If you have issues viewing or accessing this file contact us at NCJRS.gov.

108812

U.S. Department of Justice
National Institute of Justice

108812

This document has been reproduced exactly as received from the person or organization originating it. Points of view or opinions stated in this document are those of the authors and do not necessarily represent the official position or policies of the National Institute of Justice.

Permission to reproduce this copyrighted material has been granted by

U.S. Department of Health and Human Services/Public Health Service

to the National Criminal Justice Reference Service (NCJRS)

Further reproduction outside of the NCJRS system requires permission of the copyright owner.

108812

CENTERS FOR DISEASE CONTROL

January 8, 1988 / Vol. 36 / No. 52

MMWR

MORBIDITY AND MORTALITY WEEKLY REPORT

NCJRS

JAN 25 1988

ACQUISITIONS

Current Trends

Update: Serologic Testing for Antibody to Human Immunodeficiency Virus

Tests to detect antibody to human immunodeficiency virus (HIV), the virus that causes acquired immunodeficiency syndrome (AIDS), were first licensed by the Food and Drug Administration (FDA) in 1985, primarily as screening tests for blood and plasma donation. Since that time, millions of HIV antibody tests have been performed in laboratories of blood and plasma collection centers, in counseling and testing centers, and in clinical facilities as well as for purposes such as screening active duty military personnel and applicants for military service. Assuring accurate test results requires continued attention to both the intrinsic quality of the tests and the performance of the technical personnel doing the tests.

Given the medical and social significance of a positive test for HIV antibody, test results must be accurate, and interpretations of the results must be correct. For these reasons, the Public Health Service has emphasized that an individual be considered to have serologic evidence of HIV infection only after an enzyme immunoassay (EIA) screening test is repeatedly reactive* and another test such as Western blot (WB) or immunofluorescence assay has been performed to validate the results (*1*).†

*The terms "reactive" or "nonreactive" are used to describe serum or plasma specimens that give reactive or nonreactive test results and to describe the test results from EIA or WB tests before final interpretation. The terms "positive" and "negative" are used to describe the interpretation of EIA test results indicating that the specimen tested is 1) repeatedly reactive (positive) or 2) nonreactive or not repeatedly reactive (negative). The terms "positive," "indeterminate," and "negative" are used to describe the interpretation of WB test results that indicate that the specimen tested is reactive with a specific pattern of bands (positive), reactive with a nonspecific pattern of bands (indeterminate), or nonreactive (negative).

†Blood and plasma are not accepted for transfusion or further manufacture when the EIA screening test is positive, regardless of the results of other tests that may be performed.

A notice regarding changes in telephone numbers throughout the Centers for Disease Control and the Agency for Toxic Substances and Disease Registry appears on page 852.

Serologic Testing – Continued

Licensed test kits currently available in the United States for HIV antibody testing comprise seven EIAs and one WB. All of these tests use HIV antigens derived from disruption of whole virus cultured in human-derived cell lines. In addition, many laboratories produce their own WB test reagents using viral antigen purchased from commercial sources. A variety of other test procedures are in use or under development or are being evaluated for licensure.

Criteria for interpretation of a reactive anti-HIV EIA test are based on data from clinical studies performed under the auspices of each manufacturer. Since licensure of the first EIA test kits in 1985, the manufacturers have worked to improve the sensitivity, specificity, and reproducibility of their assays.[5] Clinical data submitted by the manufacturers to FDA for licensure indicate that the sensitivity and specificity of the EIA tests currently marketed in the United States are >99.0%. Other laboratories performing comparative analyses of licensed anti-HIV EIA test kits have found similar or slightly lower sensitivity and specificity (*2-5*). In routine use, both the sensitivity and specificity of the tests depend on the quality of testing in the laboratory. In addition, false-positive test results are observed when nonspecific serologic reactions occur among uninfected persons who have immunologic disturbances or who have had multiple transfusions. False-negative test results are observed among persons who have recently become infected with HIV and who have not yet developed detectable antibody (*6*).

Repeating each initially reactive EIA test increases the specificity of the test sequence by reducing the possibility that technical laboratory error caused the reactive result. In the American Red Cross Blood Services laboratories, a specificity of approximately 99.8% has been consistently achieved during screening of donated blood (*7*, unpublished data). However, in a population with a low prevalence of infection, even a specificity of 99.8% does not provide the desired predictive value[†] for a positive test. For this reason, it is particularly important not to rely solely on EIA testing to determine whether a person is infected with HIV. Rather, EIA test results should be validated with an independent supplemental test of high specificity conducted by a laboratory with high performance standards. In the United States, the validation test used most often is the WB. Some laboratories also use radioimmunoprecipitation assays and indirect immunofluorescence assays.

For the licensed WB test, interpretation of reactive and nonreactive tests is based on data from clinical studies submitted to FDA for licensure. The manufacturer states that, for a test to be considered positive with this WB, antibody must be reactive with multiple virus-specific protein bands, i.e., p24, p31, and either gp41 or gp160 (Table 1). If fewer bands are present, the test is considered indeterminate; it is interpreted as negative only if no bands are present on the blot. When the manufacturer's stringent criteria are used for interpreting test results, the probability of either a false-positive or a false-negative result is extremely small. In clinical trials for licensure of this WB, however, as many as 15% to 20% of tests on persons at low risk for HIV infection were described as indeterminate. Sera from persons recently infected with HIV also may produce an indeterminate WB pattern. For such

[5]Sensitivity is the probability that the test result will be reactive if the specimen is a true positive; specificity is the probability that the test result will be nonreactive if the specimen is a true negative; and reproducibility (reliability) is the ability to replicate qualitative results with the same or similar test procedures on blindly paired samples.

[†]The predictive value of a positive or negative test is the probability that the test result is correct.

Serologic Testing — Continued

persons, a repeat WB on a second specimen obtained after the initial specimen often yields a positive blot pattern within 6 months. Conversely, follow-up testing of uninfected persons whose serum had an indeterminate blot pattern on initial testing usually will show no change in the banding pattern. Serum from some HIV-infected persons who have advanced immunodeficiency may have an indeterminate pattern because of a loss of antibodies to non-*env* proteins (*8*). To reinstate donors with a history of a positive EIA test, blood and plasma centers may use only results from the licensed WB test performed in the FDA-approved test sequence.

The performance characteristics of the unlicensed tests used by many laboratories, whether WB, immunofluorescence assays, or other procedures, have not been uniformly subjected to the same rigorous scrutiny required for licensure by FDA. Recommendations for standardization have been published (*9*), but the extent to which these are followed is unknown. Information about production standards, inter-lot variability, or validation of criteria used for interpretation often is not available. Absence of standardization and appropriate quality controls may result in a lower sensitivity or specificity and, thus, a higher probability of inaccurate results (*10*).

Despite the existence of a licensed WB test, many laboratories continue to use unlicensed WB tests because of cost and the stringent criteria required for interpreting the licensed test. The potential problems in using and interpreting unlicensed WB tests have been openly debated (*11,12*). Although unlicensed WB tests can be highly accurate and reproducible when done with appropriate quality controls in laboratories with established performance standards (*9*), not all laboratories meet acceptable performance standards. Ten of 19 laboratories bidding for contracts to perform WB tests for the Department of Defense failed the required proficiency panel on one or more occasions (*13*). Two of the laboratories satisfying the performance standards were awarded contracts by the U.S. Army. Both of these laboratories use well-validated techniques for WB that yield virus-specific bands at p17, p24, p31, gp41, p53, p55, and p64. The U.S. Army considers these WBs to be positive if bands are present either at gp41 or at both p24 and p55 (*14*). In comparison with multiple

TABLE 1. Description of major gene products of human immunodeficiency virus (HIV)

Gene Product*	Description
p17	*gag*† protein
p24	*gag* protein
p31	Endonuclease component of *pol*§ translate
gp41	Transmembrane *env*¶ glycoprotein
p51	Reverse transcriptase component of *pol* translate
p55	Precursor of *gag* proteins
p66	Reverse transcriptase component of *pol* translate
gp120	Outer *env* glycoprotein
gp160	Precursor of *env* glycoprotein

*Number refers to molecular weight of the protein in kilodaltons; measurement of molecular weight may vary slightly in different laboratories.
†*gag* = core.
§*pol* = polymerase.
¶*env* = envelope.

Serologic Testing -- Continued

validation procedures, WBs in these contract laboratories have an estimated specificity of 99.4%, and the laboratories have consistently performed accurately on all pre- and post-award quality assurance serum panels (*14*). These and other laboratories have demonstrated that the achievable false-positive rate of sequentially performed EIA and WB tests can be <0.001% (<1/100,000 persons tested) (*13,15*).

The College of American Pathologists (CAP), in conjunction with the American Association of Blood Banks, conducts an open proficiency testing program** for laboratories performing HIV antibody tests. Each quarter, more than 600 laboratories that participate voluntarily report results from testing five coded samples of plasma that have various known levels of anti-HIV reactivity or that are nonreactive.

In the CAP survey conducted in October 1987, the results of EIA tests at the participating laboratories correlated well with results from the referee laboratories (Table 2). For the three reactive samples (W-21, W-23, W-24), correlation ranged from 99.5% to 100%. For the single nonreactive sample that could be adequately evaluated (W-25), correlation was 98.3%. The nonreactive W-22 sample that was sent with the October 1987 serum panel had been prepared with a pool of processed plasma that caused an unexplained, nonspecific reaction with one of the EIA test kits. Consequently, the EIA results for this sample could not be evaluated.

The individual participating laboratories used their own criteria for interpreting WB results. WB results for two of the three reactive specimens were reported as indeterminate by one referee laboratory each, while results for the two nonreactive specimens in the CAP survey were reported correctly by all 10 referee laboratories (Table 3). One of the 73 participating laboratories reported a nonreactive sample (W-22, the sample that gave artifactual reactions with one of the EIA test kits) as reactive, while approximately 5% reported the two nonreactive samples as indeterminate, and 12% to 15% reported two of three reactive specimens as indeterminate.

For the three reactive samples, the results of 241 repeatedly reactive EIA tests could be compared with WB results (Table 4). For 215 (89.2%) of these, the WB tests

**The laboratories know that the samples have been supplied for proficiency testing.

TABLE 2. Comparison of responses by referee and participant laboratories on samples tested for anti-HIV by enzyme immunoassay (EIA), by sample number -- College of American Pathologists Proficiency Testing, 1987

Sample Number	Reactivity	Percentage of Laboratories Reporting Correct Result	
		Referee Laboratory*	Participant Laboratory†
W-21	Reactive	100.0	99.8
W-22§	Nonreactive	80.0	51.4
W-23¶	Reactive	100.0	99.5
W-24¶	Reactive	100.0	100.0
W-25	Nonreactive	100.0	98.3

*Results reported by 15 laboratories selected because of extensive experience and excellent long-term performance in proficiency testing programs.
†Results reported by 601 other laboratories that voluntarily participated.
§Sample W-22 was prepared with a pool of processed plasma that caused an artifactual, nonspecific reaction with one EIA test kit.
¶Samples W-23 and W-24 were identical.

Serologic Testing -- Continued

were reported as positive; for 23 (9.5%), the WBs were reported as indeterminate; and, for 3 (1.2%), they were reported as negative. Of 58 WB results performed on nonreactive samples found nonreactive by EIA, 55 (94.8%) were reported as negative by WB, and 3 (5.2%) were reported as indeterminate. None of the nonreactive samples were read as positive by WB.

Because criteria used to interpret WB varied by laboratory, banding patterns reported in the 299 WB tests conducted in the October 1987 survey were examined (Table 5). Two or more virus-specific protein bands were reported in 215 blots, 208 (96.7%) of which were interpreted as positive. Eighteen (60.0%) of 30 blots with only a single virus-specific protein band were considered positive. When the single protein band was from the *env* gene, 12 (85.7%) of 14 were read as positive. These data demonstrate that different laboratories may report different WB results for samples with the same banding patterns.

Results of CAP proficiency tests from more than 500 laboratories participating in the 1986 and 1987 surveys indicate the following performance for the anti-HIV EIA test. Of 6,946 tests on reactive samples, 99.5% were reported as positive. Of 1,142

TABLE 3. Comparison of responses on samples tested for anti-HIV by Western blot (WB) by referee and participant laboratories,* by sample number -- College of American Pathologists Proficiency Testing, 1987

		Interpretation of WB Test Results (Percentage of Responses)					
		Positive Test		Indeterminate Test		Negative Test	
Sample Number	Reactivity	Referee Laboratory	Participant Laboratory	Referee Laboratory	Participant Laboratory	Referee Laboratory	Participant Laboratory
W-21	Reactive	100.0	100.0	0.0	0.0	0.0	0.0
W-22	Nonreactive	0.0	1.6	0.0	4.9	100.0	93.4
W-23	Reactive	90.0	80.8	10.0	15.1	0.0	4.1
W-24	Reactive	90.0	84.9	10.0	12.3	0.0	2.8
W-25	Nonreactive	0.0	0.0	0.0	5.6	100.0	94.4

*Results reported by the 10 referee and 73 participant laboratories that performed both EIA and WB tests.

TABLE 4. Relationship between results on samples tested for anti-HIV by enzyme immunoassay (EIA) and Western blot (WB), by sample number -- College of American Pathologists Proficiency Testing, 1987

		Results by EIA*		Results by WB*		
Sample Number	Reactivity	Positive	Negative	Positive	Indeterminate	Negative
W-21	Reactive	76	0	76	0	0
W-23	Reactive	83	0	69	13	1
W-24	Reactive	82	0	70	10	2
W-25	Nonreactive	0	58	0	3[1]	55
Total		241	58	215	26	58

*Number of responses reported by both referee and participant laboratories. Sample W-22 was excluded because of an artifact of the sample.

[1]One sample by WB had only p24 bands reported; one sample had both p24 and p32 bands reported; and one sample had no bands reported.

Serologic Testing — Continued

tests on nonreactive samples, 98.3% were interpreted as negative. Based on results from 601 laboratories on a pair of identical reactive samples (W-23 and W-24), reproducibility was 99.5%.

For the WB test, calculations were based only on positive or negative results divided by the total number of tests in the October 1987 CAP survey (Table 4). For the reactive samples, 89.2% of 241 results were correctly interpreted as positive, and, for the nonreactive samples, 94.8% of 58 results were correctly interpreted as negative. Reproducibility, which was based on 83 tests on a pair of identical reactive samples (W-23 and W-24), was 95.2%. The performance of the referee laboratories was more accurate for the EIA and much more accurate for the WB than was the performance of the participating laboratories. The performance of the licensed and unlicensed WB tests could not be compared because the data were not collected.

Reported by: HF Polesky, MD, College of American Pathologists. Div of Blood and Blood Products, Center for Biologics Evaluation and Research, Food and Drug Administration. Div of Assessment and Management Consultation, Training and Laboratory Program Office; AIDS Program, Center for Infectious Diseases, CDC.

Editorial Note: Quality laboratory testing for HIV antibody is a critically important element for surveillance and detection of HIV infection. The laboratory testing process requires quality assurance for each step including: 1) collection, labeling, and transport of specimens; 2) laboratory reagents and procedures; 3) interpretation of analytical results; and 4) communication from the laboratory scientist to the clinician and then to the person being tested. Quality performance is promoted by using licensed or standardized tests in proper sequence and by developing consensus about interpretation of analytical results.

Proficiency testing benefits participating laboratories by identifying problems with particular types of samples, with particular tests, or with interpretation of results.

TABLE 5. Distribution and interpretation of HIV-specific protein band patterns on Western blot* (WB) — College of American Pathologists Proficiency Testing, 1987

HIV-Specific Bands†	WB as Interpreted by Referee and Participant Laboratories					
	Positive		Indeterminate		Negative	
	No.	(%)	No.	(%)	No.	(%)
None	0	(0.0)	9	(7.1)	118	(92.9)
Single Band	18	(60.0)	9	(30.0)	3	(10.0)
gag	6	(42.9)	7	(50.0)	1	(7.1)
pol	0	(0.0)	2	(100.0)	0	(0.0)
env	12	(85.7)	0	(0.0)	2	(14.3)
Multiple Bands	208	(96.7)	4	(1.9)	3	(1.4)
gag, pol	8	(80.0)	1	(10.0)	1	(10.0)
gag, env	125	(98.4)	0	(0.0)	2	(1.6)
pol, env	2	(40.0)	3	(60.0)	0	(0.0)
gag, pol, env	73	(100.0)	0	(0.0)	0	(0.0)
Total	**226**	**(91.8)**	**22**	**(5.9)**	**124**	**(33.3)**

*Samples tested and reported include reactive samples W-21, W-23, and W-24 and nonreactive samples W-22 and W-25.

†Bands may be any proteins or glycoproteins that are products of the genes listed. HIV-specific gene products are shown in Table 1.

Serologic Testing — Continued

However, results of proficiency testing programs should be interpreted cautiously. Data from proficiency testing measure only the operational performance of participating laboratories but cannot be used to measure the sensitivity or specificity of a given test. Samples provided for testing in the HIV antibody surveys may be pooled human plasma samples with known levels of anti-HIV reactivity, or they may be dilutions of a single reactive plasma sample in HIV-negative serum. They are rarely fresh serum specimens from a person who is or is not infected with HIV. Some samples are selected because they exhibit nonspecific reactivity or are otherwise difficult to test and interpret; they are not typical of the vast majority of specimens that will be handled by the participating laboratories. For instance, in normal practice, samples W-22 and W-25 would not be tested by WB because the EIA was nonreactive. The nonspecific reactivity of the type that occurred with specimen W-22 cannot always be predicted; a similar unexplained nonspecific reaction occurred in a proficiency testing program conducted by CDC (*16*) and with several samples used by the American Association of Bioanalysts (unpublished data).

The number of specimens commonly used in proficiency testing programs (five in each CAP survey) sent to each laboratory also limits the application of survey results. This number of specimens is not sufficient to measure adequately the performance of any single laboratory. The number of specimens tested per month in different laboratories varies enormously, and no attempt is made in the survey to select a representative sample of laboratories performing the test; those that choose to participate in the survey do so voluntarily.

Laboratories in the surveys reported indeterminate WB results on some reactive and nonreactive samples. An indeterminate result is not a final result; it requires additional laboratory testing on the same specimen and often entails asking the person from whom the specimen was obtained to provide one or more additional specimens. The final interpretation of an indeterminate result frequently will also require additional epidemiologic, clinical, or corroborating laboratory information.

Even among the diverse laboratories participating in the CAP survey, none performing the EIA and WB tests in sequence would have reported false-positive test results. However, performance and interpretation of WB tests vary among laboratories. The Public Health Service is convening a meeting to address these issues. A nationwide performance evaluation program for HIV antibody testing has been started by CDC's Training and Laboratory Program Office and Center for Infectious Diseases (*17*). The first sample shipment, consisting of reference materials, was mailed in November 1987 to more than 700 participating U.S. laboratories.

The predictive values of both positive and negative test results for HIV antibody are extremely high in laboratories that have good quality control and high performance standards and that use licensed EIA tests and the licensed WB or other well-standardized tests. Physicians or other health-care providers who request HIV antibody tests and who counsel persons about test results must have a clear understanding of the significance of the test results and the potential pitfalls of the testing process. When test results are indeterminate or inconsistent with other information, additional information should be obtained to try to confirm whether the person is infected with HIV. The counseling procedure should include a careful assessment of the person's potential risks or exposures to HIV. As for all medical tests, results should be interpreted in concert with all the historic, epidemiologic, clinical, and other pertinent laboratory information available.

Serologic Testing — Continued

References

1. Centers for Disease Control. Public Health Service guidelines for counseling and antibody testing to prevent HIV infection and AIDS. MMWR 1987;36:509-15.
2. Burkhardt U, Mertens Th., Eggers HJ. Comparison of two commercially available anti-HIV ELISAs: Abbott HTLV III EIA and DuPont HTLV III-ELISA. J Med Virol 1987;23:217-24.
3. Mortimer PP, Parry JV, Mortimer JY. Which anti-HTLV-III/LAV assays for screening and confirmatory testing? Lancet 1985;2:873-7.
4. Reesink HW, Huisman JG, Gonsalves M, et al. Evaluation of six enzyme immunoassays for antibody against human immunodeficiency virus. Lancet 1986;2:483-6.
5. Gürtler LG, Eberle J, Lorbeer B, Deinhardt F. Sensitivity and specificity of commercial ELISA kits for screening anti-LAV/HTLV III. J Virol Methods 1987;15:11-23.
6. Kessler HA, Blaauw B, Spear J, Paul DA, Falk LA, Landay A. Diagnosis of human immunodeficiency virus infection in seronegative homosexuals presenting with an acute viral syndrome. JAMA 1987;258:1196-9.

(Continued on page 852)

TABLE I. Summary — cases of specified notifiable diseases, United States

Disease	52nd Week Ending			Cumulative, 52nd Week Ending		
	Jan. 2, 1988	Dec. 27, 1986	Median 1982-1986	Jan. 2, 1988	Dec. 27, 1986	Median 1982-1986
Acquired Immunodeficiency Syndrome (AIDS)	514	434	N	20,940	13,405	N
Aseptic meningitis	84	186	209	10,949	10,934	10,379
Encephalitis: Primary (arthropod-borne & unspec)	15	24	35	1,266	1,228	1,320
Post-infectious	3	1	3	104	104	104
Gonorrhea: Civilian	8,574	13,242	14,160	751,600	887,936	887,936
Military	124	213	322	15,887	16,969	21,107
Hepatitis: Type A	447	439	654	24,491	23,043	23,043
Type B	375	523	755	25,170	25,842	25,842
Non A, Non B	41	67	N	2,882	3,494	N
Unspecified	42	82	149	3,067	4,368	5,755
Legionellosis	12	21	N	863	832	N
Leprosy	3	2	8	206	262	251
Malaria	12	19	26	882	1,103	1,034
Measles: Total*	2	20	47	3,588	6,235	2,579
Indigenous	2	20	N	3,166	5,925	N
Imported	-	-	N	422	310	N
Meningococcal infections: Total	52	59	75	2,857	2,491	2,689
Civilian	52	58	75	2,856	2,488	2,685
Military	-	1	-	1	3	7
Mumps	35	312	84	12,299	6,011	3,348
Pertussis	65	22	101	2,529	4,053	2,460
Rubella (German measles)	-	15	12	329	530	740
Syphilis (Primary & Secondary): Civilian	480	455	459	35,398	27,273	27,947
Military	8	1	7	168	164	288
Toxic Shock syndrome	3	6	N	325	358	N
Tuberculosis	532	587	745	21,668	22,212	22,212
Tularemia	2	-	4	188	168	271
Typhoid Fever	5	12	22	347	332	403
Typhus fever, tick-borne (RMSF)	2	1	11	592	744	833
Rabies, animal	43	60	100	4,507	5,318	5,394

TABLE II. Notifiable diseases of low frequency, United States

	Cum. 1987		Cum. 1987
Anthrax	1	Leptospirosis (Hawaii 13)	50
Botulism: Foodborne (Fla. 1)	15	Plague	11
Infant	46	Poliomyelitis, Paralytic	-
Other (Ore. 1)	3	Psittacosis (Ore. 1, Ga. 1, Minn. 1, Iowa 3)	86
Brucellosis (Tex. 1)	116	Rabies, human	-
Cholera	5	Tetanus (Kan. 1)	40
Congenital rubella syndrome	5	Trichinosis	37
Congenital syphilis, ages < 1 year	339	Typhus fever, flea-borne (endemic, murine)	37
Diphtheria	3		

*There were no cases of internationally imported measles reported for this week.