JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1988, p. 371–374
0095-1137/88/020371-04$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 26, No. 2

# Comparable Sensitivities for Detection of Human Immunodeficiency Virus by Sensitive Reverse Transcriptase and Antigen Capture Enzyme-Linked Immunosorbent Assays

MOON H. LEE, KOUICHI SANO,† FATIMA E. MORALES, AND DAVID T. IMAGAWA*

*Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, California 90509*

Received 19 August 1987/Accepted 2 November 1987

**The sensitive overnight reverse transcriptase and the antigen capture enzyme-linked immunosorbent assays were tested for their ability to detect human immunodeficiency virus. The two assays were able to quantitate as few as 75 to 130 virus particles. Thus, both tests offer similar sensitivities for determining the presence of the virus.**

The study of human immunodeficiency virus (HIV) has increased rapidly since it is the etiologic agent associated with acquired immune deficiency syndrome. Numerous methods have been used to detect the virus. One widely used test assays for the reverse transcriptase (RT) of the virion (1, 6). We have recently reported a sensitive overnight RT assay that exploited the unusual stability of the HIV polymerase, allowing better detection of the virus (4). Various immunological tests have also been used for the detection of HIV. A sensitive method appears to be the use of specific antibody for the capture of viral antigen and its subsequent detection by enzyme-linked immunosorbent assay (ELISA) (5). We report here a comparison of these two sensitive tests and our findings that both have similar abilities to detect HIV.

The virus for our studies was prepared by growing an HIV isolate (human T-cell lymphotropic virus type III) (1) in a continuous T-cell line (CEM) and quantitated by counting stained viruslike particles in a Hitachi H-600 electron microscope (4). The RT assay was done at 37°C for 22 h by combining 50 μl of virus sample with 50 μl of a concentrated (2×) RT assay buffer (3). The 2× buffer was conveniently made up in a large volume, aliquoted, frozen at −20°C, and thawed just before use. Per 25 ml (500 assays), it consisted of 2.5 ml of 1 M Tris hydrochloride (pH 7.9), 1.5 ml of 0.2 M dithiothreitol, 0.5 ml of 60 mM reduced glutathione, 1.5 ml of 0.2 M $MgCl_2$, 4.0 ml of 2 M KCl, 2.5 ml of 10 mM ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 0.5 ml of 10% Triton X-100, 2.5 ml of 40% ethylene glycol, 2.5 ml of poly(rA · dT) template (10 U/ml), 2.0 ml of [$^3$H]TTP (New England Nuclear Research Products, Boston, Mass.; 2.5 mCi/ml in 10 mM Tricine buffer), and 5.0 ml of sterile distilled water. The chemicals were obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind., or Sigma Chemical Co., St. Louis, Mo. The antigen capture ELISA (AC ELISA) used commercially prepared kits (Abbott Laboratories, North Chicago, Ill., and Du Pont New England Nuclear) and was performed by solubilizing the virus sample in 0.5% Triton X-100 detergent and following the instructions for each kit. Both AC ELISAs are based on the same principle, using antibody to sandwich antigen for detection in a test sample. The Abbott Laboratories kit uses human anti-HIV antibody-coated polystyrene beads for virus antigen capture. Its subsequent detection is by rabbit anti-HIV antibody and goat anti-rabbit immunoglobulin G-horseradish peroxidase conjugate, with *O*-phenylenediamine as the substrate for the enzyme. For virus antigen capture, the Du Pont kit uses specific rabbit anti-HIV p24 antibody fixed in 96 wells of a microdilution plate. The detection system is biotinylated rabbit anti-HIV p24 antibody and streptavidin-horseradish peroxidase conjugate, with *O*-phenylenediamine as the substrate. Both manufacturers recommend an overnight incubation for the antigen capture step. The subsequent incubation times for the detection of antigen is 6.5 h for the Abbott assay and 1.75 h for the Du Pont assay. To semiautomate the test procedures, the Abbott system requires a dispensing pump for delivery of the rinse solution and an aspirator device for washing the beads contained in a plastic test tube; the Du Pont system requires a multichannel port manifold apparatus for dispensing buffer and washing the microdilution plate. Both require a spectrophotometer capable of reading $A_{492}$. For laboratories not set up for ELISA, the companies have been willing to loan the necessary equipment for use with their assay kits.

Identical samples of different dilutions of quantitated virus were assayed in duplicate by both the RT assay and AC ELISA. The sensitivity of the RT assay is shown in Fig. 1. As reported previously (4), the viral polymerase activity is linearly related to the number of virus particles (slope of 1.3 for a full logarithmic plot), and therefore virus can be quantitated from RT activity. The rate of [$^3$H]TMP incorporated into DNA product by $1.3 \times 10^2$ to $1.3 \times 10^4$ virus particles ranged from $1.2 \times 10^4$ to $2.5 \times 10^6$ cpm in the 22-h incubation period. The sensitivities of the AC ELISAs and the RT assay are shown in Table 1. The RT assay detected 130 virion particles. Thus, among the retroviruses, detection by this polymerase assay is most sensitive for HIV. In a previous study by Tereba and Murti (9), the minimum detectable number of various avian oncornaviruses ranged from 560 to $3 \times 10^6$. The AC ELISAs were slightly more sensitive, with both kits detecting 75 virus particles. With the viral lysate standard (200 ng of HIV p24 protein per ml) supplied with the Du Pont test kit, the amount of antigen detected for $7.5 \times 10^1$ to $1.3 \times 10^4$ virions ranged from 0.04 to 12.2 ng/ml. The viral antigen measured is linearly related to virus particle number, and therefore the number of virions can be determined from the optical density (OD) reading (Fig. 2). Thus, this immunological assay is capable of detecting low numbers of virus particles and low levels of viral

* Corresponding author.
† Present address: Department of Microbiology, Osaka Medical College, Takatsuki, Osaka, Japan.

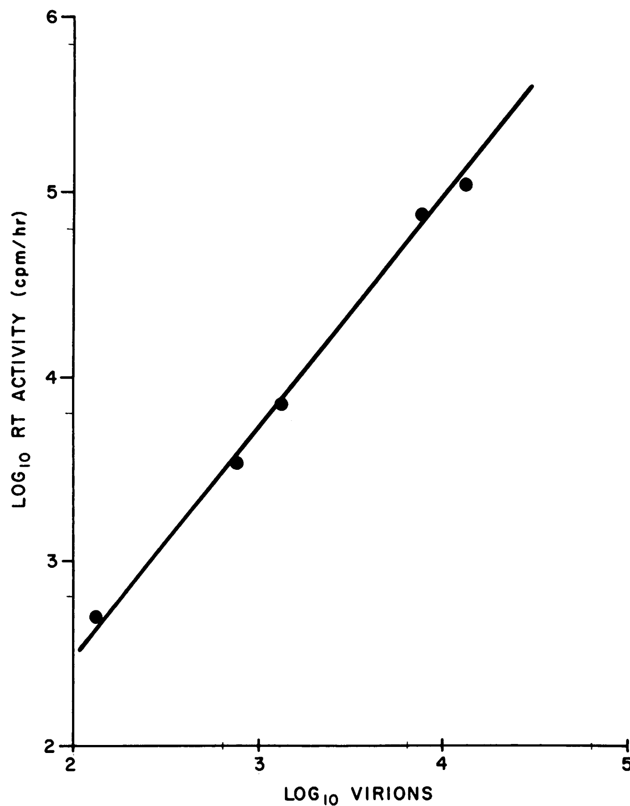


FIG. 1. Sensitivity of RT assay for detection of HIV. The number of viruslike particles was determined by counting in a Hitachi H-600 electron microscope, and dilutions of duplicate samples were made in RPMI 1640 culture medium and assayed for RT activity at 37°C for 22 h.

antigen. Both the RT assay and AC ELISA showed adequate specificity for the detection of virus, since the limiting number of virus particles approached background levels for each assay, as determined from negative-control lymphocyte culture fluids from a normal blood donor (Table 1). There was a good correlation between the two assays, since the plot of the RT assay results versus the AC ELISA results is linear (Fig. 3). These results show the sensitive detection of HIV by both assays, and thus either test can be used with the assurance that it is as good as the other. Thus, which assay to use will be determined by the preference of the user and the advantages and disadvantages of each assay.

The RT assay has been routinely used for over 15 years for the study of retroviruses (8). It is a well-characterized polymerase assay that uses a radioactive tritiated triphosphate nucleotide precursor for incorporation into DNA by the action of the viral polymerase. The assay consists of a reaction assay buffer that is easily made, with the template primers for the enzyme available from several manufacturers (e.g., Pharmacia, Inc., Piscataway, N.J., and Boehringer Mannheim Biochemicals). An advantage of the RT assay is its ability to both detect and quantitate virus over a wide

TABLE 1. Detection of HIV

| Sample | RT assay | | Antigen capture ELISA | | | | |
|---|---|---|---|---|---|---|---|
| | | | Abbott Laboratories | | Du Pont New England Nuclear | | |
| | cpm ($10^4$) | Result[a] | OD | Result[a] | OD | p24 (ng/ml) | Result[a] |
| Virus particles | | | | | | | |
| $1.3 \times 10^4$ | 250.0 | + | >2.000 | + | >2.000 | 12.20[b] | + |
| $7.5 \times 10^3$ | 170.0 | + | >2.000 | + | >2.000 | 6.00[b] | + |
| $1.3 \times 10^3$ | 15.7 | + | >2.000 | + | 1.475 | 1.20 | + |
| $7.5 \times 10^2$ | 7.6 | + | 1.637 | + | 0.810 | 0.60 | + |
| $1.3 \times 10^2$ | 1.2 | + | 0.376 | + | 0.129 | 0.09 | + |
| $7.5 \times 10^1$ | 0.3 | − | 0.227 | + | 0.058 | 0.04 | + |
| $1.3 \times 10^1$ | 0.4 | − | 0.067 | − | 0.019 | 0 | − |
| 7.5 | 0.8 | − | 0.048 | − | 0.029 | 0 | − |
| 1.3 | 0.2 | − | 0.038 | − | 0.016 | 0 | − |
| Controls | | | | | | | |
| Donor lymphocyte culture | 0.2 | − | 0.035 | − | 0.014 | 0 | − |
| HTLV-III[c] | 150.0 | + | >2.000 | + | >2.000 | >1.0 | + |
| HIV p24 antigen standards (Du Pont New England Nuclear) | | | | | | | |
| A | NA[d] | | ND[e] | | 1.238 | 1.000 | + |
| B | NA | | ND | | 0.694 | 0.500 | + |
| C | NA | | ND | | 0.357 | 0.250 | + |
| D | NA | | ND | | 0.178 | 0.125 | + |
| E | NA | | ND | | 0.096 | 0.063 | + |
| F | NA | | ND | | 0.040 | 0.032 | + |

[a] The cutoff values for a positive result were set at $10^4$ cpm for the RT assay (2.5 times the value for a negative control, which ranged from $0.2 \times 10^4$ to $0.4 \times 10^4$ cpm) and 0.085 and 0.028 OD units for Abbott and Du Pont New England Nuclear AC ELISAs, respectively, determined with a negative control sample in accordance with instructions in the kits.

[b] Concentration calculated from measurement of virus at higher dilution.

[c] HTLV-III, Human T-cell lymphotropic virus type III.

[d] NA, Not applicable.

[e] ND, Not determined.

range. We have shown the enzyme activity to be linearly related to at least a threefold log range of virus (4). Previously, the assay was not useful for testing a large number of samples, since it required the tedious collection of DNA product onto glass fiber filters for detection. This has been overcome by the development of a micromethod assay in microdilution plates and the use of a cell harvester for semiautomated collection of DNA (7). We are now using this micromethod and find, as reported, that it gives results comparable to those of the standard assay method and enables a large number of samples to be easily handled.

The AC ELISA is a recent immunologic test developed for HIV detection. It is a simple assay that is semiautomated since it uses an ELISA detection system and thus can easily test a large number of samples. This assay has the advantage of being able to detect virus antigen in sera and plasma, clinical samples for which an enzyme assay such as RT cannot be used because of interfering proteins or nucleases. The AC ELISA has been able to detect viral antigen directly in the plasma of acquired immune deficiency syndrome patients (2). This assay does not use a radioactive isotope and is therefore ideal for laboratories not wishing to work with radioactivity. A disadvantage of the assay is that it cannot be readily used to quantitate virus in certain samples. For instance, in individuals that progress to disease, there appear to be increased levels of viral antigen in their plasma that may not reflect actual virus numbers (2). Also, during in vitro culture, viral replication could stop but viral antigen may persist. Another consideration in the choice of an assay is the cost per test. Since the RT assay materials are readily available and can be easily assembled reproducibly, it represents the lowest cost. The AC ELISA is more difficult to prepare, and care must be taken to ensure consistent results from assay to assay. Most laboratories would probably prefer to purchase it as a prepared standardized test kit. These commercially prepared kits represent a four- to eight-fold higher cost per test. However, this higher cost reflects the convenience of a prepared test with quality control that gives good consistent results.

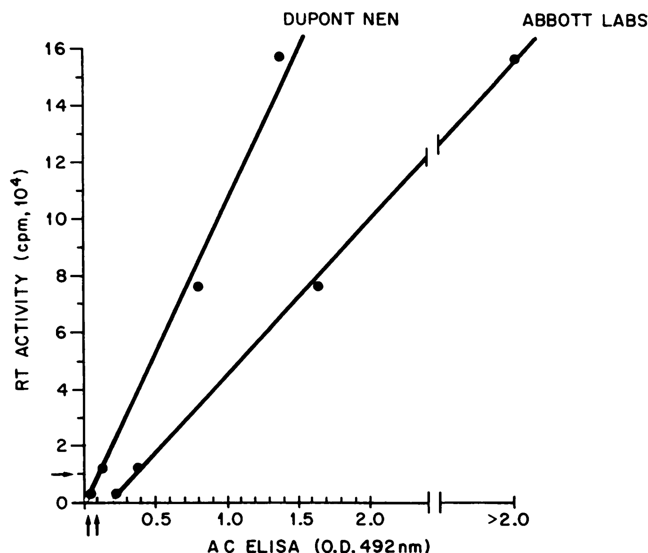


FIG. 3. Correlation of RT assay with antigen capture ELISA from Abbott Laboratories and Du Pont New England Nuclear (NEN). The viral enzyme activity was measured as described in the legend to Fig. 1. The antigen assays were done in accordance with the instructions enclosed with each kit. The arrows on the *x* and *y* axes indicate the cutoff values for a positive result in each assay (RT assay, $\geq 1.0 \times 10^4$ cpm; Du Pont New England Nuclear and Abbott Laboratories AC ELISAs, $\geq 0.028$ and $\geq 0.085$ OD units, respectively, read at 490 nm), as determined with a sample similarly prepared from the culture supernatant of a blood donor.

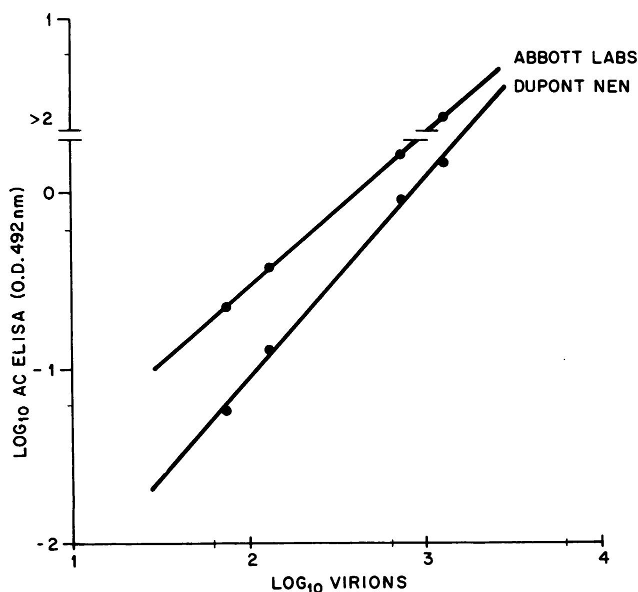


FIG. 2. Sensitivity of the antigen capture ELISA for detection of HIV. Virus samples were prepared and diluted as described in the legend to Fig. 1 and assayed for HIV antigen by using test kits supplied by Abbott Laboratories and Du Pont New England Nuclear (NEN). The break in the *y* axis represents a result reading off scale in the assay (greater than 2 OD units).

We are using both assays in our laboratory and find they work well. We routinely use the RT assay to screen our culture supernatants for virus, and positive results are then confirmed by the AC ELISA. We then report the culture as positive for HIV when both tests concur. Thus, we believe the usefulness of the two assays lies in their ability to verify each other, giving adequate assurance of the presence of virus or virus antigen and eliminating false-positive results.

We thank Henry Ly for technical assistance with the assays, A. Tereba for sharing his retrovirus expertise, and L. Zamboni and J. Seto for use of their electron microscope laboratories. The human T-cell lymphotropic virus type III strain was graciously furnished by Z. Salahuddin and R. Gallo.

This work was supported by Public Health Service research contract NOI AI-32511 from the National Institute of Allergy and Infectious Diseases to the Multicenter AIDS Cohort Study (University of California at Los Angeles; Northwestern University, Chicago, Ill.; Johns Hopkins University, Baltimore, Md.; and the University of Pittsburgh, Pittsburgh, Pa.).

## LITERATURE CITED

1. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science **224**:500–503.
2. **Goudsmit, J., F. De Wolf, D. A. Paul, L. G. Epstein, J. M. A. Lange, W. J. A. Krone, H. Speelman, E. C. Wolters, J. Van Der Noordaa, J. M. Oleske, H. J. Van Der Helm, and R. A. Coutinho.** 1986. Expression of human immunodeficiency virus antigen

(HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. Lancet **ii**:177–180.

3. **Hoffman, A. D., B. Banapour, and J. A. Levy.** 1985. Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. Virology **147**: 326–335.
4. **Lee, M. H., K. Sano, F. E. Morales, and D. T. Imagawa.** 1987. Sensitive reverse transcriptase assay to detect and quantitate human immunodeficiency virus. J. Clin. Microbiol. **25**:1717–1721.
5. **McDougal, J. S., S. P. Cort, M. S. Kennedy, C. D. Cabridilla, P. M. Feorino, D. P. Francis, D. Hicks, V. S. Kalyanaraman, and L. S. Martin.** 1985. Immunoassay for the detection and quantitation of infectious human retrovirus, lymphadenopathy-associated virus (LAV). J. Immunol. Methods **76**:171–183.
6. **Rey, M. A., B. Spire, D. Dormont, F. Barre-Sinoussi, L. Montagnier, and J. C. Chermann.** 1984. Characterization of the RNA dependent DNA polymerase of a new human T lymphotropic retrovirus (lymphadenopathy associated virus). Biochem. Biophys. Res. Commun. **121**:126–133.
7. **Spira, T. J., L. H. Bozeman, R. C. Holman, D. T. Warfield, S. K. Phillips, and P. M. Feorino.** 1987. Micromethod for assaying reverse transcriptase of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus. 1987. J. Clin. Microbiol. **25**:97–99.
8. **Temin, H. M., and D. Baltimore.** 1972. RNA-directed DNA synthesis and RNA tumor viruses. Adv. Virus Res. **17**:129–186.
9. **Tereba, A., and K. G. Murti.** 1977. A very sensitive biochemical assay for detecting and quantitating avian oncornaviruses. Virology **80**:166–176.