri, E. M. Fenyo, H. Wigzell, P. Rossi, and J. Albert.** 1991. Polymerase chain reaction, virus isolation and antigen assay in HIV-1 antibody positive mothers and their children. AIDS **5:**1173–1178.
14. **Tien, P. C., T. Chiu, A. Latif, S. Ray, M. Batra, C. H. Contag, L. Zijenah, M. Mbizvo, E. L. Delwart, J. I. Mullins, and D. Katzenstein.** 1999. Primary subtype C HIV-1 infection in Harare, Zimbabwe. J. Acquir. Immune Defic. Hum. Retrovirol. **20:**147–153.
15. **Van der Groen, G., I. Van kerchoven, G. Vercauteren, and P. Piot.** 1991. Simplified and less expensive confirmatory HIV testing. Bull. W. H. O. **69:**747–752.
16. **Williams, P., P. Simmonds, P. L. Yap, P. Balfe, J. Bishop, R. Brettle, R. Hague, D. Hargeaves, J. Inglis, A. L. Brown, J. Puetherer, S. Rebus, and J. Mok.** 1990. The polymerase chain reaction in the diagnosis of vertically transmitted HIV infection. AIDS **4:**393–398.
17. **The Working Group on Mother-to-Child Transmission of HIV.** 1995. Rates of mother to child transmission of HIV in Africa, America, and Europe: results from 13 perinatal studies. J. Acquir. Immune Defic. Hum. Retrovir. **8:**506–510.
18. **Zijenah, L., M. T. Mbizvo, J. Kasule, K. Nathoo, K. Mahomed, M. Munjoma, S. Madzime, Y. Maldonado, and D. Katzenstein.** 1998. Mortality in the first two years among infants born to HIV-infected women in Harare, Zimbabwe. J. Infect. Dis. **178:**109–113.