JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1992, p. 2509–2510
0095-1137/92/092509-02$02.00/0

Vol. 30, No. 9

Evaluation of an Indirect Immunofluorescence Assay for Confirmation of Human Immunodeficiency Virus Type 1 Antibody in U.S. Blood Donor Sera

MARIAN T. SULLIVAN,[1]* HERMANN MUCKE,[2] SCOTT D. KADEY,[1] CHYANG T. FANG,[1] AND ALAN E. WILLIAMS[1]

American Red Cross Holland Laboratory, Rockville, Maryland 20855,[1] and Waldheim Pharmazeutika, Vienna, Austria[2]

Received 13 March 1992/Accepted 8 June 1992

An indirect immunofluorescence assay (IFA) was evaluated as a confirmatory test for antibody to human immunodeficiency virus type 1 in U.S. blood donor sera previously found to be repeatedly reactive by enzyme immunoassay. IFA results were 100% concordant with a licensed Western blot (immunoblot) for 53 negative and 49 positive samples. Four samples which exhibited antibody to viral proteins from more than one gene, yet were indeterminate by Western blot by the manufacturer's criteria, were also reactive by IFA, whereas 49 additional indeterminate samples were IFA negative.

The most common initial laboratory step in human immunodeficiency virus type 1 (HIV-1) antibody detection is the enzyme immunoassay (EIA), which, despite its high specificity, has a low positive predictive value when used to screen large populations with a very low prevalence of infection (2). Thus, specimens repeatedly reactive by EIA must always be confirmed by testing with a supplemental assay, typically the Western blot (WB) (immunoblot), which results in a diagnostic pattern of bands that is more specific than the EIA for antibodies to HIV-1 viral proteins.

Prior to U.S. Food and Drug Administration licensure of the Fluorognost HIV-1 Indirect Immunofluorescence Assay (IFA) on 5 February 1992, the WB had been the sole Food and Drug Administration-licensed HIV confirmatory test since 1987. However, some drawbacks of WB include the continuing controversy over interpretation, lot-to-lot variability (6), the need for overnight incubation, relatively high cost, and frequent occurrence of indeterminate band patterns.

Supplemental tests that employ other methodologies include the radioimmunoprecipitation assay (RIPA), which is technically difficult and best suited to research laboratories, and the IFA. Several studies have reported that IFA is a sensitive and specific method for screening and/or confirming HIV EIA reactive sera (3–5). IFA testing allows the user to quickly visualize a result by microscopy and immediately distinguish specific from nonspecific fluorescence by comparing stained cells with uninfected control cells.

This evaluation provides performance data for a broad geographical sample of a low risk U.S. population. The evaluation panel consisted of unselected HIV-1 EIA repeatedly reactive sera or plasma submitted sequentially by American Red Cross Blood Services Regions to the American Red Cross reference laboratory for HIV confirmatory testing. The samples were collected during the period from 27 September to 1 November 1991, were stored at 4°C, and were never frozen prior to being tested.

Immunofluorescence testing was performed with the Fluorognost HIV-1 IFA (Waldheim Pharmazeutika, Vienna, Austria) according to the package insert. Stained slides were viewed on a Nikon Optiphot fluorescence microscope at a magnification of ×400. The EIA procedure used by the regional blood centers was the HIV-1 EIA (Abbott Laboratories, North Chicago, Ill.). WB testing was performed with the Food and Drug Administration-licensed Cambridge-Biotech HIV-1 Western Blot Kit (distributed by Ortho Diagnostic Systems, Raritan, N.J.). Samples were interpreted as WB positive when they exhibited antibody to p24, p31, and either gp41 or gp120/160. Samples displaying any pattern of viral or nonviral bands which did not meet the criteria for positivity were classified as indeterminate. Samples designated as negative had no bands detectable on WB. On the basis of these criteria, the coded panel included 53 serum samples classified as WB indeterminate, 53 classified as negative, and 49 samples classified as WB positive.

RIPAs (Advanced BioScience Laboratories, Inc., Rockville, Md.) were performed on all WB-indeterminate samples plus four WB-positive and three WB-negative controls under code. Viral antigens were produced in HIV-1-infected Molt-3 cells and labelled with [^{35}S]methionine (7). RIPA reactivity was based on visible bands corresponding to p24, p39 (intermediate Gag precursor), p53 (Gag precursor), gp120, and gp160.

The IFA detected HIV-1 antibody in all 49 WB-positive samples (sensitivity, 100%). Each of 53 WB-negative samples from EIA repeatedly reactive blood donors was IFA nonreactive. All of the IFA-positive samples produced a clear fluorescence signal that could be readily interpreted as positive. The IFA-negative samples were easily interpreted as well, with little or no nonspecific staining. Figure 1 illustrates the typical fluorescence pattern for HIV-infected cells incubated with HIV-1 antibody-positive sera.

Table 1 summarizes the results for all samples tested by IFA and shows the distribution of the indeterminate samples according to the WB band patterns represented. Of the 53 samples classified as WB indeterminate, 49 were IFA negative and 4 were IFA positive. The four samples found to be IFA positive but WB indeterminate had insufficient reactivity to be classified as WB positive by the manufacturer's criteria; however, each would be considered positive by current Centers for Disease Control and Association of State

* Corresponding author.

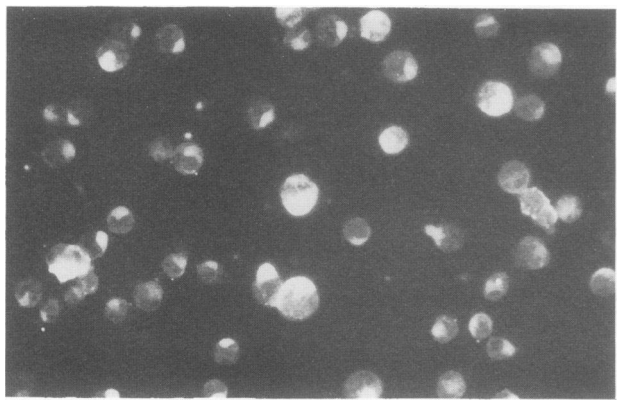

FIG. 1. Typical Fluorognost HIV-1 IFA staining pattern observed with HIV-infected Pall cells incubated with low-titer HIV-1 antibody-positive serum. Magnification, ×400. (Photo was overexposed to increase cellular details.)

and Territorial Public Health Laboratory Directors WB criteria (any two of three bands: p24, gp41, and gp120/160) (1). In addition to the displayed reactivity on WB, the first case also showed evidence of p24, p53, gp120, and gp160 and the second case showed evidence of p53, gp120, and gp160 on RIPA. The third case had weak reactivity to gp120 and gp160, while the final case demonstrated no RIPA reactivity.

TABLE 1. Comparison of Fluorognost HIV-1 IFA with licensed WB for EIA repeatedly reactive blood donor sera

WB band(s)	Viral product(s) represented	WB interpretation[a]	No. of samples tested	IFA result[a]
24, 31, 41 (or 120/160) (at least)	Gag Pol Env	Pos	49	Pos
None	None	Neg	53	Neg
17	Gag	Ind	7	Neg[b]
24	Gag	Ind	16	Neg
55	Gag	Ind	2	Neg
51	Pol	Ind	1	Neg
66	Pol	Ind	7	Neg
17, 24	Gag	Ind	1	Neg
24, 55	Gag	Ind	1	Neg[b]
51, 66	Pol	Ind	2	Neg
120/160	Env	Ind	2	Neg
55, 66	Gag Pol	Ind	1	Neg
17, 55, 66	Gag Pol	Ind	1	Neg
24, 66	Gag Pol	Ind	1	Neg
24, 51	Gag Pol	Ind	1	Neg
24, 51, 66	Gag Pol	Ind	1	Neg
24, 120/160	Gag Env	Ind	1	Pos
41, 51, 120/160	Pol Env	Ind	1	Pos
17, 24, 51, 55, 120/160	Gag Pol Env	Ind	1	Pos
17, 24, 41, 51, 55, 66, 120/160	Gag Pol Env	Ind	1	Pos
Nonviral	None	Ind	5	Neg

[a] Pos, positive; Neg, negative; Ind, indeterminate.
[b] One sample from this group was IFA reactive initially but RIPA negative. When retested in duplicate by IFA, the sample was repeatedly negative and was so classified in the final analysis.

All RIPA results for WB-indeterminate samples were negative with the exception of three of the four IFA-positive samples noted above and two other samples which were IFA negative. Of the two IFA-negative samples, the first displayed only nonviral bands on WB and p39 and p53 on RIPA. The second had p51 and p66 WB reactivity and exhibited p24, p39, p53, weak gp120, and gp160 on RIPA. Both RIPAs were repeated with the same results. The four WB-positive samples included as RIPA controls all displayed bands at p24, p39, p53, gp120, and gp160.

Considering only those samples classified as either positive or negative by WB, the Fluorognost HIV-1 IFA displayed sensitivity and specificity equivalent to those of the licensed WB when evaluated in a low prevalence population. The IFA was superior to the WB with respect to ease of use and rapidity. Although some technical training was necessary in order to correctly interpret fluorescence patterns, differentiation of positive and negative results was not problematic because of the virtual absence of nonspecific staining.

One drawback of the procedure is the lack of an objective printout, allowing the transcribed test results to become the only permanent record. Photography is one convenient option, however, for laboratories requiring permanent evidence of results. In addition, running samples in duplicate may minimize the effect of errors in interpretation or transcription. Alternatively, the development of an objective image recognition system would help to circumvent this problem. Another limitation is the inability to differentiate the component(s) of the virus to which the serological response is targeted, which may be necessary for certain applications. In conclusion, the Fluorognost HIV-1 IFA is a straightforward, accurate assay which may be useful as a confirmatory test for HIV-1 infection in some laboratory settings.

REFERENCES

1. **Centers for Disease Control.** 1989. MMWR interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. Morbid. Mortal. Weekly Rep. **38:**1–7.
2. **Dodd, R. Y., and C. T. Fang.** 1990. The Western immunoblot procedure for HIV antibodies and its interpretation. Arch. Pathol. Lab. Med. **114:**240–245.
3. **Gallo, D., J. L. Diggs, G. R. Shell, P. J. Dailey, M. N. Hoffman, and J. L. Riggs.** 1986. Comparison of detection of antibody to the acquired immune deficiency syndrome virus by enzyme immunoassay, immunofluorescence, and Western blot methods. J. Clin. Microbiol. **23:**1049–1051.
4. **Lennette, E. T., S. Karpatkin, and J. A. Levy.** 1987. Indirect immunofluorescence assay for antibodies to human immunodeficiency virus. J. Clin. Microbiol. **25:**199–202.
5. **Mahony, J., K. Rosenthal, M. Chernesky, S. Castriciano, E. Scheid, M. Blajchman, and D. Harnish.** 1989. Agreement study between two laboratories of immunofluorescence as a confirmatory test for human immunodeficiency virus type 1 antibody screening. J. Clin. Microbiol. **27:**1234–1237.
6. **Midthun, K., L. Garrison, M. L. Clements, H. Farzadegan, B. Fernie, T. Quinn, and the NIAID AIDS Vaccine Clinical Trials Network.** 1990. Frequency of indeterminate Western blot tests in healthy adults at low risk for human immunodeficiency virus infection. J. Infect. Dis. **162:**1379–1382.
7. **Veronese, F. D., A. L. DeVico, T. D. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sarngadharan.** 1985. Characterization of gp41 as the transmembrane protein coded by the HTLV-III/LAV envelope gene. Science **229:**1402–1405.