NOTES

Diagnosis of Human Immunodeficiency Virus Type 1 Infection with Different Subtypes Using Rapid Tests

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We evaluated six rapid tests for their sensitivity and specificity in diagnosing human immunodeficiency virus type 1 (HIV-1) infection using 241 specimens (172 HIV-1 positive, 69 HIV-1 negative) representing different HIV-1 subtypes (A [$n = 40$], B [$n = 47$], C [$n = 28$], E [$n = 42$], and F [$n = 7$]). HIVCHEK, Multispot, RTD and SeroStrip were 100% sensitive and specific. Capillus failed to identify two of eight subtype C specimens (overall sensitivity of 98.85%), while the SUDS test (the only test approved by the Food and Drug Administration) gave false-positive results for 5 of 69 seronegative specimens (specificity of 93.24%). Our results suggest that although rapid tests perform well in general, it may be prudent to evaluate a rapid test for sensitivity and specificity in a local population prior to its widespread use.

Simple and rapid tests are gaining importance in diagnosing human immunodeficiency virus type 1 (HIV-1) infection, and their sensitivity and specificity are similar to those of the standard enzyme immunoassay (EIA) and Western blot (WB) algorithm (2, 3, 12, 13, 15, 16, 18). The use of rapid tests can result in significant shortening of reporting time and is cost-effective in many settings (1, 4, 6, 10, 11, 14, 17, 19). Due to simplicity and short turnaround time, these tests are increasingly used in developing countries where non-B subtype viruses predominate. However, most of these tests employ recombinant proteins or synthetic peptide antigens derived from subtype B viruses. Therefore, there is a concern that use of rapid tests with a limited antigen set (e.g., a synthetic peptide) may compromise sensitivity and specificity.

The immunodominant gp41 site (9) is the most commonly used region for designing peptide-based assays. Although it is quite conserved, changes in key amino acids in this region do occur and could result in low-affinity binding and missed diagnoses. In fact, using our in-house gp41 peptide-based EIA, occasional subtype D infections from Uganda were not detected (unpublished data). Rapid tests, by nature, employ short incubation times which could further compromise their performance. Failure of rapid tests to diagnose HIV-1 infection could have adverse consequences with regard to counseling, treatment, and prevention of transmission and could also discourage their widespread use for surveillance or diagnosis.

The goal of this study was to evaluate the performance of a number of rapid tests using serum specimens from individuals infected with different HIV-1 subtypes. The panel ($n = 241$) consisted of unlinked specimens from 172 HIV-1-infected and 69 uninfected individuals. HIV status was confirmed by the EIA-WB algorithm. All 172 specimens from HIV-1-infected people were repeatedly reactive on EIA and were WB positive.

Seropositive specimens included HIV-1 subtypes A ($n = 40$), B ($n = 47$), C ($n = 8$), D ($n = 28$), E ($n = 42$), and F ($n = 7$). The specimens were acquired from various global locales in Asia, Africa, and the Americas. They were selected for this study because they were available in the volume needed for this evaluation (at least 0.5 ml). The subtype was determined by V3 sequence analysis or by V3 peptide-specific serology (5) when sequencing information was not available. Six rapid tests were evaluated: SUDS HIV-1 test (Murex Corporation, Norcross, Ga.; Food and Drug Administration-approved test), Capillus HIV-1/2 (Cambridge Diagnostics, Galloway, Ireland), HIVCHek 1+2 (Ortho Diagnostic Systems, Raritan, N.J.), Multispot HIV-1/HIV-2 (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France), Recombigen HIV-1/HIV-2 RTD (Cambridge Diagnostics), and SeroStrip HIV1/2 (Saliva Diagnostic Systems, Vancouver, Wash.). SUDS, Multispot, RTD, and HIVChek are flow-through devices with antigen applied to the membrane. Capillus is a latex agglutination test, while SeroStrip is in a dipstick format. All the testing was done singly, and appropriate positive and negative controls were included in each run. The tests were conducted and interpreted by experienced technicians.

Results of our study are summarized in Table 1. Five of six tests evaluated were 100% sensitive in detecting HIV-1 antibodies in all 172 seropositive specimens. Capillus HIV-1/2, a capillary agglutination assay, missed two of eight subtype C specimens, with an overall sensitivity of 98.85%. Interestingly, RTD, a flowthrough device that uses the same antigen (recombinant envelope protein) as Capillus, identified all eight subtype C specimens. This suggests that the format of the assay can be important in determining performance of the assay. Specificity of all the assays, except that of SUDS, was 100%. SUDS was positive for 5 of 69 confirmed seronegative individuals, resulting in a specificity of 93.24%. Higher proportions of WB-indeterminate sera and of EIA-positive WB-negative sera in certain parts of the world may further impact the specificity of the rapid tests.

Our data suggest that most of these tests are suitable for

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TABLE 1. Sensitivity and specificity of rapid tests with specimens of different HIV-1 subtypes

Subtype (no. of specimens)	No. with rapid test result[a]											
	Capillus		HIVChek		Multispot		RTD		SeroStrip		SUDS	
	P	N	P	N	P	N	P	N	P	N	P	N
A (40)	40	0	40	0	40	0	40	0	40	0	40	0
B (47)	47	0	47	0	47	0	47	0	47	0	47	0
C (8)	6	2	8	0	8	0	8	0	8	0	8	0
D (28)	28	0	28	0	28	0	28	0	28	0	28	0
E (42)	42	0	42	0	42	0	42	0	42	0	42	0
F (7)	7	0	7	0	7	0	7	0	7	0	7	0
Negative (69)	0	69	0	69	0	69	0	69	0	69	5	64
Total (241)	171	71	172	69	172	69	172	69	172	69	177	64
Sensitivity (%)	98.85		100		100		100		100		100	
Specificity (%)	100		100		100		100		100		93.24	

[a] P, positive; N, negative.

diagnosis of HIV-1 infection in individuals infected with divergent subtypes. However, as observed with Capillus HIV-1/2, variants of certain subtypes could lead to misdiagnosis. A study by Constantine et al. (7) reported successful identification of all HIV-1 group M subtype sera by seven rapid tests, although only 21 sera of different subtypes were tested. Another evaluation of seven rapid tests similarly showed problems in detecting antibodies from patients infected with subtype C or D (8). Lower sensitivity of rapid tests with certain subtypes could adversely affect their use in screening and confirmatory-testing algorithms. Generation of recombinant viruses and continuing genetic variation in HIV-1 may pose additional diagnostic challenges, especially for performance of rapid tests. Actual sensitivity and positive and negative predictive values may be affected by the relative distribution of various subtypes in a region. Moreover, whether detection of antibodies during the early phase of infection will be equivalent using rapid tests with different subtypes remains to be seen. Commercially available seroconversion panels are all of subtype B, and no such panels are available for non-B subtypes.

Ongoing evaluation of rapid tests using specimens from recently infected individuals in different parts of the world may be necessary. The performance of these tests in detecting antibodies to group O viruses and to HIV-2 also needs to be investigated, especially if these tests are to be used in areas with a high prevalence of infection with these virus types.

In summary, the use of a rapid test in a specific geographic area should be validated to ensure that the test is adequately sensitive to circulating HIV-1 subtypes. Four of the six rapid tests evaluated were found to be 100% sensitive and specific, thus providing assurance that rapid tests can be used in diagnosis of infection with various HIV-1 subtypes.

REFERENCES

1. Asihene, P. J., R. L. Kline, M. W. Moss, A. V. Carella, and T. C. Quinn. 1994. Evaluation of rapid test for detection of antibody to human immunodeficiency virus type 1 and type 2. J. Clin. Microbiol. 32:1341–1342.
2. Behets, F., K. Bishagara, A. Disasi, S. Likin, R. W. Ryder, C. Brown, and T. C. Quinn. 1992. Diagnosis of HIV infection with instrument-free assays as an alternative to ELISA and Western blot testing strategy: an evaluation in Central Africa. J. Acquir. Immune Defic. Syndr. 5:878–882.
3. Brattegaard, K., J. Kouadio, M.-L. Adom, R. Doorly, J. R. George, and K. M. De Cock. 1993. Rapid and simple screening and supplemental testing for HIV-1 and HIV-2 infections in West Africa. AIDS 7:883–885.
4. Burgess-Cassler, A., G. B. Angulo, S. E. Wade, P. C. Torres, and W. Schramm. 1996. A field test for the detection of antibodies to human immunodeficiency virus types 1 and 2 in serum or plasma. Clin. Diagn. Lab. Immunol. 3:480–482.
5. Cheingsong-Popov, R., S. Osmanov, C.-P. Pau, G. Schochetman, F. Barin, H. Holmes, G. Francis, H. Ruppach, U. Deitrich, S. Lister, and J. Weber. 1998. Serotyping of HIV-1 infections: definition, relationship to viral genetic subtypes, and assay evaluation. AIDS Res. Hum. Retrovir. 14:311–318.
6. Conradie, J. D., and D. R. Tait. 1992. SimpliRED—a rapid and reliable test for HIV. S. Afr. Med. J. 81:624–625.
7. Constantine, N. T., L. Zekeng, A. K. Sangare, L. Gurtler, R. Saville, H. Anhary, and C. Wild. 1997. Diagnostic challenges for rapid human immunodeficiency virus assays: performance using HIV-1 group O, HIV-1 group M, and HIV-2 samples. J. Human Virol. 1:45–51.
8. Engelbrecht, S., G. J. de Jager, and J. van Rensburg. 1994. Evaluation of commercially available assays for antibodies to HIV-1 in serum obtained from South African patients infected with HIV-1 subtypes B, C, and D. J. Med. Virol. 44:223–228.
9. Gnann, J. W., J. B. McCormick, S. Mitchell, J. A. Nelson, and M. B. Oldstone. 1987. Synthetic peptide immunoassay distinguishes HIV-1 and HIV-2 infections. Science 237:1346–1349.
10. Kassler, W. J., C. Haley, W. K. Jones, A. R. Gerber, E. J. Kennedy, and J. R. George. 1995. Performance of a rapid, on-site human immunodeficiency virus antibody assay in a public health setting. J. Clin. Microbiol. 33:2899–2902.
11. Kelen, G. D., T. A. Bennecoff, R. Kline, G. B. Green, and T. C. Quinn. 1991. Evaluation of two rapid screening assays for the detection of human immunodeficiency virus-1 in infection in emergency department patients. Am. J. Emerg. Med. 9:416–420.
12. Malone, J. D., E. S. Smith, J. Sheffield, D. Bigelow, K. C. Hyams, S. G. Beardsley, R. S. Lewis, and C. R. Roberts. 1993. Comparative evaluation of six rapid serological tests for HIV-1 antibody. J. Acquir. Immune Defic. Syndr. 6:115–119.
13. Mortimer, J. 1992. An alternative approach to confirming anti-HIV reactivity: a multi-country collaborative study. Bull. W. H. O. 70:751–756.
14. Pagcharoenpol, P., A. Burgess-Cassler, and W. Schramm. 1996. Simplified testing for antibodies to human immunodeficiency virus. J. Clin. Microbiol. 34:973–974.
15. Stetler, H. C., T. C. Granade, C. A. Nunez, R. Meza, S. Terrell, L. Amador, and J. R. George. 1997. Field evaluation of rapid HIV serologic tests for screening and confirming HIV-1 infection in Honduras. AIDS 11:369–375.
16. Urassa, W. K., U. Bredberg-Raden, E. Mbena, K. Palsson, E. Minja, R. A. Lema, K. Pallangyo, R. S. Mhalu, and G. Biberfeld. 1992. Alternative confirmatory strategies in HIV-1 antibody testing. J. Acquir. Immune Defic. Syndr. 5:170–176.
17. Vercauteren, G., G. Beelaert, and G. van der Groen. 1995. Evaluation of an agglutination HIV-1+2 antibody assay. J. Virol. Methods 51:1–8.
18. Weekly Epidemiological Record. 1998. The importance of simple/rapid assays in HIV testing. WHO/UNAIDS Recommendations. Wkly. Epidemiol. Rec. 73:321–328.
19. Wilkinson, D., N. Wilkinson, C. Lombard, D. Martin, A. Smith, K. Floyd, and R. Ballard. 1997. On-site HIV testing in resource-poor settings: is one rapid test enough? AIDS 11:377–381.