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SUBTITLE: Sixth Annual Conference on Human Retrovirus Testing, Kansas City, Missouri, March 5-7, 1991

PRINCIPAL INVESTIGATOR: Jerome R. Cordts

CONTRACTING ORGANIZATION: Association of State & Territorial Public Health Laboratory Directors  
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# REPORT DOCUMENTATION PAGE

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**REPORT  
AND  
RECOMMENDATIONS**



**SIXTH ANNUAL CONFERENCE ON  
HUMAN RETROVIRUS TESTING**

**KANSAS CITY, MISSOURI**

**MARCH 5-7, 1991**

*The views, opinions and/or findings contained herein are those of the author(s) and should not be construed as an official Department of the Army position or decision unless so designated by other documentation.*

### ASTPHLD

The Association of State and Territorial Public Health Laboratory Directors is an organization representing state and territorial public health laboratory directors throughout the United States. The Association maintains a Headquarters office and seven Area Resource Offices, and operates a National Laboratory Training Network that forms alliances among federal, state, and local health agencies and private sector organizations to develop and promote the delivery of localized laboratory training programs based on documented need. The Association also organizes and presents scientific conferences and symposia relevant to the testing activities of public health laboratories. ASTPHLD operates exclusively as a scientific and educational organization.

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# THE SIXTH ANNUAL CONFERENCE ON HUMAN RETROVIRUS TESTING

The constant evolution of testing methodologies for the human retroviruses demands that periodic reviews of the capabilities of these methodologies used in the public health laboratories be conducted. To this end, the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) sponsored the Sixth Conference on Human Retrovirus testing March 5-7, 1991 in Kansas City, MO. Approximately 285 conference participants viewed 36 poster presentations, heard 12 plenary session speakers discuss a variety of topics relevant to retrovirus testing, and participated in 6 panel sessions concentrating on the most relevant topics of human retrovirus testing.

Panel Session 1 was entitled "Testing for HIV-1". Topics discussed included rapid testing methods, Western blots, testing of non-traditional samples, surrogate markers, recombinant/synthetic based assays, Western blot interpretation criteria, indirect fluorescent antibody methods, and viral detection methods.

Panel Session 2, entitled "Polymerase Chain Reaction", discussed methodologies used in PCR, quality assurance, proficiency testing, and test interpretation and reporting.

Panel Session 3 centered on developments within "Flow Cytometry". The topics covered flow cytometry for disease staging, monitoring anti-viral therapy, training, quality assurance, reporting criteria, and interpretation.

Panel Session 4 addressed "Testing for HIV-2 and HTLV-I/ HTLV-II". Topics covered HTLV-I and HTLV-II differentiation, the confirmation methodologies and criteria for HIV-1 and HIV-2 as opposed to HTLV-I/II viruses, HIV-1 and HIV-2 combination tests and alternate rapid testing methods.

Panel Session 5 concentrated on "The Diagnosis of HIV Infection in Newborns". The topics covered, in a very specific sense, the use of flow cytometry, PCR, and viral culture to enhance detection of infection in newborns. Discussions also covered the use of ELISPOT, in vitro assays and analysis for IgA.

Panel Session 6 dealt with "Standards of Practice and Reporting". A new topic covered during this session was the "Interpretation/Reporting of Reactions in Vaccinated Individuals".

There continues to be intense interest among laboratorians in the methodologies discussed in these six panel sessions due to the increased attention the medical and scientific community has focused on early intervention and treatment programs for HIV antibody positive patients. Title III of the Ryan White CARE Act outlines provisions for monitoring the immune status of HIV antibody positive patients in order to assess the stage of the AIDS disease. Laboratory analysis, in all aspects of this monitoring process, is extremely important. Laboratory findings form the basis of the therapies currently available for retrovirus infected individuals.

It is intended that the recommendations derived from the Conference on Human Retrovirus Testing be utilized universally to set laboratory standards in methodologies, policies, quality assurance, interpretation and reporting.



# 1991 SURVEY RESULTS

To assess the current status of testing for Human Retroviruses, a survey was sent to 54 state and territorial public health laboratories during the third quarter of 1990. The survey requested data for fiscal year 1990 (July, 1989 -June, 1990). In previous years, calendar year data were requested.

A milestone was achieved in gathering the data for this most recent survey. All 54 state and territorial public health laboratories and the city of New York contributed to the survey data. This had not occurred in any previous year. The summary of these data follows.

The total number of EIA tests reported by the survey participants, as indicated in Figure 1, totalled in excess of 4 million. These data cannot be compared directly to the data from previous years because they include data from 99 California clinical laboratories and blood banks and 37 county public health laboratories. However, in evaluating these data, it can be noted that the EIA reactive rate in this national sample is 3%. Western blot confirmation of the reactives was 58%; 9% of the Western blots were indeterminate. Using IFA, 79% of the reactives were confirmed and 1.5% were indeterminate.

TEST	TOTAL	REACTIVE	IND
E I A	4,424,943	132,376	N/A
W B	131,890	77,075	11,625
I F A	35,221	27,831	559

Figure 1 Testing reported by the members of ASTPHLD includes 37 county (local) public health laboratories and 99 clinical laboratories in California.

TEST	TOTAL	REACTIVE	IND
E I A	3,175,534	111,479	N/A
W B	91,375	75,051	6,990
I F A	33,934	26,664	555

Excludes local/clinical California laboratories

Figure 2 Testing volume adjusted from Figure 1 to exclude the 37 local public health and 99 clinical laboratories in California.

Removing the California data generates Figure 2, demonstrating 3.175 million EIA tests. These data exhibit a slightly higher reactive rate (3.5%). The Western Blot test confirmed 82% reactive and 7.5% were indeterminate. In utilizing the IFA, 78% of the original EIA reactive tests were confirmed while only 1.5% were indeterminate.

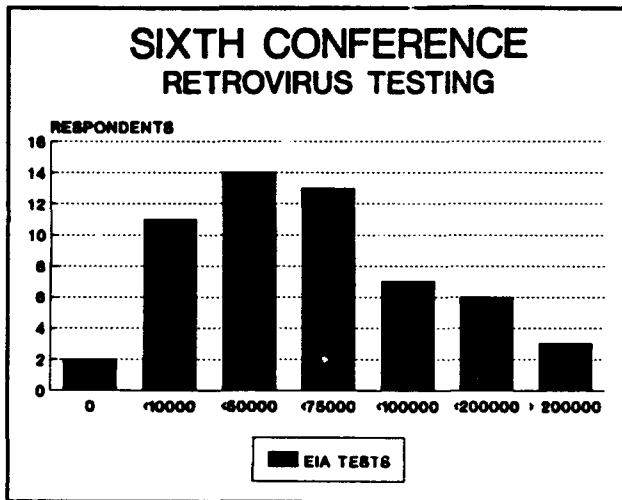


Figure 3 Total number of tests performed by reporting laboratories

Figure 3 indicates the total number of EIA tests done by each of the responding laboratories. These data indicate that, of the responding laboratories: approximately 48% conducted less than 50,000 tests, approximately 35% between 50,000 and 100,000 tests, and 16% conducted more than 100,000 tests for retrovirus.

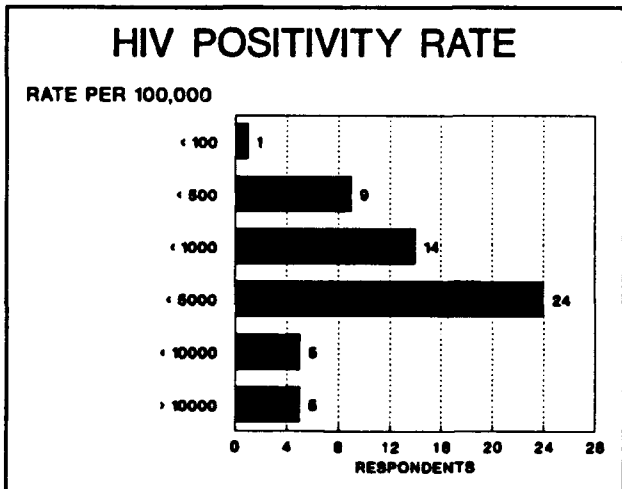


Figure 4 HIV positivity rates for tests in public health laboratories

The HIV positivity rates reported by public health laboratories are shown in Figure 4. Of the responding laboratories, 42% have a low positivity rate of less than 1%, 42% of the responding laboratories indicate a positivity rate between 1 and 5% and 17% have a positivity rate in excess of 5%. The pie chart depicted in Figure 5 indicates the tests that are used to confirm EIA reactives. These data show that the Western blot test was

used most frequently, (73% of all confirmatory tests conducted). The IFA confirmatory system had been used for 26% of the reactive tests, while approximately 1% of the confirmations were conducted with either PCR or tissue culture techniques.

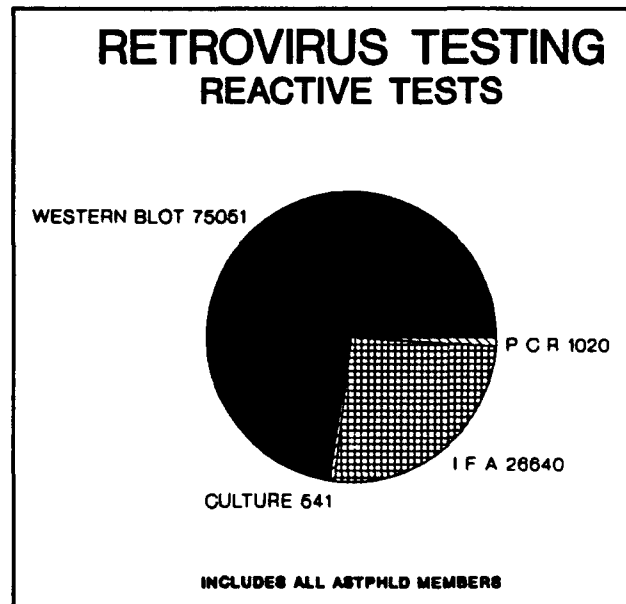


Figure 5 Distribution of confirmatory methods for reactive EIA

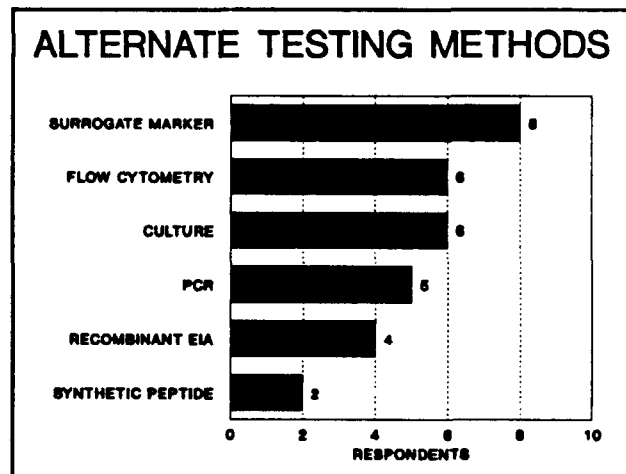


Figure 6 Other test methods used in public health laboratories to follow up on patients with HIV

Figure 6 reveals that alternate testing methodologies are being used by more laboratories. Surrogate markers, which include p24 antigen, neopterin and beta-2-microglobulin, are used by eight laboratories. Flow cytometry, culture, PCR, recombinant antigen and synthetic peptides are also being utilized, but in fewer numbers of laboratories.

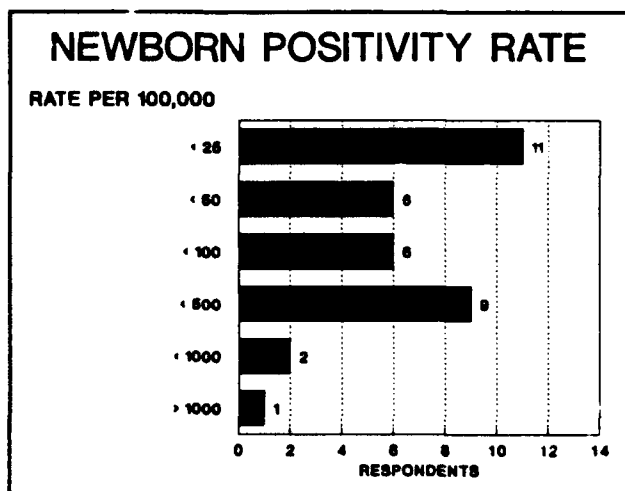


Figure 7 The HIV positivity rate for newborns

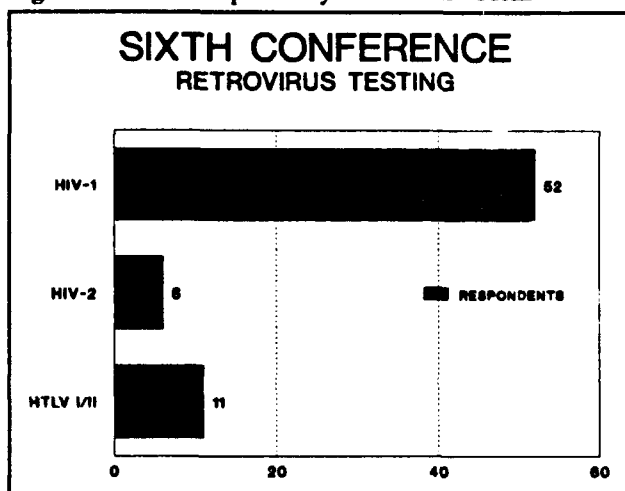


Figure 8 Summary of the public health laboratory retrovirus testing (Note: in 2 states the HIV-1 test is not performed in the state laboratory).

Public health laboratories continue to support the initiative of the Centers for Disease Control in gathering data for the family of seroprevalence surveys. Figure 7 indicates that 35 laboratories conducted HIV tests in populations of women of childbearing age. The data indicate that the positivity rate in this population is substantially below that seen in the adult population shown in Figure 4. Of 35 labs reporting, only 1 laboratory indicated a positive rate greater than 1%.

Figure 8 outlines the retrovirus testing being conducted in each of the responding laboratories. A total of 52 responding laboratories indicated that they conduct tests for HIV-1. Pennsylvania and Minnesota state public health laboratories do not conduct HIV testing. The data in this figure indicate 6 laboratories also conduct HIV-2 testing, and 11 laboratories perform tests for HTLV-I/II.

### RETROVIRUS TESTING HIV-2

TEST	TOTAL	REACTIVE	IND
EIA	4,460	114	N/A
WB	194	22	32
IFA	17	0	1

Figure 9 The testing volume for HIV-2, including the California local laboratories

### RETROVIRUS TESTING HTLV I/II

TEST	TOTAL	REACTIVE	IND
EIA	523,524	1946	N/A
WB	1,702	407	195
IFA	1,942	1,063	112

Figure 10 Testing volume for HTLV-I/II includes the 37 local public health and 99 clinical laboratories in California. Of the total EIA tests 515,093 were performed by 35 clinical laboratories in California.

Figure 9 shows the number of HIV-2 tests conducted. Of the 4,460 EIA tests, only 114 (2.5%) were found to be reactive - similar to the rate seen for HIV-1. However, the Western blot confirmation rate was very different from HIV-1, in that only 11% of the EIA reactive tests were confirmed, while 16% were indeterminate. Laboratories using IFA methodologies had no reactives and only 1 indeterminate. These data appear to indicate the need to refine testing conducted for HIV-2.

Figure 10 demonstrates that of the approximately 500,000 tests conducted for HTLV-I/II less than 1% were reactive. Those reactive specimens showed a 25% confirmatory rate Western blot, while 11% were indeterminate. In utilizing IFA methodology, 55% were confirmed and 6% were indeterminate.

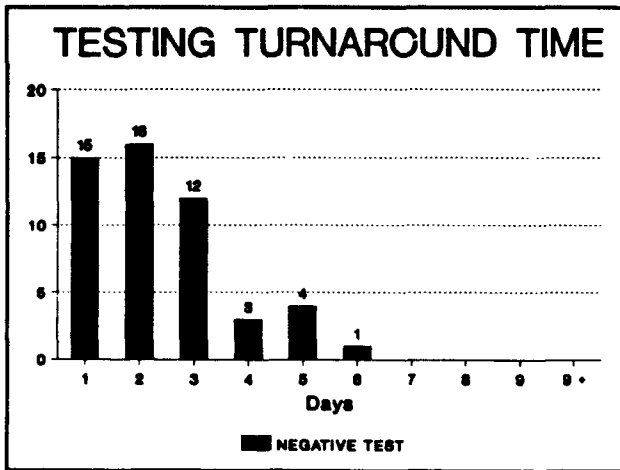


Figure 11 Over 80% of the public health laboratories report non-reactive EIA results within three days.

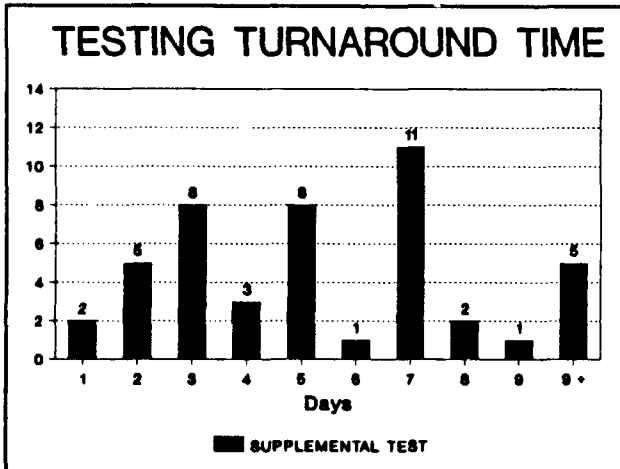


Figure 12 The time required to report reactive EIA varies greatly according to individual state procedures.

Figure 11 shows the average testing turnaround time when the results of the EIA tests are negative. Most laboratories indicate that results are reported within 3 calendar days. The testing turnaround time for an EIA reactive specimen is longer and more inconsistent (Figure 12). It is clearly evident that extended periods of time are needed to confirm results; the largest number of laboratories (11) indicated that 7 calendar days were needed to report a confirmed positive result. This extended turnaround time is a source of frequent discussion between the state public health laboratory and the AIDS program in each of the states and territories. Many requests have been made to consider various approaches to reduce the reporting turnaround time for confirmation of reactive EIA results.

The principal manufacturers of EIA testing reagents are listed in alphabetical order in Figure 13. The leading suppliers of the Western blot reagents are indicated in Figure 14.

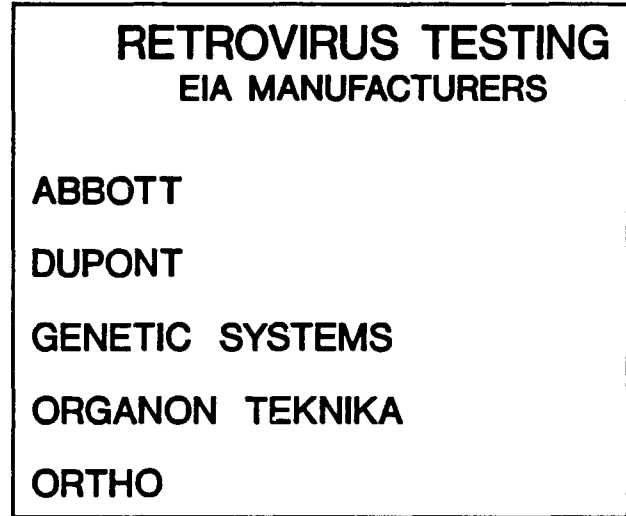


Figure 13 Suppliers of EIA reagents used by the state public health laboratories.

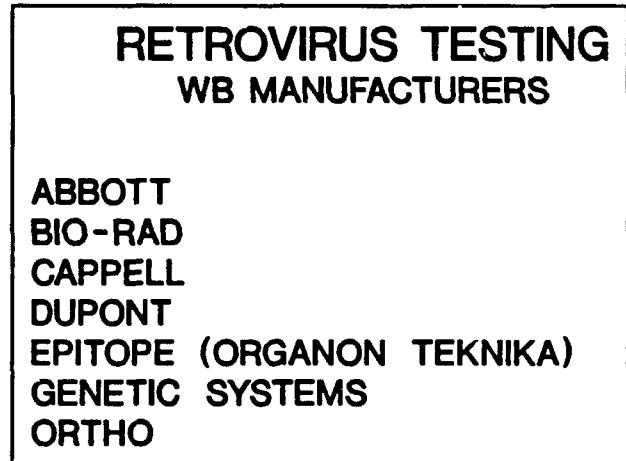


Figure 14 Suppliers of Western Blot reagents used by the state public health laboratories.

HIV-1 TESTING US ARMY FY90			
TEST	TOTAL	REACTIVE	IND
E I A	1,304,224	6,012	N/A
W B	6,875	2,278	863
CULTURE	2,417	1,092	
P C R	657		
FLOW	7,500		

Figure 15 US Army testing of HIV-1

HTLV I/II TESTING US ARMY FY90		
TEST	TOTAL	POSITIVE
CULTURE	15	8
P C R	177	
FLOW	100	

Figure 17 The US confirmatory tests for HTLV-I/II

HIV-2 TESTING US ARMY FY90			
TEST	TOTAL	REACTIVE	IND
E I A	1,590	16	N/A
W B	16	0	1
P C R	5		
FLOW	10		

Figure 16 US Army testing for HIV-2

Figures 15, 16 & 17 represent respectively the HIV-1, HIV-2, and HTLV-I/II testing done by the US Army. Although not a member of ASTPHLD, the US Army testing laboratories represent a considerable amount of testing performed on members of the armed forces. These data are significant in that this is an excellent cross section of the young adult population of the United States. These data are not included with the survey data.

# TESTING (HIV-1)

## PANEL SESSION 1

- ROOM:** EMPIRE A/B
- TIME:** 8:30 TO 10:00 AND 10:45 TO 12:00, WEDNESDAY, MARCH 6, 1991
- CHAIR:** J Mehsen Joseph, Ph.D., Director, Laboratories Administration, Maryland Department of Health and Mental Hygiene, Baltimore, Maryland
- MEMBERS:** Cynthia K Cossen, Public Health Microbiologist, Viral and Rickettsial Diseases Laboratory, Department of Health, Berkeley, California; J Richard George, Ph.D., Chief, Developmental Technology Section, Centers for Disease Control, Atlanta, Georgia; Stephen Josephson, Ph.D., Director, Clinical Microbiology/Virology, Rhode Island Hospital, Providence, Rhode Island (*Rapporteur*)
- TOPICS:** Rapid testing methods; Blots; Testing of non-traditional samples; Surrogate markers; Recombinant/synthetic based assays; Simultaneous detection methods; Western Blot criteria; IFA; Viral detection methods

### RECOMMENDATIONS

- 1.01 Rapid tests for the detection of HIV-1 antibody not be a waived test under CLIA regulations. It is essential that a proficiency testing program be developed to ensure the quality of testing.
- 1.02 At the present time, we cannot recommend the use of urine or saliva specimens for HIV antibody testing. Available data, however, indicate that these specimens may be acceptable. We recommend that manufacturers and interested investigators publish in peer review journals and submit data to the FDA which would allow urine and saliva to be used for HIV antibody testing.
- 1.03 We encourage manufacturers to evaluate non-traditional markers such as p17 antigen and develop quantitative assays for staging.
- 1.04 FDA licensed recombinant or synthetic peptide ELISA tests for the detection of HIV-1 antibody can be used in an HIV-1 algorithm. The following algorithm has been proposed as an alternative to the existing algorithm for the detection of HIV-1 antibody. This algorithm should be evaluated during the coming year and data be submitted at the next meeting.
  1. HIV-1 ELISA A
    - a. Negative - REPORT
    - b. Reactive - GO TO 2.
  2. HIV-1 ELISA B
    - a. Negative - REPORT
    - b. Reactive - GO TO 3. or 4.
  3. HIV-1 IFA
    - a. Positive - REPORT
    - b. Negative or Non-specific - GO TO WB and/or Follow-up specimen
  4. HIV-1 WB
    - a. Positive - REPORT
    - b. Indeterminate - GO TO Follow-up specimen
    - c. Negative - Report or Repeat
- 1.05 The ASTPHLD criteria for Western blot interpretation should be reevaluated in light of recent reports of uninfected individuals with envelope only or envelope and gag bands. The issue of contamination by another specimen may be a contributing factor and must be addressed.

- 1.06 Quantitative antigen assays for measuring virus burden are needed to monitor disease progression and efficacy of therapy. Further development of better quantitative assays should be encouraged. It is recommended that a national reference standard for antigen quantitation be developed for use by manufacturers for standardizing their antigen detection kits.
- 1.07 IFA has a place in HIV diagnostic schemes as a supplemental test. Some of the currently available commercial kits show promise for use as confirmatory tests.
- 1.08 Laboratories with significant experience with HIV IFA may use their own IFA based protocols for confirmation of screening test results.
- 1.09 Non-specific IFA results should be clarified whenever possible. The use of absorption will resolve many of these problems and manufacturers are encouraged to develop standard protocols for this and to make cells available for this purpose. Other supplemental tests should be used when absorption cannot resolve the antibody status of a specimen. A positive IFA can be reported as positive, however a negative or a non-specific result should be followed by an additional supplemental test.
- 1.10 The IFA should not be used as a screening procedure.
- 1.11 IFA kit manufacturers are encouraged to submit their products to the FDA for licensure. Public health and other laboratories are encouraged to actively participate in gathering data needed to support these applications.
- 1.12 The following references should be used for Delta Value Calculations:  
 Crofts, N. et.al. *Evaluation of enzyme-linked immunosorbent assays: a method of data analysis*, J. Virol. Methods, 22:51-59 (1988)  
 Maskill, W.J. et al. *An evaluation of competitive and second generation ELISA*, J. Virol. Methods, 22:61-73 (1988)

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*Dwight Aseltine*

*Arthur Back*

*Jeanne Baldwin*

*J Dean Barry*

*Vonnita Barton*

*Lisa Bauman*

*Carol Bell*

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*Barry Bennett*

*Sharon Blumer*

*Rosemary Boker*

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*Sandy Buhler*

*Kay Buchanan*

*Tom Callaway*

*Laurie H Cartwright*

*Jack Cassorla*

*Joan C Catignani*

*Donald C Chambers*

*Lorraine M Clarke*

*Carlyn L Collins*

*Jeanne Connelly*

*Sandra Currin*

*Susanne D DesRosiers*

*P David Dotson Jr*

*Harold Dowda*

*Arlette Fauquex*

*Dennis Ferrero*

*Bill Fleming*

*Dana Gallo*

*Jill Senne Giesick*

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*Marjorie Giles*

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*Sylvia Kelly*

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*Martial LaCroix*

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Judy Sheldon  
R Ken Shockley  
Mark Sieczkarek  
Mel Smith  
Catherine Spruill  
Richard C Steece  
Susan Stramer  
Gary E Tegtmeier*

*Gary Toedter  
Darrell Trofimuk  
Richard Walkenbach  
Barbara J Weiblen  
Steve Weiss  
Barbara Werner  
Jane Westerman  
Judith A Wethers  
John A Wilhelm  
J Steven Williams  
Jo Ann L Yee  
Madonna L Young  
Suzanne Zanto*

*Eleven ASTPHLD laboratories are planning to initiate flow cytometry within the next 12 months. Of the laboratories now performing flow cytometry two routinely test all HIV positives.*



# POLYMERASE CHAIN REACTION

## PANEL SESSION 2

- ROOM:** CHOUTEAU
- TIME:** 8:30 TO 10:00 AND 10:45 TO 12:00, WEDNESDAY, MARCH 6, 1991
- CHAIR:** Haynes W. (Chip) Sheppard, Ph.D., Research Scientist, California Public Health Foundation, Berkeley, California
- MEMBERS:** Anne M Comeau, Ph.D., Co-Principal Investigator, Newborn HIV Project, Theobald Smith Research Institute, Jamaica Plain, Massachusetts (*Rapporteur*); Harold Dowda, Ph.D., Director, Diagnostic Microbiology, South Carolina Department of Health and Environmental Control, Columbia, South Carolina; Robert Martin, Dr.P.H., Director of Laboratories, Michigan Department of Health, Lansing, Michigan; Susan Mottice, Ph.D., Director, Microbiology, Utah State Health Department, Salt Lake City, Utah; John Pfister, RM (AAM), Retrovirus Supervisor, State Laboratory of Hygiene, Madison, Wisconsin
- TOPICS:** Quality assurance; Methodology; Applications; Interpretation/Reporting; Proficiency Testing

### RECOMMENDATIONS

- 2.01 For specimens obtained "on site", with same day processing, EDTA, Heparin, or ACD are suitable. For specimens which are shipped from another site, samples in ACD may be suitable for up to 5 days. For specimens in EDTA or heparin, the maximum transport time is 48 hours.
- 2.02 Every effort should be made to obtain whole blood samples 24-48 hrs after venipuncture. Samples should be transported at room temperature.
- 2.03 Dried blood spots appear to be suitable for PCR testing and may be stable for long periods of time. Further data are awaited regarding the sensitivity and specificity of PCR testing from dried blood spots.
- 2.04 Magnesium concentration must be optimized for each set of primers. Current primers: 1.5-2.5mM
- 2.05 Annealing and extension conditions must be optimized in each laboratory.
- 2.06 The number of cycles in the amplification process should be between 30 and 35 for HIV detection.
- 2.07 Liquid oligonucleotide hybridization (OH) is a highly sensitive and specific detection method and is recommended.
- 2.08 A variety of non-radioactive detection methods are now available and some have sensitivity and specificity comparable to OH detection. Further comparative testing is recommended.
- 2.09 In order to obtain the authority to perform and report PCR results, the PCR sub-committee recommends initiation and continuation of discussions with those who hold patents affecting the use of PCR for the purpose of insuring continuation of public health investigations of the diagnosis, etiology, and pathogenesis of diseases of public health importance.
- 2.10 Laboratories performing PCR should employ a minimum of two primer pairs for initial testing and/or resolution of discrepant results.
- 2.11 Primers with documented sensitivity and specificity should be used.
- 2.12 A minimum of two separate PCR reactions (two primer pairs or duplicates of one primer pair) should be performed for each specimen. Splitting of the original specimen is recommended when possible.
- 2.13 Discrepant results should be resolved by additional analysis of the original specimen using the same or different primer pairs. If discrepant results cannot be resolved by repeat testing, the results should be reported as indeterminate and another specimen should be requested.
- 2.14 The overall interpretation of PCR testing should be reported as HIV DNA detected, HIV DNA not detected, or Indeterminate.
- 2.15 Complete testing algorithms need not be reported.

- 2.16 Appropriate positive, negative, and reagent controls should be included with every PCR test.
- 2.17 All published guidelines for minimizing the contamination of specimens with amplified PCR product should be rigorously followed.
- 2.18 Biochemical sterilization of PCR products should be instituted in all PCR laboratories as soon as possible.
- 2.19 The committee recommends that each laboratory performing PCR conduct validation studies on an appropriate number of well characterized test samples and maintain appropriate records of such test results.
- 2.20 The development of validation panels for distribution to PCR laboratories by the private sector, NIH, and CDC is recommended.
- 2.21 The development of standardized reagents and controls in the form of commercial kits is encouraged.
- 2.22 The immediate initiation of a proficiency testing program for PCR is recommended.
- 2.23 The application of PCR, in combination with other tests, to the diagnosis of infants born to seropositive mothers is recommended. Additional studies on the use of PCR in the first 3 months of life are needed.
- 2.24 The use of PCR for retroviral diagnosis in "high risk" seronegative adults is not recommended as a routine procedure.
- 2.25 The use of PCR for the resolution of indeterminate serology in adults is not recommended as a routine procedure.
- 2.26 PCR may be helpful in the diagnosis of rare individuals with defective antibody production.
- 2.27 PCR is useful for the differentiation of viral sub-types (e.g., HTLV-I/II or HIV-1 vs HIV-2).
- 2.28 The determination of viral burden by PCR may be helpful in the prognosis of HIV disease. However, quantitative PCR requires complex procedures which are not established in most laboratories.
- 2.29 The use of PCR to monitor the effects of antiviral therapy on viral burden is an important area of future study but has not been validated at this time.

## Attendees

*Julie Ackerman*  
*Marion Alleman*  
*David W Anderson*  
*Connie Ballard*  
*Vonitta Barton*  
*R Bauer*  
*Sara T Beatrice*  
*John Boffo*  
*Robin Botchlet*  
*Larry Briggs*  
*Laurence C Briggs*  
*Nerissa Cabrera*  
*James M Conroy*  
*C Darrow*  
*S Delaney*  
*Chyang Fang*  
*Carol Ferrera*  
*Bagher Forghani*  
*Fritz Friedhoff*  
*Myriam Garcia*  
*Pat Garrett*

*Ron Genevie*  
*G van der Groen*  
*K Gupta*  
*Nancy J Haley*  
*Cindy Handwerk- Leber*  
*W Harry Hannon*  
*Margaret Hanson*  
*Pat Hays*  
*Wanda K Jones*  
*Pia Kabin*  
*David Kanatazar*  
*Shyh-Ching Lo*  
*Carl Ludvigsen*  
*H Craig Lyerla*  
*Lisa McFarland*  
*J Todd McPherson*  
*Bruce McCreedy*  
*Jim Mahony*  
*Dave Marshman*  
*Jeff Massey*  
*Frank J Michalski*

*Gregory Milman*  
*Barry Mitchell*  
*Patricia Necessary*  
*Larry Nelson*  
*Peter Neumann*  
*E Nichol*  
*Ken Ostrander*  
*Gary Ory*  
*Terri Ozegovich*  
*Jane Rachel*  
*Barry Reed*  
*Mike Richey*  
*Pete Rogers*  
*Becky Rorapaugh*  
*Teresa Salas*  
*Carol Schimek*  
*Nick Schmitz*  
*G Schochetman*  
*Caterina Sellitto*  
*M Steller*  
*Roy Stevens*

*Pat Stewart  
Henry Strother  
Steven Stump*

*Roger Taylor  
John Vernasco  
D B Villareal  
Michael Wandall*

*Jeffrey S Webb  
Judith Wilber  
Dave Wyllie*

*Thirty nine (75%) of ASTPHLD laboratories participated in the CDC Family of Serosurveys during FY90.*

# FLOW CYTOMETRY

## PANEL SESSION 3

**ROOM:** EMPIRE C  
**TIME:** 8:30 TO 10:00 AND 10:45 TO 12:00, WEDNESDAY, MARCH 6, 1991  
**CHAIR:** Jonathan M Kagan, Ph.D., Chief, Clinical Sciences Section, Treatment Research Program, Division of Acquired Immunodeficiency Syndrome (AIDS), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland  
**MEMBERS:** A Russell Gerber, M.D., Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia; Janet K A Nicholson, Ph.D. Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia (*Rapporteur*);  
**TOPICS:** Quality assurance; Training; Monitoring antiviral therapy; Reporting criteria and interpretation; Disease staging

### RECOMMENDATIONS

- 3.01 Each public health laboratory should evaluate the need to establish flow cytometry capability based on an assessment of criteria including: prevalence of HIV infection and cumulative incidence of AIDS within the area served, ability to monitor and evaluate the quality of flow cytometry and pertinent hematology results, cost effectiveness, and the availability of other early HIV intervention services. Additional data are needed before specific recommendations can be made on non-retroviral public health and environmental applications of flow cytometry.
- 3.02 Flow cytometry training should be mandatory for all relevant personnel including instrument operators, laboratory supervisors and laboratory directors.
- (a) All instrument operators should, in addition to flow cytometer manufacturer's training, receive supplementary training through additional courses or workshops offered by independent organizations. In-house training at experienced laboratories may be an acceptable alternative.
  - (b) Clinical flow cytometry training courses for laboratory directors and supervisors should be developed.
  - (c) The ASTPHLD recommends the development of certification programs for laboratory technicians trained in flow cytometry.
  - (d) The ASTPHLD recommends the development and implementation of accreditation standards for all training courses and workshops in clinical flow cytometry.
- 3.03 The ASTPHLD should assemble and make available updated information on clinical flow cytometry training courses and workshops.
- 3.04 Before accepting specimens for clinical flow cytometric analysis, each laboratory must have in place a comprehensive quality assurance protocol that includes standardization, quality control procedures, and proficiency testing.
- (a) Standardization of instrument optical alignment, spectral sensitivity, and fluorescence compensation must be performed daily.
  - (b) Quality control includes daily monitoring and recording of instrument performance and cell preparation methodologies. Reagent stability should be assessed with lot changes and as otherwise needed.
  - (c) Proficiency testing within a nationally recognized program on a quarterly basis is required as an integral component of comprehensive quality assurance.
- 3.05 The ASTPHLD endorses the National Committee for Clinical Laboratory Standards (NCCLS) Proposed Guideline, H42-P, Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes.

3.06 The determination of absolute counts for lymphocyte subsets requires both hematologic and flow cytometric measures.

For hematologic measures:

- (a) Determination of the absolute lymphocyte count requires both a white blood cell count (WBC) and a differential (including percent lymphocytes). The ASTPHLD endorses the NCCLS Tentative Standard (1984), H-20T, *Leukocyte Differential Counting*. The optimal specimen for these hematologic measures is EDTA-preserved whole blood (lavender top tube) less than six hours old.
- (b) Recognizing that laboratories may receive hematologic specimens more than 6 hours old, the ASTPHLD recommends consideration of the following options:
  - (1) Hematologic analysis may be performed within six hours locally and a second specimen (drawn simultaneously) for flow cytometry may be transported to the flow cytometry laboratory. It may be desirable to obtain a fresh smear for quality assurance.
  - (2) Laboratories can verify the maximum age of specimens for which hematologic results are comparable to fresh specimens.
- (c) The laboratory performing the hematology should maintain a documented intralaboratory coefficient of variation less than five percent for the white blood cell (WBC) count.
- (d) Laboratories should evaluate and characterize intralaboratory bias and establish confidence intervals for the WBC and the differential lymphocyte counts.
- (e) Automated differentials are strongly recommended. Manual differentials should count at least 400 cells.

For flow cytometric measures:

- (a) The optimal specimen for lymphocyte immunophenotyping by flow cytometry is either an EDTA-preserved whole blood specimen less than six hours old or an heparinized whole blood specimen up to 24 hours old.
  - (b) Recognizing that laboratories may receive suboptimal (old) flow cytometric specimens, ASTPHLD recommends that laboratories verify the maximum age of specimens for which immunophenotyping results are comparable to fresh specimens.
  - (c) Optimally, specimens should be maintained at room temperature (18-22 C) until tested.
- 3.07 Whole blood lysis and two-color immunofluorescence are the methods of choice for flow cytometric immunophenotyping.
- 3.08 The ASTPHLD recommends the following two-color monoclonal antibody panel for routine immunophenotyping:

Monoclonal Antibody Combination <sup>a</sup>	Cell Type Enumerated
1. IgG <sub>1</sub> /IgG <sub>2</sub>	Isotype controls
2. CD45/CD14	% lymphocytes in gating region <sup>b</sup>
3. CD3/CD4	T-helper/inducer subset <sup>c</sup>
4. CD3/CD8	T-suppressor/cytotoxic subset <sup>c</sup>
5. CD3/CD16 + CD56	Total T cells/Total NK cells <sup>d</sup>
6. CD19	Total B-Cells

<sup>a</sup> FITC/PE-labeled reagents

<sup>b</sup> Lymphocytes will be CD45<sup>bright</sup>CD14<sup>negative</sup>

<sup>c</sup> Indicated cell type will be positive for both antibodies

<sup>d</sup> Total T cells = all cells expressing CD3; Total NK cells = all cells which are CD3 negative but positive for CD16 and/or CD56.

- 3.09 Lymphocyte light scatter gates must be validated by anti-CD45 (pan-leukocyte) and anti-CD14 (monocyte) reactivity.
- (a) Optimally, non-lymphocyte contamination within the gate should not exceed 5 percent. 85% is the lower limit of acceptable lymphocyte representation in the lymphocyte gate.
  - (b) At least 95 percent of lymphocytes should be contained within the light scatter gate.
- 3.10 Lymphocyte subset percentage values from the flow cytometer should be corrected by dividing the observed percentage by the percentage of lymphocytes (CD45<sup>bright</sup>CD14<sup>negative</sup>) in the lymphocyte gating region.
- 3.11 For most specimens, the total of the corrected CD3<sup>positive</sup> (Total T), CD19<sup>positive</sup> (Total B), and CD3<sup>negative</sup> CD56<sup>positive</sup> &/or CD16<sup>positive</sup> (Total NK) percentages should sum to between 95 and 105 percent.
- 3.12 Each laboratory must establish age- and population-appropriate reference ranges in accordance with validated statistical criteria. It should be noted that pediatric reference ranges may differ substantially from the reference ranges for adult populations.
- 3.13 The manufacturers of flow cytometry instrumentation and reagents are urged to cooperatively expedite the development of:
- (a) Improved lymphocyte gating reagents
  - (b) Automated sample preparation technology
  - (c) Flow cytometers capable of determining absolute numbers for lymphocyte subsets
  - (d) Improved laboratory quality control reagents
  - (e) Anticoagulants and preservatives suitable for both hematologic and flow cytometric measurements
- 3.14 Laboratory reports should optimally include lymphocyte subset percentages, absolute values and laboratory reference ranges.
- (a) Laboratory reports should specify the immunophenotype (CD designation) for all lymphocyte subsets reported therein (e.g., T-helper/inducer = CD3<sup>positive</sup>/CD4<sup>positive</sup>).
  - (b) Values for lymphocyte subsets should be corrected for the lymphocyte representation in the gating region.
  - (c) Absolute values for lymphocyte subsets should be reported unless hematologic results are suspect.
- 3.15 The ASTPHLD strongly supports efforts by the Centers for Disease Control to establish a national lymphocyte immunophenotyping performance evaluation program including training and education programs.
- 3.16 The ASTPHLD strongly supports the efforts of the NCCLS and the NIAID Flow Cytometry Advisory Committee in setting standards for clinical flow cytometric immunophenotyping.
- 3.17 The ASTPHLD encourages the development of alternative (non flow cytometric) methods for the enumeration of CD4<sup>+</sup> lymphocytes.
- 3.18 The ASTPHLD endorses universal precautions and the flow cytometry safety guidelines outlined in NCCLS document H42-P, Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes.

### Attendees

*Greg Ballish*  
*Mark Connelly*  
*G David Cross*  
*Giaocchino DeChirico*  
*Kenneth P Dressler*  
*Margarita Garcia*  
*Jane Getchell*

*George F Grady*  
*Sherry Harris*  
*Gerald L Hoff*  
*Timm Hurst*  
*Monica Krieger*  
*Robert Lindner*  
*Kathleen Meckstroth*

*James L Pearson*  
*Michael Peddecord*  
*Mary Richardson*  
*William Slanta*  
*Ann Toledo*  
*Wallis M Velleca*  
*John G Warhol*

# TESTING (HIV-2 and HTLV-I/II)

## PANEL SESSION 4

- ROOM:** EMPIRE A/B
- TIME:** 1:30 TO 3:00, WEDNESDAY, MARCH 6, 1991
- CHAIR:** Chyang Fang, Ph.D., Director, National Reference Laboratory for Infectious Diseases, American Red Cross, Rockville, Maryland
- MEMBERS:** J Richard George, Ph.D., Chief, Developmental Technology Section, Centers for Disease Control, Atlanta, Georgia; Jonathan Kaplan, M.D., Retrovirus Diseases Branch, Centers for Disease Control, Atlanta, Georgia; Helen Lee, Ph.D., Director, Transfusion Biology Research and Development, Abbott Laboratories, North Chicago, Illinois (*Rapporteur*); Roy Stevens, Ph.D., Director of Laboratories for Diagnostic Immunology, New York State Department of Health, Albany, New York; Barbara Werner, Ph.D., Director, Clinical Investigation and Virology, State Laboratory Institute, Boston Massachusetts
- TOPICS:** HTLV-I and HTLV-II differentiation; Confirmation methodology and criteria for HIV-1/HIV-2 and HTLV-I/II; HIV-1/HIV-2 combination tests; Rapid testing methods

### RECOMMENDATIONS

- 4.01 We encourage manufacturers to develop second generation confirmatory tests to replace the current WB/RIPA procedures with assays utilizing recombinant proteins and/or synthetic peptides in formats that allow objective reading.
- 4.02 The inclusion of p19 + gp46/61 pattern as confirmatory for HTLV-I/II seropositivity should be further evaluated.
- 4.03 The utility of p21e in the current confirmation criteria needs to be validated; the specificity of recombinant p21e, in particular, should be further evaluated.
- 4.04 Since HTLV-I and HTLV-II infections have different disease manifestations, we recommend that public health laboratories carry out, if possible, routine differentiation of HTLV-I from HTLV-II in HTLV-I/II seropositive samples.
- 4.05 We encourage the development and evaluation of recombinant and/or synthetic peptide reagents for the differentiation of HTLV-I from HTLV-II antibodies.
- 4.06 We recognize that current HTLV-I screening assays will occasionally fail to detect HTLV-II antibodies and encourage systematic epidemiological and laboratory studies to determine the sensitivity of HTLV-I reagents for HTLV-II antibody detection. If a sizeable percentage of HTLV-II antibodies are missed, the development of a more sensitive second generation HTLV assay is recommended.
- 4.07 National surveillance for HIV-2 infections should be continued.
- 4.08 HIV-1 seronegative or indeterminate persons with AIDS related symptoms or those at risk for HIV-2 infection should be considered as candidates for HIV-2 antibody testing.
- 4.09 Upon the availability of HIV-1/2 combination tests, the public health laboratories should consider the importance of adopting the combination assays based on epidemiological data pertinent to the local area.
- 4.10 The ASTPHLD Committee On Human Retrovirus Testing should appoint a committee composed of representatives from public health laboratories and manufacturers to determine the characteristics of standard HIV-2 confirmatory tests. For example, for Western blot, these would include the virus strain, purification and processing of antigens, and interpretive criteria.

## Attendees

Julie Ackerman  
Steve Alexander  
David Anderson  
Tom Armstrong  
Arthur Back  
Connie Ballard  
Vonnita Barton  
R Bauer  
Lisa Bauman  
James Beebe  
Sharon Blumer  
John Boffo  
Rosemary Boker  
Kay Buchanan  
Nerissa Cabrera  
Laurie H Cartwright  
Jack Cassorla  
Lorraine M Clarke  
Cindy Collins  
Jeanne Connelly  
Cynthia Cossen  
Steve Dahlan  
Clem Darrow  
P David Dotson Jr  
Kenneth Dressler  
Arlette Fauquex  
Carol Ferrera  
Bill Fleming  
Dana Gallo  
Jill Giesick  
James A Gibson  
Marjorie Giles  
Robert K Gleeson

Tim Granade  
Guido van der Groen  
K Gupta  
Nancy J Haley  
Cindy Handwerk-Leber  
Sherry Harris  
Sharon Henderson  
Frank R Hunt  
Jong-Ho Jean  
James E Johnson  
Stephen Josephson  
Farrah Kamalian  
J W D Kay  
Kim Kelly  
Sylvia Kelly  
Howard Kim  
Monica Krieger  
Martial LaCroix  
Tsun-Kong Lee  
Debbie Lepine  
James P Lugo  
Bruce McCreedy  
Peyton S Metzel  
Patricia Necessary  
Larry Nelson  
Richard H Newhouse  
Naziha Nuwayhid  
Debbie Oronzio  
Ken Ostrander  
Gary Ory  
Terri Ozegovich  
Percy S Pan  
Elizabeth Parker

Max R Proffitt  
Jane Rachel  
Steven Raia  
Barry Reed  
Karen L Richards  
Mary Richardson  
Donald Ritter  
Becky Rorapaugh  
Jane Ruppel  
Keith Sayre  
Nick Schmitz  
Richard T Schumacher  
Luc Segers  
Caterina Sellitto  
Judy Sheldon  
Catherine Spruill  
Richard C Steece  
M Steller  
Susan Stramer  
Steve Stump  
Gary E Tegtmeier  
Ann Toldeo  
Darrell Trofimuk  
John Vernasco  
Michael Wandall  
John Warhol  
Stephanie Wells  
Jane Westerman  
John A Wilhelm  
J Stephen Williams  
Dave Wyllie  
Jo Ann L Yee  
Madonna L Young

*Ninety two percent of the ASTPHLD laboratories follow the recommendations of ASTPHLD-CDC in performing and interpreting the Western Blot.*



# DIAGNOSIS OF HIV INFECTION IN NEWBORN

## PANEL SESSION 5

- ROOM:** CHOUTEAU  
**TIME:** 1:30 TO 3:00, WEDNESDAY, MARCH 6, 1991  
**CHAIR:** Sara Beatrice, Ph.D., Director of Retrovirology and Immunology, New York City Department of Public Health, New York, New York  
**MEMBERS:** Anne M Comeau, Ph.D., Co-Principal Investigator, Newborn HIV Project, Theobald Smith Research Institute, Jamaica Plain, Massachusetts; Francis Lee, M.D., Assistant Professor of Pediatrics, Pediatric Infectious Diseases, Emory University, Atlanta, Georgia; Barbara Weiblen, M.S. Senior Scientist, Newborn HIV Project, Theobald Smith Research Institute, Jamaica Plain, Massachusetts  
**RAPPORTEUR:** James Conroy, Research Scientist, New York State Department of Health, Albany, New York  
**TOPICS:** Flow Cytometry; Polymerase chain reaction and Culture Assays; ELISPOT and In Vitro Assays; IgA and Other Serologic Assays

### RECOMMENDATIONS

- 5.01 The committee recommends the continuation of prospective studies for evaluation of relevant tests for the early diagnosis of infection especially to identify rapid progressors. Prospective studies should include comparison analysis of PCR, culture, Elispot and HIV specific IgA antibodies.
- 5.02 PCR appears to be the most sensitive test for diagnosis of HIV infection in the first three months of life.
- 5.03 Negative results obtained on specimens from infants less than one month of age must be regarded as tentative. Additional specimens should be tested from these infants at ages greater than one month. Positive results obtained within the first month of life must be confirmed with tests on an independent specimen as soon as possible (preferably in the first two months of life). All positive PCR results regardless of age should be confirmed with tests on an independent specimen.
- 5.04 Virus culture is an effective tool for diagnosis of HIV infection in young children. A negative result does not rule out infection; a positive result should be confirmed.
- 5.05 ELISPOT is a promising new test for the diagnosis of infection in infants in the first 3 months of life. It may complement PCR testing during the neonatal period. The committee recommends further testing on early specimens to evaluate its predictive value.
- 5.06 IgA HIV antibody tests are of limited diagnostic value in neonates but may be positive by three months of age. Because the IgA test is a simple modification of existing technology, it may be useful as a supplemental test in regional laboratories.

### Attendees

*Dwight Aseltine  
Jeanne Baldwin  
Greg Ballish  
J Dean Barry  
Vonnita Barton  
Ramon Benet-Ferran  
Sandy Buhler  
Tom Callaway  
Joan C Canignani*

*Mark Connelly  
Sandra Currin  
Harold Dowda  
Jane Getchell  
George F Grady  
Louis E Guskey  
W Harry Hannon  
Pat Hays  
Richard L Heberling*

*Stephen Josephson  
Jerry Kudlac  
Robert Lindner  
Shyh-Ching Lo  
Patsy McCarty  
J Todd McPherson  
Dave Marshman  
Robert Martin  
Jeff Massey*

*Donald Mayo  
Frank Michalski  
William J Miller  
Gregory Milman  
Barry Mitchell  
Phillip P Mortimer*

*Peter Neumann  
Martha Redus  
Marion Reuter  
Mike Richey  
Bill Roberts  
Teresa Salas*

*Mark E Schwerlzer  
Haynes W Sheppard  
Pat Stewart  
Gary Toedter  
Richard Walkenbach  
Steve Weiss*

*Michigan is the only state to require prenatal HIV testing.*

# STANDARDS OF PRACTICE

## PANEL SESSION 6

- ROOM:** EMPIRE C
- TIME:** 1:30 TO 3:00, WEDNESDAY, MARCH 6, 1991
- CHAIR:** Dale Lawrence, M.D., Chief, Clinical Development Section, Vaccine Research and Development Branch, Basic Research and Development Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Rockville, Maryland
- MEMBERS:** Susan Mottice, Ph.D., Director, Microbiology, Utah State Health Department, Salt Lake City, Utah; Lt. Col. Chester Roberts, Ph.D., Chief, Diagnostic Retrovirology, Walter Reed Army Institute of Research, Rockville, Maryland; Judith Wethers, M.S., Director, Testing Services, Retrovirology Laboratory, State of New York Department of Health Laboratories, Albany, New York
- RAPPORTEUR:** Keith Lawrence, Administrator, New York City Department of Health, New York, New York
- TOPICS:** Reporting; Testing of vaccinated individuals

### RECOMMENDATIONS

- 6.01 This workshop reaffirms the reporting procedures recommended by the 5th Consensus Conference, with the addition by reference to Section 9.02.f.Item2:  
"A narrative laboratory interpretation, including a reference to immune response elicited by vaccines."
- 6.02 We welcome the drafting by CAP of anti-HIV test reporting guidelines and encourage all ASTPHLD attendees to review and provide feedback to CAP.
- 6.03 Persons interpreting Western blot tests should be aware that participation by initially seronegative volunteers in clinical trials of HIV/AIDS vaccines will likely result in the acquisition of detectable immune responses to HIV. These responses may include the development of a positive ELISA test and Western blot bands reflecting the antigenic formulation and immunogenicity of the vaccine. Some vaccines may induce Western blot bands which meet published criteria for a positive test.
- 6.04 During succeeding years, the testing of various types of vaccine formulations can be anticipated. To preclude misclassification of a vaccinated volunteer with a positive HIV antibody test result as infected, it will be necessary that interpretation take into account the past HIV vaccine immunization history.  
To assist in this, the following procedures should be implemented by HIV/AIDS vaccine investigators:
- a: At the conclusion of a trial, volunteers should be provided with information on the vaccine formulation(s) administered along with, as a minimum, the results of their ELISA and Western blot tests post-vaccination.
  - b: Participants should have long-term accessibility to this information with safeguards to protect confidentiality.
- 6.05 The NIH is supported in its effort to develop for publication, in concert with the CDC and other relevant agencies, an MMWR advisory acknowledging the unique circumstances surrounding HIV/AIDS vaccine study participation.

## Attendees

*Marian Alleman  
Carol Bell  
Barry Bennett  
Robin Botchlet  
Carlryn L Collins  
G David Cross  
Susanne D DesRosiers  
Dennis Ferrero  
Fritz Friedhoff  
Myriam Garcia  
Pat Garrett  
A Russell Gerber  
Bob Gleeson  
Samuel B Gregorio  
Brigitte Griffith*

*Margaret Hanson  
Theresa L Hodges  
Gerald Hoff  
Xinyue Hou  
Timm Hurst  
Mary Isaac  
David Kantazar  
Warren L Kleinsasser  
Sadie Lehr  
Bill Link  
Carl Ludvigsen  
H Craig Lyerla  
Ron Lynch  
Kathleen Meckstroth  
Rick Moody*

*Sherian Nestor  
James Pearson  
Michael Peddicord  
Theodore E Plucinski  
Wolf Prenskey  
Ken Schockley  
Mark Sieczkarek  
Mel Smith  
Henry Strother  
Roger Taylor  
Wallis M Velleca  
Jeffery S Webb  
Judith A Wethers  
Judith Wilber  
Suzanne Zanto*

*Twenty eight percent of ASTPHLD laboratories routinely request a second sample to confirm a positive test result.*

# POSTER PRESENTATIONS

(The presenter is listed in bold print.)

- 1 **J E Johnson**  
Comparison of Whole Cell Viral Lysate (VL), Synthetic Peptide (SP), and Recombinant Protein (RCP) EIA's for Detection of HIV-1 Antibody
- 2 **J P Montana, L Gosting, C A Cole, N Monji, P Su, P F Coleman**  
Detection of Antibodies to HIV-1 and HIV-2 by a Single, Rapid Peptide-Based Immunoassay
- 3 **C Ferera, N Dock, J Huprikar, J Phair, M Kreiger**  
Use of a Peptide-Based, Rapid Immunoassay for Antibody to HIV-1 to Clarify True Antibody Status of EIA Repeat Reactive, Western Blot Indeterminates
- 4 **W Link**  
Effect of Non-Specific Glycoprotein Bands on Interpretation of HIV-1 Western Blots
- 5 **Brigitte P Griffith, Thomas M Chacko**  
Comparative Performance of Peptide, Recombinant and Viral Lysate Based Enzyme Immunosorbent Assays for the Detection of HIV-1 Antibody
- 6 **Emerson W Chan, Werner Schulze, Michael Leuther, Kevin Knigge**  
Monitoring IgG, IgA, and IgM Responses to HIV-1/HIV-2 Infections by Modified Abbott Matrix Assays
- 7 **Susan Pederson, L J Croy, L Adams, S Cobel, C Ferrera, M Krieger, K Shriver**  
Development of an Enzyme Immunoassay for Simultaneous Detection of Antibodies to HIV-1 and HIV-2
- 8 **K Richards**  
Performance of the Microtrak HIV-1 (env & gag) Recombinant Antigen Enzyme Immunoassay - Summary of Field Trial Data
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# DELTA VALUE CALCULATION

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## Evaluation of enzyme-linked immunosorbent assays: a method of data analysis

Nick Crofts, William Maskill and Ian D. Gust

*National HIV Reference Laboratory, Fairfield Hospital, Fairfield Victoria, Australia*

(Accepted 12 April 1988)

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### Summary

Diagnostic tests are usually evaluated in terms of simple qualitative measures of sensitivity and specificity. When comparing different quantitative assays such as ELISAs, it is often more useful to deal with actual values (sample optical density/cut-off optical density ratio (OD ratio)) rather than the qualitative relationship to the cut-off, i.e. positive or negative. This allows for a statistical approach to the questions of sensitivity and specificity. The National HIV Reference Laboratory of Australia has developed an approach for determining statistical estimates of sensitivity and specificity in terms of delta ( $\delta$ ). Delta is defined as the distance of the mean OD ratio of the sample population from the cut-off measured in standard deviation units. This paper discusses the derivation of this measurement and its usefulness when evaluating ELISA tests.

ELISA; Evaluation; Diagnostic test; Delta

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### Introduction

In Australia, the responsibility for evaluating diagnostic tests for HIV infection falls upon the National Reference Laboratory (NRL) located at Fairfield Hospital, Melbourne. In the course of evaluating a number of enzyme linked immunosorbent assays (ELISA) it became clear that methods of comparison, traditional in the field of serology, were unable to reliably distinguish tests of high specificity and sensitivity, and that new methods of analysis were required.

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The two most common problems encountered with evaluations of ELISA tests are failure to consider the effects of sample size, and the selection of a sample that is unrepresentative of the population in which the test is to be used.

Failure to consider the effects of sample size has greatest effect when attempting to rank or distinguish between tests of high sensitivity and specificity. When considering sampling error and the confidence limits for the sensitivities and specificities, there often is no statistically significant difference between the estimates obtained for the different tests being evaluated due to an inadequate sample size. For example, to distinguish between tests having specificities which differ by 0.1% with any degree of statistical confidence, more than 15000 specimens would need to be tested. In addition, information is lost when analysing data from evaluations in a qualitative manner since the individual readings for each sample are ignored by treating all positive (and negative) results as equivalent. The answers to important questions when evaluating ELISA assays can only be obtained by analysing actual test values. For example, do the false positive samples have readings close to the cut-off? If they do, and if the test shows a clear separation of the readings for the antibody positive and negative samples then the problem with the test is simply to do with the position of the cut-off; if it were to be slightly adjusted, the test might appear extremely good. Alternatively, do the false positive readings lie in an area where most of the positive results also lie? If this is the case, the test is fundamentally flawed. A method of analysing the effect of different settings of the cut-off is the Receiver Operating Characteristic (ROC) curve which has been applied in many settings (Swets and Pickett, 1982; Lusted, 1971).

#### *Alternative approaches*

To establish how a quantitative assay performs on a particular sample of specimens, or to compare different assays on one sample of specimens, it is more useful to deal with actual values than their qualitative relationship to the cut-off. This allows a statistical analysis of test efficacy which uses all information produced by an evaluation.

The statistical approach, which has been used by some workers (Weiss et al., 1985), is often marred by the assumption that the results are normally distributed. This assumption often underlies methods of calculation of the cut-off for a particular test, for example, the mean of the negatives plus three standard deviations. If the distribution of results is non-normal, this approach can give very misleading results. The ELISA methodology gives a sigmoidal response curve when optical density is plotted against concentration of antibody. When this test method is applied to a population in which antibody concentrations are normally distributed, the resulting curves tend to be log normal. If appropriate transformations are not performed before summary analysis, results can be misleading.

It would, however, be useful to have a method of analysing quantitative diagnostic tests which can be easily summarized in meaningful parameters. The ROC curve fulfils these criteria (Staquet et al., 1980), but does not immediately demonstrate important relationships to the non-statistician. Here we outline an approach developed at the NRL, for evaluations of anti-HIV screening tests.

*Approach developed at the NRL*

Most evaluations have consisted of testing panels of specimens 'known' (on the basis of information derived from a variety of clinical and serological sources) to be positive for antibody to HIV, obtained from subjects with as wide a spectrum of disease and from as diverse epidemiological groups as possible; and of specimens 'known' to be negative for anti-HIV, again from as representative a sample as possible.

When these panels are tested by indirect ELISA, and the frequency of results of a particular reading are plotted against that reading, curves of the general shape shown in Fig. 1A are obtained. In fact, each represents a family of curves, because a different curve is generated for each test run; there is a shift to left or right along the x-axis, due to variation in the cut-off value which varies from run to run. The distribution of positive results should extend far to the right of the graph (Fig. 1A, -▲-) but because there is often an upper limit to the readings obtainable with ELISA readers, it may be compressed to the left (Fig. 1A, -+ -). Plotting the variation (shift) between runs is a useful quality control device, but for evaluating tests it is more satisfactory to standardise each reading by assigning the cut-off an ar-

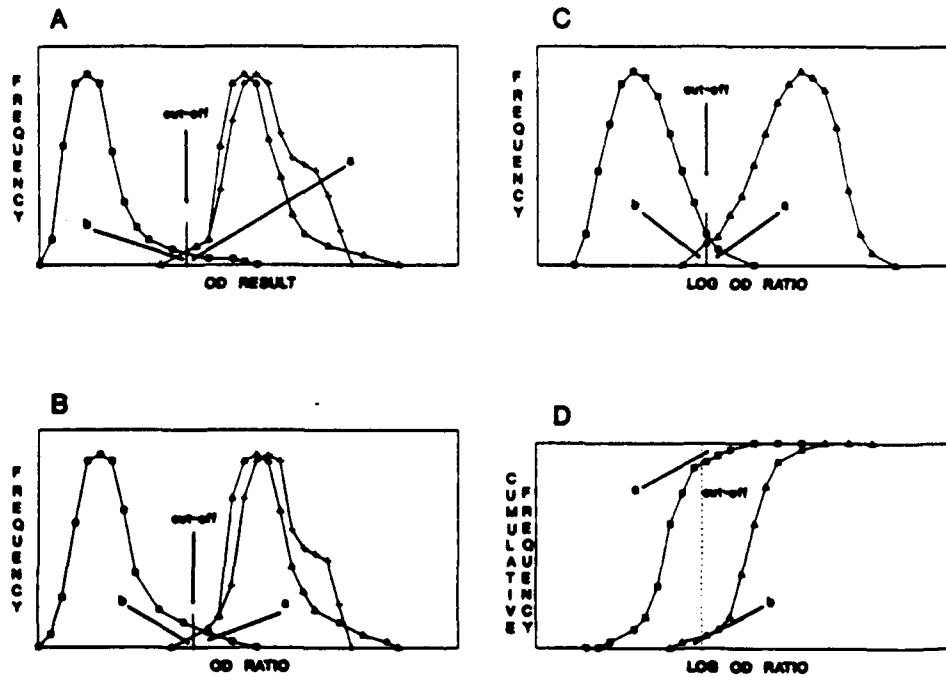


Fig. 1. Diagrammatic representations of curves obtained by plotting values produced by testing by ELISA populations of known negatives (-□-) and known positives (-▲-); A, B and C as percentages. D as cumulative percentages; a = false positive rate, b = false negative rate. The expected OD values for positive samples (-▲-). Compressed OD values for positive samples obtained from ELISA readers (-+ -) (see text).

bitrary value of 1, and plotting each reading in relation to this value. When this is done all the curves can then be treated as one population. This standardisation is normally performed by dividing each reading by the cut-off; this produces an OD ratio for each sample tested.

Comparisons between curves can then be made. Plotting frequency of results against ratio produces curves with similar shapes as previously, but standardised against the same reference point (Fig. 1B).

Two problems remain with these standardised curves: firstly, it is often difficult to fit positive and negative results on the same graph (because the spread of positives is so much greater than that of the negatives) and secondly the curves are generally log normal or approximately log normal. The simple answer to both problems is to change the ratio from a linear to a logarithmic scale (Fig. 1C); this brings the two distributions onto one graph and 'normalises' most of the curves (i.e. they become symmetrical about their means), so that they can be described satisfactorily by their mean ( $\bar{X}$ ) and standard deviation (S).

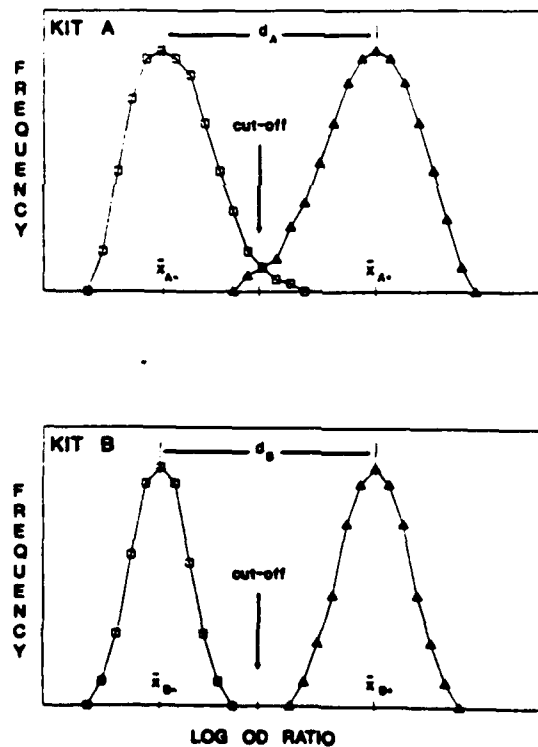


Fig. 2. Separation of negative and positive test populations using mean criteria only. Representation of curves obtained by testing populations of known negatives ( $\square$ ) and known positives ( $\blacktriangle$ ) by two different ELISAs, A and B;  $\bar{x}_{A-}$  = mean of negatives for kit A;  $\bar{x}_{A+}$  = mean of positives for kit A;  $\bar{x}_{B-}$  = mean of negatives for kit B;  $\bar{x}_{B+}$  = mean of positives for kit B;  $d_A$  = distance between means for kit A;  $d_B$  = distance between means for kit B.

The same data can also be presented as cumulative percentages (Fig. 1D). Both these latter approaches (Fig. 1C, D) demonstrate clearly how effectively the test separates the positive and negative populations, and illustrate the percentage of false positive (a) and false negative (b) results which can be expected.

It would be convenient to have a single measure which summarises how effective a test is at separating positive and negative populations so that different tests could be rated by a single figure. One such measure might be the difference between the means of the positive and negative populations on the assumption that the greater the difference, the better the kit is at separating the two populations. This measure is not reliable as it fails to take into account differences in the distribution of results which may be obtained with different tests as shown in Fig. 2. In this example both kits produce the same value for the difference between the means of the antibody positive and negative populations; however, kit B is obviously the better test as there is no overlap of the two populations.

A measure which takes into account the spread of results as well as the distance between means, is the 'index of detectability' ( $d'$ ) (Lusted, 1971); this is denoted by Armitage as 'Delta' ( $\Delta$ ) (Armitage, 1971).

This measure requires two assumptions; that the distributions are normal and that the variances of the two distributions are approximately equal. While the first assumption is met when ELISA generated curves are log transformed, the second is rarely met.

Because of this we are forced to use two measures - one for the positives and one for the negatives - to describe the efficacy of the test fully. These measures we call 'delta' ( $\delta$ ) measures; they are defined as the distance of the mean of the distribution from the cut-off in standard deviation units (Fig. 3). Thus delta for the

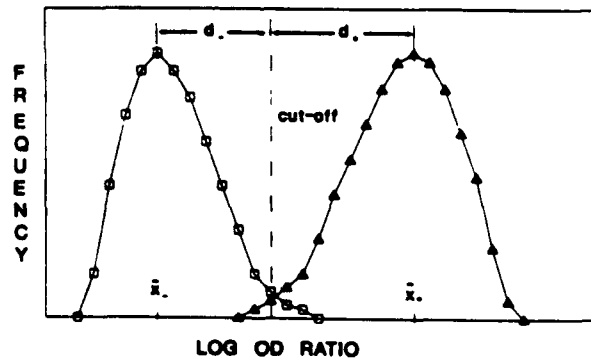


Fig. 3. Separation of negative and positive test populations using mean and standard deviation criteria. Representation of distributions of log OD ratios obtained by testing populations of known negatives (-□-) and known positives (-▲-);  $\bar{X}_n$  = mean of negatives;  $\bar{X}_p$  = mean of positives;  $d_n$  = distance from mean of negatives to cut-off;  $d_p$  = distance from mean of positives to cut-off;  $S_n$  = standard deviation of negatives;  $S_p$  = standard deviation of positives;  $\delta$  = delta, the distance of the mean of the sample population from the cut off in standard deviation units. Delta for positive samples:  $\delta_p = d_p/S_p = (\bar{X}_p - \text{cut-off})/S_p$ . Delta for negative samples:  $\delta_n = d_n/S_n = (\text{cut-off} - \bar{X}_n)/S_n$ .

negative population,  $\delta_- = \delta_-/S_-$ ; as the cut-off is defined as zero, this is equivalent to  $\delta_- = \bar{X}/S_-$ . Similarly, the delta for the positive population,  $\delta_+ = d_+/S_+ = \bar{X}_+/S_+$ .

The advantages of expressing results in these terms, graphically and with the summary measure of  $\delta_+$  and  $\delta_-$  for the test, lie in the immediate ease of comparison of the ability of the test to distinguish positives from negatives and the correctness of the placement of its cut-off, and the fact that delta measures take into account each individual reading, and thus statistically summarise the results more accurately than do the traditional qualitative measures of sensitivity and specificity.

In addition, if a panel of specimens is tested with a particular kit, the mean and 95% confidence limits derived from the log distribution can be plotted on a linear scale as a Hi-Lo graph. This method is particularly useful for comparing the results obtained by a variety of tests on the same panel of sera and for quality control purposes. Fig. 4 shows the steps involved. With this style of presentation, three or more distributions can be depicted for comparison, and separation between positive and negative populations easily seen.

The delta value is particularly useful when attempting to distinguish between tests

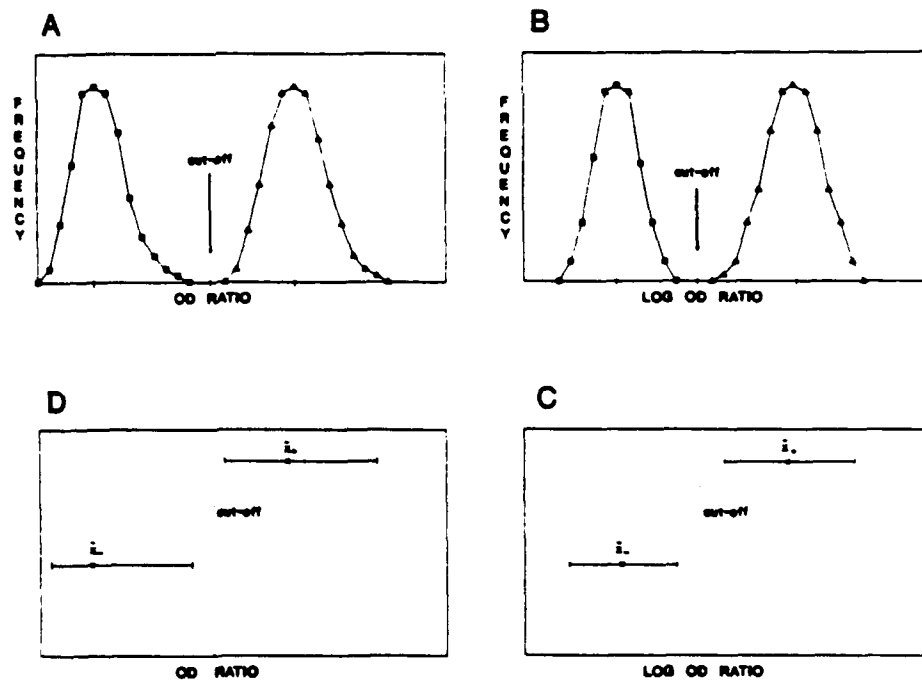


Fig. 4. Hi-Lo graph presentation. Representation of steps involved in forming Hi-Lo graphs on a linear scale from linear distribution of positives (- $\Delta$ -) and negatives (- $\square$ -) derived from ELISA testing, taking account of log normalisation. Step 1: A $\rightarrow$ B log transformation of OD ratio data; Step 2: B $\rightarrow$ C mean and 95% confidence limits of log transformed data; Step 3: C $\rightarrow$ D conversion to linear scale of mean and 95% confidence limits.

of high sensitivity and specificity. For example two ELISA tests, each correctly classifying all antibody positive and antibody negative samples of a coded panel of sera, would both be reported as showing 100% sensitivity and specificity. However, test A gave a delta of 4.60 for the antibody positive samples (i.e.  $\delta_+$  4.60) whereas test B produced a delta of 3.01 for the same samples (i.e.  $\delta_+$  3.01). According to statistical tables, the probability of test A is 99.99% for correctly identifying an antibody positive sample whereas test B has a probability of 99.87% for obtaining the correct result. There is less chance of test A producing a false negative result (0.01%) than test B (0.13%) when testing the same population.

The relationship of delta and the probability of a test producing the correct result can similarly be applied to the data for the antibody negative samples of the serum panel tested by both assays mentioned above. The test with the largest value for  $\delta_-$  would be the test with the highest probability of obtaining the correct result when testing antibody negative subjects and the assay with the least chance of producing a false positive result.

Therefore,  $\delta_+$  determines the probability of obtaining the correct result when testing antibody positive samples and can be viewed as a statistically derived estimate of sensitivity and  $\delta_-$  determines the probability of obtaining the correct result when testing antibody negative samples and can be viewed as a statistically derived estimate of specificity.

The values of these principles in the evaluation of both first and second generation assays for anti-HIV is demonstrated in the following paper (Maskill et al., 1988).

### Conclusion

Several principles of evaluation of diagnostic tests must be taken as axiomatic. These include consideration of sample size; consideration of what use the test will be put to, from which comes knowledge of what population it will be used in; and thus guidance in forming evaluation samples so as to be representative of this population.

With these requirements satisfied, it is important to consider the internal dynamics of the test, and thus the meaning of the results in relation to the biological characteristics they are derived from. Analysis and presentation of results of evaluations should then attempt to reflect these dynamics, and make clear the relationship between test and phenomena described. It is hoped that the method described in this paper will assist others in achieving these goals.

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JVM 00751

## An evaluation of competitive and second generation ELISA screening tests for antibody to HIV

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(Accepted 12 April 1988)

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### Summary

Two competitive anti-HIV ELISA screening assays (Behring and Wellcozyme) and two second generation assays using antigens generated by recombinant DNA technology (Abbott) and synthetic peptides (Biochrom) were evaluated against common panels of anti-HIV positive sera and sera known or thought likely to give false positive reactions. The assays were also tested on fresh sequential blood donations. Conventional estimates of sensitivity and specificity did not reveal a significant difference between the assays. Statistical analyses using  $\log_{10}$  transformed data to determine delta values (the distance of the mean optical density (OD) ratio from the cut-off measured in standard deviation units) showed the Abbott assays to have the highest probability (>99.99%) of detecting anti-HIV positive samples and the Behring assay as having the highest probability (>99.99%) of correctly identifying anti-HIV negative specimens. The combined data from conventional estimates of sensitivity and specificity and delta values suggests that the Abbott assay is the test of choice for screening purposes.

Anti-HIV; ELISA; Evaluation; Delta

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### Introduction

Since the discovery of the human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS), many tests for the de-

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tection of antibody to this virus (anti-HIV) have been developed and introduced into clinical practice (Weiss et al., 1985; Maskill et al., 1986).

The first generation screening tests were almost exclusively enzyme-linked immunosorbent assays (ELISA) in which disrupted virus particles were bound to a solid phase (usually the well of a microtitre plate) and the presence of antibodies in test sera detected by direct binding of labelled anti-human antibody.

Although these tests have achieved sensitivities and specificities in excess of 99%, because of the scale on which these tests are used in blood banks, false positive results, though rare, have major implications. These include permanent deferral of potential blood donors (even though the results of screening tests cannot be confirmed by supplementary assays), and the need to utilize a range of expensive supplemental tests to assist in the management of subjects who may or may not be infected (Kuhnl et al., 1985; Crofts and Gust, 1987). Many first generation assays have also shown inadequate sensitivity especially for specimens taken at the early stages of infection (Saah et al., 1987).

A need therefore exists to develop assays which are more specific than the first generation of screening tests and are capable of detecting antibodies in the early stages of infection.

Two approaches have been followed in the development of improved assays. Firstly, indirect ELISAs have been replaced by competitive ELISAs in which patients' antibodies compete with labelled human anti-HIV for binding sites on HIV antigen fixed to a solid phase (Cheinsong-Popov et al., 1984). Secondly, purified viral lysates have been replaced by synthetic peptides (Gnann et al., 1987) or by antigens generated by recombinant DNA technology (Frosner et al., 1987).

We have evaluated four of the latest anti-HIV screening assays to become available in Australia, which represent each of these approaches. The assays are the Abbott recombinant HTLV-III EIA (Abbott Laboratories, North Chicago, IL), the Biochrom synthetic peptide anti-HIV EIA (Biochrom Berlin, F.R.G.), the Behring competitive anti-HIV EIA (Behringwerke AG, Marburg, F.R.G.) and the Wellcozyme monoclonal competitive anti-HIV EIA (Wellcome Diagnostics U.K.). These evaluations were carried out by a number of Australian Reference Laboratories and Blood Transfusion Services, in a study coordinated by the National HIV Reference Laboratory (NRL) at Fairfield Hospital, Melbourne.

We present here the results of the evaluations undertaken at the NRL and the Blood Transfusion Services.

## Methods

### *The strategy of evaluation*

Each assay was used to test a coded panel of 600 sera prepared by the NRL, and 1700-4500 fresh sequential donations in blood transfusion laboratories. Data from these studies was forwarded to the NRL and analysed using a DEC Microvax computer and in-house programs developed from Datatrieve (DEC, Sydney, Australia).

### *The panel*

The panel of 600 sera selected by the NRL was made up of duplicate aliquots of 300 specimens presented under code. This panel comprised sera from 148 anti-HIV positive subjects which belonged to one of three clinical categories: Category A, patients with AIDS (50 sera); Category B, patients with clinical or laboratory evidence of disease, e.g. ARC (71 sera); Category C, infected individuals with no signs or symptoms of infection nor evidence of deficient cell mediated immunity (27 sera). Sera were also included from 52 subjects not infected with HIV but whose sera either had given or potentially could give, false positive reactions on existing screening assays. Of the 52 sera, 40 had produced false positive EIA results with existing licensed assays and 12 sera were from patients or individuals in which EIA false positive reactions are common (patients infected with syphilis, legionella, brucella, Epstein Barr virus (EBV), herpes simplex virus (HSV), malaria, pregnant women, and pools of sera positive for rheumatoid factor, anti-streptolysin O (ASO), cold agglutinins, OX Weil-Felix, and a pool of sera which react with only the p55 band by Western blots (WB), all of which were obtained from healthy blood donors who had signed a declaration form stating that they were not members of any group considered to be at risk of infection with HIV). In addition, two sera not containing antibody to HIV and eight known to contain antibody were presented neat and in nine doubling dilutions titrated in a pool of normal human sera (NHS).

Subjects whose sera were included in the panel were designated as infected or uninfected on the basis of all available clinical, epidemiological and laboratory data including absolute and differential lymphocyte counts and both screening and supplementary assays for anti-HIV.

Serological analysis included an in-house WB assay using the LAV strain of HIV cultured in CEM cells (Commonwealth Serum Laboratories, Parkville, Australia) and three different radioimmunoprecipitation assays (RIP). Two of the RIP assays used purified <sup>125</sup>I-labelled p24 viral antigen provided by Dr. L. Arthur (Frederick Cancer Research Facility, National Cancer Institute, MD, U.S.A.). The remaining RIP assay employed a recombinant <sup>125</sup>I-labelled gp41 viral antigen (Centocor, Malvern, PA, U.S.A.).

One of the <sup>125</sup>I p24 RIP assays and the <sup>125</sup>I gp41 RIP assay were performed by staff of the Medical Virology Division, Institute of Medical and Veterinary Science, Adelaide.

### *Criteria for Western blot interpretation*

Sera showing no reactivity or reactivity to non-viral bands were reported as negative. Sera showing reactivity to any of the glycoprotein antigens (gp41-45, gp 120, gp160) and to at least three other viral proteins (p12, p18, p24, p34, p40, p53, p55, p68) were reported as positive. Sera showing reactivity at viral specific bands which did not fulfill the criteria for a positive were reported as indeterminate.

### *Performance of the tests*

The 600 coded specimens were tested at the NRL by four technicians, with different pairs performing each assay.

In each blood bank, the assays were performed in parallel with the assay that the blood bank was currently using. Any specimen reactive on either assay was forwarded to the NRL for further testing.

Each assay was performed according to the manufacturer's package insert. These are briefly described below:

The Abbott assay utilized a single bead coated with both recombinant DNA (rDNA) HIV core and envelope antigens derived from an *E. coli* expression vector. Coated beads were incubated with diluted (1:40) test sera for 30 min at 40°C, washed three times with 5 ml of distilled H<sub>2</sub>O, and incubated with goat anti-human IgG, conjugated with horseradish peroxidase, for 30 min at 40°C. This was followed by further washing, addition of the substrate (*o*-phenylenediamine) and incubation at room temperature for 30 min. The reaction was stopped by the addition of 1 M H<sub>2</sub>SO<sub>4</sub> and the optical density read at 492 nm.

The Biochrom assay is a conventional indirect ELISA; however, the antigen immobilized to the solid phase microtitre wells is a mixture of synthetic peptides which correspond to epitopes of HIV proteins p18, p24, gp41 and gp120. Diluted sera (1:40) were added to the wells of microtitre plates and incubated for 1 h at 37°C. The plates were washed four times with diluted wash buffer, incubated for 1 h at 37°C with sheep anti-human IgG conjugated with alkaline phosphatase. This was followed by further washing and the addition of the *p*-nitrophenyl-phosphate substrate and incubation at room temperature for 30 min. The reaction was stopped by addition of 3 M NaOH and the optical density read at 405 nm.

In the Behring competition assay, detergent-treated, heat-inactivated viral lysate prepared from a HIV infected H9 cell line was coated onto microtitre wells. Twenty-five microlitres of undiluted test serum and 100 µl of human anti-HIV conjugated to horseradish peroxidase were added to each well and incubated for 1 h at 37°C. Following 4 washes with diluted wash solution and addition of tetramethyldiaminobiphenyl dihydrochloride, the plates were incubated at room temperature for 30 min. The reaction was stopped by the addition of 0.5 M H<sub>2</sub>SO<sub>4</sub> and the optical density read at 450 nm.

The Wellcozyme competition monoclonal assay differs from the Behring assay in that viral antigen is prepared from an HIV infected CEM cell line, disrupted by sonication, inactivated by treatment with β-propiolactone, and attached to the wells of microtitre plates with mouse monoclonal antibody. Test serum (50 µl) and human anti-HIV (75 µl) conjugated to horseradish peroxidase were added to each well and incubated for 1 h at 45°C. After washing four times with diluted wash fluid, 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide were added to each well and the plates incubated for 20 min at room temperature. The reaction was stopped by the addition of 2 M H<sub>2</sub>SO<sub>4</sub> and the optical density read at 450 nm.

#### *Data analysis*

Data were provided to the NRL in the form of optical density readings and cut-offs for each plate or run. Optical density (OD) ratios were calculated by dividing each reading by the relevant cut-off. For the Behring and Wellcozyme assays the reciprocal value was calculated.

The delta ( $\delta$ ) values for the anti-HIV positive and negative sample populations were calculated by dividing the mean OD ratio ( $\log_{10}$ ) by the standard deviation of each population. This value provides a means of comparing how well the assays separate positive and negative populations from the cut-off. The higher the positive delta value obtained with anti-HIV positive sera, the higher the probability that the test will correctly identify such specimens; similarly, the higher the negative delta value obtained with a panel of anti-HIV negative sera, the higher the probability that the test will correctly identify antibody negative specimens. This measure has similarities to the indices described by Lusted ( $d'$ ) (1971) and Armitage ( $\Delta$ ) (1971) but overcomes the problem of dealing with populations having different variances. For further details concerning this method of analysis please refer to the previous paper in this issue (Crofts et al., 1988).

The mean end point titre for each kit determined from the titration series is defined as the highest dilution at which the mean OD ratio for all 8 anti-HIV positive sera was found to be above the cut-off.

## Results

The  $\log_{10}$  OD ratio data obtained from the testing of the anti-HIV positive and negative samples presented in the coded panel and that of the blood donors is shown in Fig. 1.

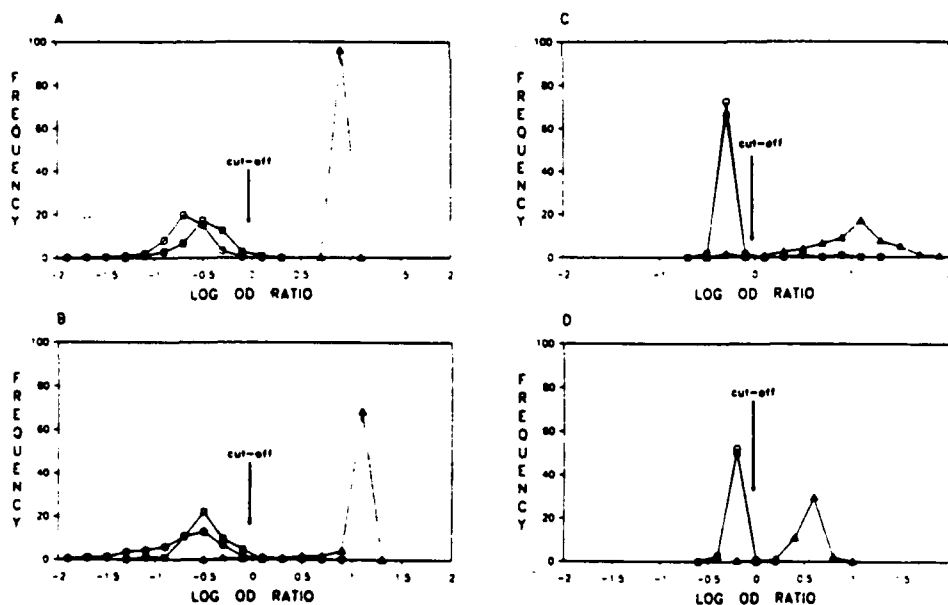


Fig. 1. Distribution of  $\log_{10}$  OD ratios for anti-HIV positive sera ( $\blacktriangle$ — $\blacktriangle$ ), known and potential false positive sera ( $\square$ — $\square$ ), and anti-HIV negative blood donor sera ( $\circ$ — $\circ$ ). A = Abbott; B = Biochrom; C = Behring; D = Wellcozyme.

With the Abbott and Biochrom assays, an artificial tightening of the spread of positive results is observed because most ELISA readers do not read above an OD of 2.0. In the Abbott assay, as all positive readings were at this upper limit, the variation in results was produced by the minor changes in the cut-off observed from run to run. For the Biochrom assay, the majority of positive results gave an OD of 2.0 or greater, producing a similar effect.

### Sensitivity

The sensitivity of the four assays was evaluated on a panel of 148 sera obtained from subjects at various stages of infection with HIV. The results obtained are shown in Table 1.

The assay using synthetic peptides (Biochrom) found 144 of 148 known positive sera repeatably reactive, found 1 specimen to be not repeatably reactive and 3 sera repeatably negative. Each of the repeatably negative sera were from a patient with Category A AIDS in the terminal stage of the disease. When examined by WB, these sera were found to have antibodies to only gp41-45 and p55. The results obtained with the assay which employs antigen generated by recombinant DNA technology (Abbott Laboratories) were identical to the results obtained in the Reference Laboratory.

The two assays based on competitive inhibition detected the majority of infected individuals and produced discordant results (between duplicate aliquots examined under code) in only 5 or 6 of the 148 pairs of sera. Only one serum sample (from a patient with Category B infection) was found to be repeatably negative by one of the competitive assays (Behring). This sample, when tested by WB, contained antibodies reactive to all major HIV antigens.

The mean and 95% confidence limits of the OD ratios for sera from each of the clinical categories of disease are shown in Fig. 2. The largest variation of OD ra-

TABLE 1

Results of testing duplicate aliquots of 148 antibody positive and 52 antibody negative sera

Clinical category	No. of sera	Results of duplicate tests											
		Abbott			Biochrom			Behring			Wellcozyme		
		++	+-	--	++	+-	--	++	+-	--	++	+-	--
<i>Infected</i>													
A	50	50	0	0	47	0	3	46	4	0	50	0	0
B	71	71	0	0	70	1	0	68	2	1	67	4	0
C	27	27	0	0	27	0	0	27	0	0	26	1	0
Total	148	148	0	0	144	1	3	141	6	1	143	5	0
<i>Uninfected (problem sera)</i>													
	52	0	1	51	1	0	51	0	5	47	0	0	52

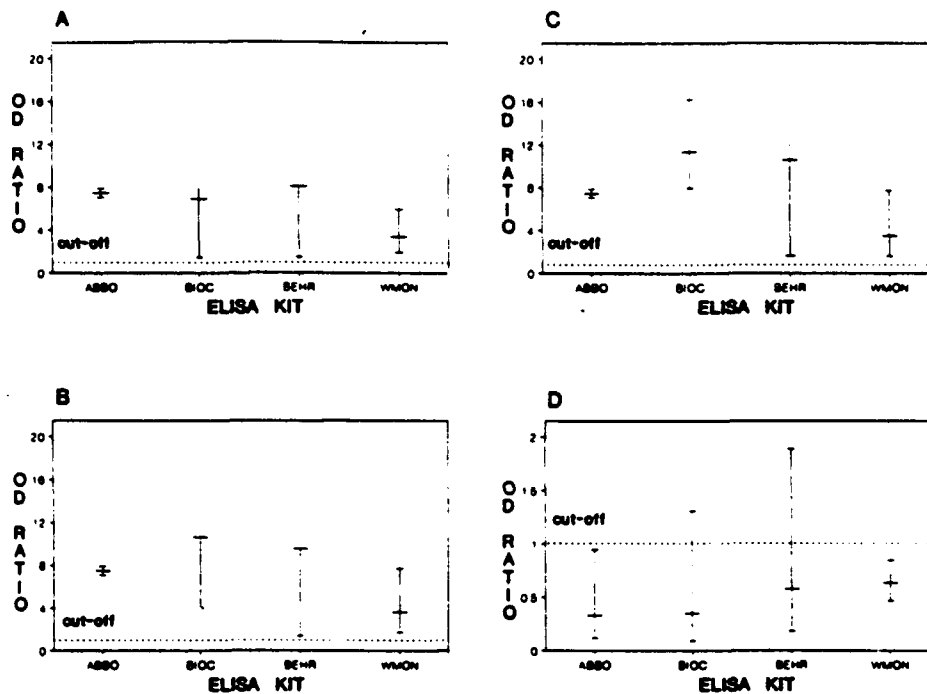


Fig. 2. Means and 95% confidence limits of the OD ratios for each assay for sera obtained from patients at different stages of disease and for the known and potential false positive reactors. A = AIDS patients; B = Symptomatic anti-HIV positive patients; C = Asymptomatic anti-HIV positive patients; D = Known and potential false positive reactors.

tios was seen with the Biochrom and Behring assays, both of which also showed a decline in the mean OD ratio with progression of disease.

The sensitivity and 95% confidence limits for the sensitivity of each assay are given in Table 2.

The assays were also evaluated for their ability to detect anti-HIV in serially diluted sera. Fig. 3 shows the mean and 95% confidence limits of OD ratio values

TABLE 2  
Sensitivity of four anti-HIV assays

Kit	Sensitivity* (%)	95% C.L.	
		Lower (%)	Upper (%)
Abbott	100.0	98.69	100.00
Biochrom	97.64	95.17	98.86
Behring	97.30	94.73	98.63
Wellcozyme	98.31	96.08	99.28

\* Calculated as the percentage of anti-HIV positive aliquots found reactive from the total number of aliquots tested (296).

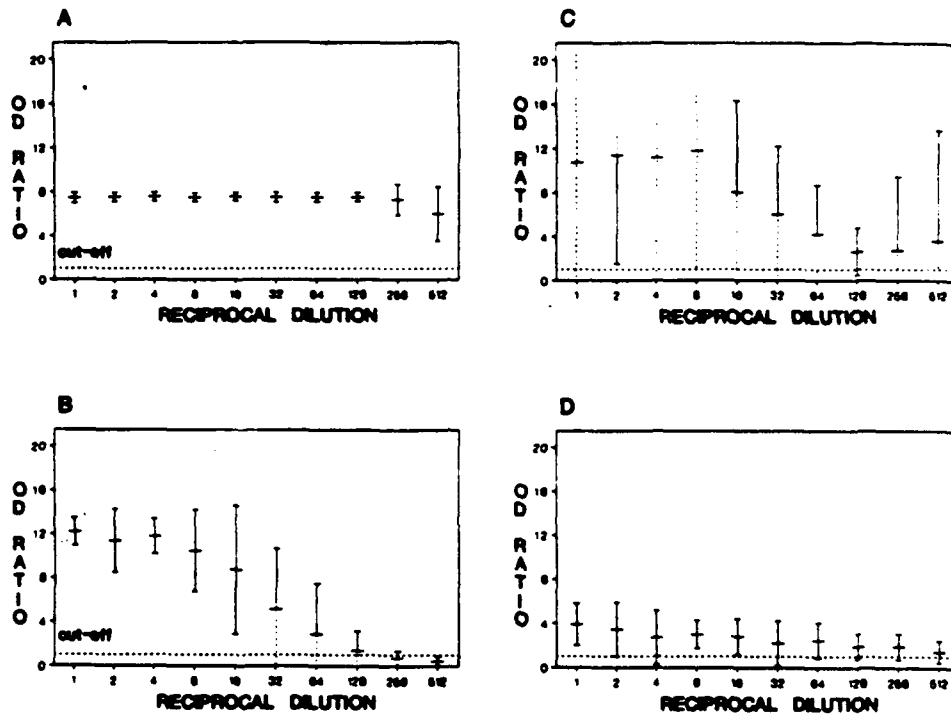


Fig. 3. Mean and 95% confidence limits of OD ratio values for each assay at each dilution for the eight anti-HIV positive sera presented in titration. A = Abbott; B = Biochrom; C = Behring; D = Wellcozyme.

obtained by each assay for each dilution of the 8 sera presented in titration.

Three assays (Abbott, Behring and Wellcozyme) gave mean titres of greater than 1:512 for all 8 sera whereas the remaining assay (Biochrom) gave a mean titre of 1:128.

### Specificity

The specificity of the 4 assays was evaluated in two ways – by testing a small panel of 52 'problem sera' thought likely to produce false positive results, and by testing several thousand freshly collected units of blood.

One assay (Biochrom) found one of the 'problem sera' repeatably reactive. This specimen was obtained from an individual at low risk of infection and was reactive by a currently licensed screening assay. The sample showed an indeterminate band profile by WB. The remaining assays did not misclassify any of these sera, although the Behring assay detected non-repeatable reactivity in one of 5 separate serum pairs and the Abbott assay detected reactivity in one aliquot of a serum pair (see Table 1).

The mean and 95% confidence limits of the OD ratios for the 'problem sera' panel for each kit are shown in Fig. 2D.



TABLE 3

Initial and repeatable reactor rates from testing blood donations and specificity estimates

Test	Donations tested	No. (%)		Specificity** %	95% C.L.	
		Initially reactive	Repeatably reactive*		Lower	Upper
Abbott	4455	8 (0.18)	1 (0.02)	99.98	99.87	99.99
Biochrom	2981	41 (1.38)	5 (0.17)	99.83	99.60	99.93
Behring	3531	7 (0.20)	1 (0.03)	99.97	99.84	99.99
Wellcozyme	1770	6 (0.34)	4 (0.23)	99.77	99.42	99.91

\* None of the repeatably reactive sera tested by Western blot were found positive.

\*\* Calculated from the number of donor sera tested minus those found repeatably reactive.

Table 3 illustrates the prevalence of initially reactive and repeatably reactive specimens detected when large panels of freshly collected plasma specimens from volunteer blood donors were tested. The frequency of repeatably reactive sera detected by the two competition assays ranged from 0.23% (Wellcozyme) to 0.03% (Behring) while the figures obtained with the assays based on rDNA (Abbott) and synthetic peptide technology (Biochrom) were 0.02% and 0.17%, respectively.

None of the blood donor specimens which were found to be repeatably reactive were positive by WB using the criteria currently recommended by the National Reference Laboratory. The specificity and 95% confidence limits for the specificity of each assay are also shown in Table 3.

#### *Delta values*

Recently we reported on a new method of comparing the performance of anti-HIV assays which involves analysis of log-transformed data (see previous paper in this issue). Briefly the performance of an assay is evaluated on a large panel of sera from subjects who are known to be infected or known to be free of infection. The ability of the test to distinguish the two populations is defined by units known as delta. Delta is the distance of the mean OD ratio of a sample population from the cut-off measured in standard deviation units.

The delta values obtained for each test for the anti-HIV positive sera from each of the clinical category of disease, for anti-HIV positive sera of all categories combined (total), for the known and potential false positive reactors and for the blood donations are given in Table 4.

#### **Discussion**

In the present study we report on four of the most recent assays to become available in Australia which represent two different approaches to improving anti-HIV ELISA screening assays. These are competition ELISAs (Behring and Wellcozyme) and second generation tests, which utilize antigens generated by recombinant DNA technology (Abbott) and synthetic peptides (Biochrom).

TABLE 4

Delta values produced by each kit for anti-HIV positive and negative serum sample populations

Kit	Antibody positive sera				Antibody negative sera	
	A*	B*	C*	Total	Problem sera	Blood donors
Abbott	72.75	72.92	72.83	72.83	-2.06	-2.64
Biochrom	2.17	6.30	13.37	3.61	-1.56	-1.68
Behring	2.17	2.18	2.76	2.26	-0.90	-5.28
Wellcozyme	4.23	3.31	3.10	3.47	-3.03	-3.39

\* Clinical category of patients from whom sera was obtained.

A comparison of the  $\log_{10}$  OD ratio distributions for known anti-HIV positive and negative sera and problem sera (see Fig. 1) demonstrates the ability of the four assays to discriminate between the three populations. The Abbott test produced clearest separation of anti-HIV positive and negative sera with no overlap between the two populations. The three other tests (Biochrom, Behring and Wellcozyme) displayed some degree of overlap due to both repeatable and non-repeatable false negative and/or false positive results. In general, non-repeatable results (i.e. one aliquot found to be positive, the other negative) are due to technical errors, whereas false positive or false negative results which occur on both occasions are due to biological causes. On this basis, very few biological false negative results were obtained by the three kits (see Table 1). Western blot analysis of the repeatable false negative sera suggests that the Biochrom assay may encounter difficulty with sera which show reactivity predominantly to HIV glycoprotein antigens such as those obtained from AIDS patients at the terminal stages of disease.

The performance of the four assays on the panel of anti-HIV positive sera suggests that the Abbott assay has the highest sensitivity and Behring the lowest (see Table 2). However, after considering the 95% confidence limits for these sensitivities, it is not possible in a sample of this size to distinguish or rank the kits from highest to lowest sensitivity.

The four assays showed some variation in OD ratio values for the sera obtained from patients at different stages of disease. With the exception of the Abbott and Wellcozyme assays, which were equally reliable in each clinical category, the performance of the other two tests (Biochrom and Behring) tended to diminish with progression of disease (see Fig. 2A, B and C).

The data from the titration of eight anti-HIV positive sera did not give end point titres for the Abbott, Behring and Wellcozyme assays but showed a mean titre of 1:128 for the Biochrom assay. The most consistent and reproducible results (as evidenced by the least variation in OD ratios at each dilution) were obtained with the Abbott and Wellcozyme assays (see Fig. 3). It should be noted that although the sera presented in titration represent a variety of different reactivities with different WB profiles, the results are not generalizable because it is not known how representative such sera are. Also, end point titre estimated on a few sera does not necessarily mirror the ability of an assay to detect antibody in patients early in the disease (Cheneboux and Delagneau, 1986).

Each assay performed well on the 'problem sera' which either had given or were thought likely to give false positive reactions with ELISA screening tests. The Wellcozyme assay found all 52 'problem sera' repeatedly negative. The Abbott, Behring and Biochrom assays found 1 to 5 sera reactive on at least one occasion while the Biochrom assay found 1 of these sera repeatedly reactive (see Table 1). The clearest separation of the 'problem sera' from the anti-HIV positive samples was obtained by the Abbott assay (see Fig. 1). The least variation of OD ratios was observed with the Abbott and Wellcozyme assay. The Wellcozyme assay produced the closest mean OD ratio to the cut-off of all four kits for the 'problem sera' but the highly reproducible performance of the assay resulted in a tight clustering of OD ratios thus producing minimal variation (see Fig. 2D).

The performance of the four assays on the large panels of freshly collected plasma specimens from volunteer blood donors suggests that the Abbott and Behring assays have the highest specificity. However, when considering the 95% confidence limits for these specificities it is not possible to distinguish or rank the kits from highest to lowest specificity.

When comparing quantitative assays such as ELISAs it is often more useful to deal with the actual values (OD ratios) rather than the qualitative relationship to the cut-off, i.e. positive or negative. This enables a statistical approach to the estimation of sensitivity and specificity.

Accordingly we have developed such an approach by determining sensitivity and specificity in terms of delta ( $\delta$ ), the distance of the mean OD ratio of antibody positive and antibody negative sample populations from the cut-off measured in standard deviation units (Crofts et al., 1988).

The delta values shown in Table 4 reflect the observations previously made concerning sensitivity, specificity, and reproducibility of the assays but have the advantage of providing a quantitative measurement for these parameters.

The Abbott assay shows the highest and most consistent positive delta values for sera obtained from patients at different stages of disease. The high delta value shown by this assay for all combined patient categories reflects the consistently high OD ratios which resulted in the clear separation of this population from the cut-off as shown in Fig. 1. The high positive delta values shown by the Abbott assay increases the confidence that can be placed in the sensitivity estimates shown in Table 2. Although assay to assay differences in these estimates are not statistically significant, the extremely large positive delta values for the Abbott assay show that there is a much greater tolerance in this test for variation of test results without the occurrence of false negatives. This provides the Abbott assay with a larger margin for variation than the other three assays when testing anti-HIV positive sera and ensures a consistent high level of sensitivity.

The decline in mean OD ratios and wide variation (low reproducibility) in OD ratios shown by the Behring and Biochrom assays with progression of disease status (see Fig. 2A, B and C) are clearly reflected in the delta values for these assays. The Behring assay produced the largest variation in OD ratios for sera from all disease stages and this is shown by the consistently low delta values for this assay. The Wellcozyme assay performed equally well on sera from all disease stages showing low variation in OD ratios.

Comparison of delta values on the 'problem sera' shows that the Wellcozyme assay clearly separated this population from the cut-off. The delta values also show that the Behring test gave the clearest separation of the anti-HIV negative blood donor sera from the cut-off as shown by the high negative delta value for this population (see Table 4). The delta value provides a quantitative measure of the separation shown in Fig. 1C. Calculations using the delta values show this assay to have a probability of greater than 99.99% for correctly identifying anti-HIV negative samples. This increases the confidence that can be placed in the specificity estimates shown in Table 3. Although the specificity estimates for the four assays are not significantly different, the large negative delta value for the Behring assay indicates that it has a greater margin for variation than the other assays without the occurrence of false positives which ensures a reproducibly high level of specificity. This advantage, however, was somewhat diminished when testing problem sera (Table 4).

Demands for improvements in sensitivity and specificity of anti-HIV screening assays have recently been made (Saah et al., 1987; Meyer and Pauker, 1987). The conventional means of determining sensitivity and specificity of assays under evaluation does not use all the available data but relies upon qualitative estimates, namely, 'how many did the test get right or wrong?'. The use of delta values described in this study provides a quantitative measure which can be used in conjunction with the conventional estimates of kit performance to determine if improvements in sensitivity and specificity have been achieved.

Of the second generation assays the Abbott assay produced the clearest separation of anti-HIV positive and negative sera and showed the highest levels of sensitivity, specificity and smallest variation of OD ratios. Large positive delta values reflect the ability of the assay to consistently produce high OD ratios for anti-HIV positive sera taken from patients at different stages of disease. Calculations using these delta values show this assay to have a probability of greater than 99.99% of detecting anti-HIV positive individuals. This is an improvement by comparison with previously reported performance levels (Gurtler et al., 1987).

The relatively low delta value obtained by the Abbott test for both the blood donor and 'problem sera' leaves only a small margin for OD ratio variation. Calculations derived from the delta values show that the false positive rate could be as high as 0.21% if adequate laboratory technique is not maintained. In this evaluation the observed false positive rate in the blood donor population was 0.02%. This is a marked improvement in comparison to previously reported performance levels (Gurtler et al., 1987).

Of the two competition assays the Wellcozyme assay showed the highest levels of sensitivity which was also reflected in the relatively higher positive delta values. By contrast, the Behring assay showed the highest level of specificity of the two assays and this was confirmed by the large negative delta values reported for the blood donor samples. The Behring assay may encounter difficulty with 'problem sera' as shown by the number of non-repeatably reactive aliquots and the very low negative delta value for these sera (see Tables 1 and 4).

In conclusion, conventional estimates of sensitivity and specificity and the use

of delta values derived from this evaluation suggest that the Abbott recombinant assay provides marked improvements in the sensitivity of previously available assays and that competitive anti-HIV ELISA methods offer the greatest potential for improvements in specificity. Although the Abbott recombinant assay showed a high level of specificity in this evaluation, careful attention to technical aspects of the procedure will be necessary in order to maintain such levels of performance.

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## Introduction

# THE SIXTH ANNUAL CONFERENCE ON HUMAN RETROVIRUS TESTING

ASTPHLD Committee on Human Retrovirus Testing

The constant evolution of testing methodologies for the human retroviruses demands that periodic reviews of the capabilities of these methodologies used in the public health laboratories be conducted. To this end, the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) sponsored the Sixth Conference on Human Retrovirus Testing March 5-7, 1991, in Kansas City, Missouri. Approximately 285 conference participants viewed 36 poster presentations, heard 12 plenary session speakers discuss a variety of topics relevant to retrovirus testing, and participated in six panel sessions concentrating on the most relevant topics of human retrovirus testing.

Panel Session 1 was entitled "Testing for HIV-1." Topics discussed included rapid testing methods, western blots, testing of nontraditional samples, surrogate markers, recombinant/synthetic-based assays, western blot interpretation criteria, indirect fluorescent antibody methods, and viral detection methods.

Panel Session 2 was entitled "Polymerase Chain Reaction." Topics discussed within this panel session included methodologies used in PCR, quality assurance, proficiency testing, and test interpretation and reporting.

Panel Session 3 concentrated on developments within its title, "Flow Cytometry." The topics covered included flow cytometry for disease staging, monitoring antiviral therapy, training, quality assurance, reporting criteria, and interpretation.

Panel Session 4 was entitled "Testing for HIV-2 and HTLV-I/HTLV-II." Topics covered included HTLV-

I and HTLV-II differentiation, the confirmation methodologies and criteria for HIV-1 and HIV-2 as opposed to the HTLV viruses, HIV-1 and HIV-2 combination tests, and alternate rapid testing methods.

Panel Session 5 was entitled "The Diagnosis of HIV Infection in Newborns." The topics covered, in a very specific sense, were the use of flow cytometry, PCR, and culture assays to enhance the detection of infection in newborns. Discussions also covered the use of ELISPOT, in vitro assays, and analysis for IgA.

Panel Session 6 was entitled "Standards of Practice and Reporting." A new topic covered during this session was the interpretation and reporting of EIA reactivities in vaccinated individuals.

There continues to be intense interest in the methodologies discussed in these six panel sessions. In addition, the increased attention by the medical and scientific community is focused on early intervention and treatment programs for HIV-positive patients. Title III of the Ryan White CARE Act outlines provisions for monitoring the immune status of HIV-positive patients in order to assess the stage of the acquired immunodeficiency syndrome (AIDS). Laboratory analysis, in all aspects of this monitoring process, are extremely important. Laboratory findings form the basis of the therapies currently available for retrovirus-infected individuals.

It is intended that the recommendations derived from the Conference on Testing for Human Retrovirus be used universally to set laboratory standards in methodologies, policies, quality assurance, interpretation, and reporting.

*This conference was supported, in part, by a financial grant from the Department of the Army, Medical Research and Development Command. The views, opinions, and/or findings contained herein are those of the author(s), and should not be construed as an official document of the Army position, policy, or decision unless so designated by other documents.*

*Committee members: David F. Carpenter, PhD, Chair, Illinois; Arthur F. DiSalvo, MD, Nevada; J. Mehsen Joseph, PhD, Maryland; James Pearson, DrPH, North Dakota; Michael G. Volz, PhD, California. Planning committee members: Robert Linder, MD, PhD, chair; Michael S. Ascher, MD; Roger Dodd, PhD; Chester F. Roberts, PhD; Charles A. Schable, MS; Judith Wethers, MS; Judith Wilber, PhD.*

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*ASTPHLD Committee on Human Retrovirus Testing. The sixth annual conference on human retrovirus testing. Infect Control Hosp Epidemiol. 1991;12:469.*

## SUMMARY OF SURVEY #8

To assess the current status of testing for human retroviruses, a survey was sent to 54 state and territorial public health laboratories during the third quarter of 1990. The survey requested data for fiscal year 1990 (July 1989-June 1990). In previous years, calendar-year data were requested.

A milestone was achieved in gathering the data for this most recent survey. All 54 state and territorial public health laboratories and the City of New York contributed to the survey data. This had not occurred in any previous year. The summary of these data follows.

The total number of enzyme immunoassay (EIA) tests as indicated in Table 1 totalled in excess of 4 million. These data cannot be compared directly with the data from previous years because these tests include data from 99 California clinical laboratories and blood banks. However, in evaluating these data, it can be noted that the EIA reactive rate in the national sample is 3%. Western blot confirmation of the reactives was 58%; 9% of the western blots were indeterminate. Using indirect fluorescent antibody (IFA), 79% of the reactives were confirmed; 1.5% were indeterminate.

Removing the California data generates Table 2, demonstrating 3.175 million EIA tests. These data indicate a slightly higher reaction rate of 3.5%. Western blot confirmation also is higher; positive in 82%; and indeterminate in 7.5%. By IFA, 78% of the original EIA reactive tests were confirmed, while only 1.5% were indeterminate. These data appear to demonstrate superior sensitivity and specificity of the IFA testing methodologies.

Figure 1 indicates the total number of tests done

by each of the responding laboratories. The data indicate that approximately 48% of the laboratories conduct less than 50,000 tests per year. Sixteen percent conduct greater than 100,000 tests per year; approximately 35% of the responding laboratories conduct 50,000 to 100,000 tests for retrovirus per year.

The human immunodeficiency virus (HIV) positivity rate reported by public health laboratories is shown in Figure 2. Of the responding laboratories, 42% have a very low positivity rate of less than 1%. Another 42% of the responding laboratories indicate a positivity rate between 1% and 5%; 17% of laboratories have a positivity rate in excess of 5%. The pie chart depicted in Figure 3 indicates the tests that are used to confirm EIA reactives. These data show that the western blot test was used most frequently (73% of all confirmatory tests conducted). The IFA confirmatory system was used for 26% of the reactive tests, while approximately 1% of the confirmations were conducted with either polymerase chain reaction (PCR) or culture techniques.

Figure 4 indicates that alternate testing methodologies are being used by more laboratories. Surrogate markers, which include p24 antigen, neopterin, and  $\beta$ -2-microglobulin, are used by eight laboratories. Flow cytometry, culture, polymerase chain reaction (PCR), recombinant antigen, and synthetic peptides also are being used, but in fewer numbers of laboratories.

Public health laboratories continue to support the initiative of the Centers for Disease Control (CDC) in gathering data for the family seroprevalence surveys. Figure 5 indicates that 35 laboratories conducted HIV testing in populations of women of childbearing age.

**TABLE 1**  
**HIV-1 RETROVIRUS TESTING\***

Test	Total	Reactive	IND†
EIA‡	4,424,943	132,376	N/A
WB**	131,890	77,075	11,625
IFA††	35,221	27,831	559

\* Includes 37 county (local) public health laboratories and 99 clinical laboratories in California.

† Indeterminant.

‡ Enzyme immunoassay.

\*\* Western blot.

†† Indirect fluorescent antibody.

**TABLE 2**  
**HIV-1 RETROVIRUS TESTING EXCLUDING LOCAL AND CLINICAL CALIFORNIA LABORATORIES**

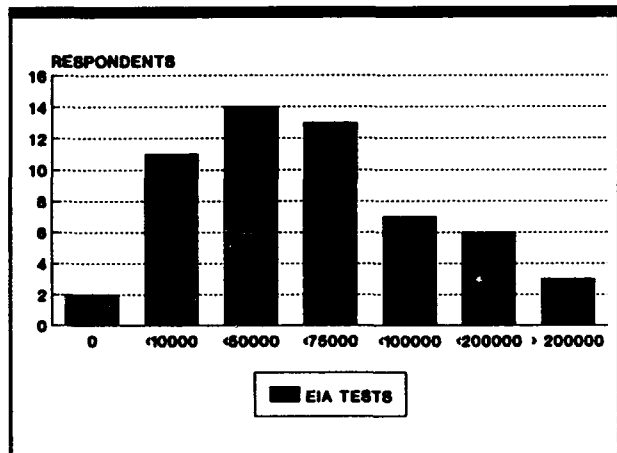
Test	Total	Reactive	IND*
EIA†	3,175,534	111,479	N/A
WB†	91,375	75,051	6,990
IFA**	33,934	26,664	555

\* Indeterminant.

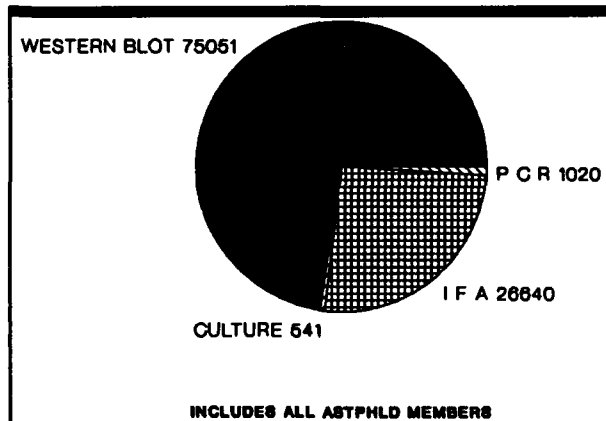
† Enzyme immunoassay.

‡ Western blot.

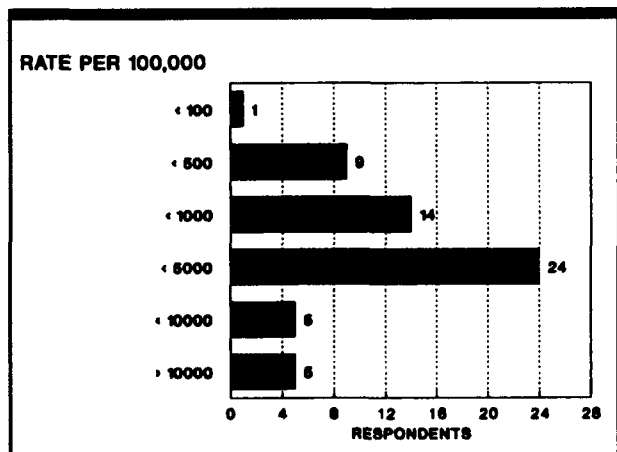
\*\* Indirect fluorescent antibody.



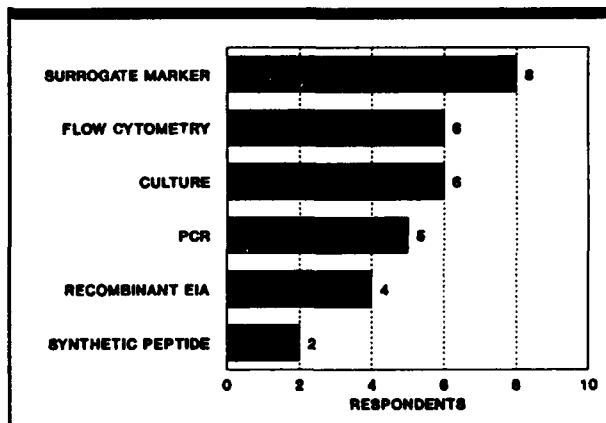
**FIGURE 1.** Total number of tests performed by reporting laboratories.



**FIGURE 3.** Distribution of confirmatory methods for reactive EIA.



**FIGURE 2.** HIV positivity rates for tests in public health laboratories.



**FIGURE 4.** Other test methods used in public health laboratories to follow up on patients with HIV.

The data indicate that the positivity rate in this population is substantially below that seen in the adult population shown in Figure 2. Of 35 labs reporting, only one laboratory indicated that the positive rate in this population was greater than 1%.

Figure 6 outlines the retrovirus testing being conducted in each of the responding laboratories. A

total of 52 responding laboratories indicated that they conduct tests for HIV-1 (Pennsylvania and Minnesota state public health laboratories do not conduct HIV testing). The data in this figure indicate that there are six laboratories conducting HIV-2 testing and 11 laboratories performing tests for HTLV-I/II.

Table 3 shows the total number of HIV-2 tests conducted. Of the 4,460 EIA tests, only 114, or 2.5% were found to be reactive. This reactive rate is similar

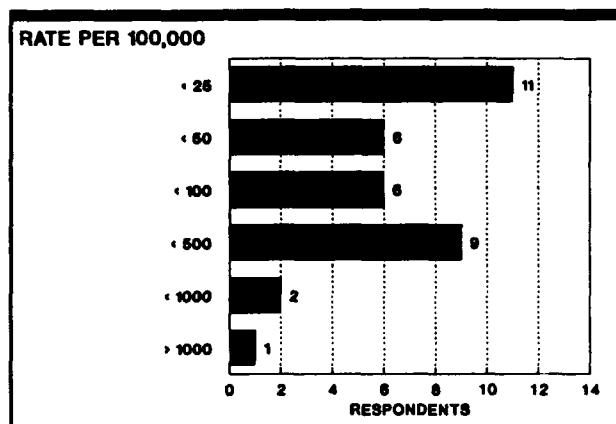


FIGURE 5. The HIV positivity rate for newborns.

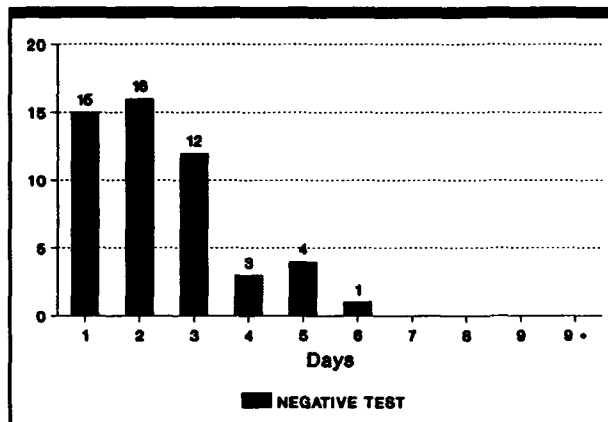


FIGURE 7. Testing turnaround time for nonreactive results.

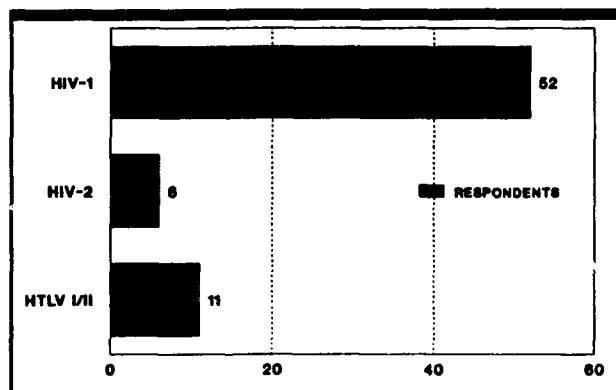


FIGURE 6. Summary of the public health laboratory retrovirus testing. Note. in two states, the HIV-1 is not performed in the state laboratory.

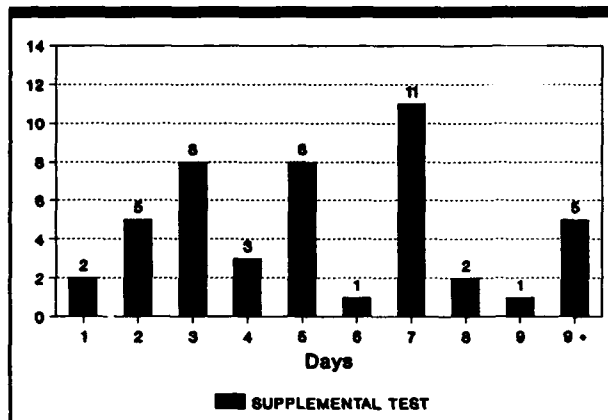


FIGURE 8. Testing turnaround time for reactive results.

TABLE 3  
HIV-2 RETROVIRUS TESTING\*

Test	Total	Reactive	IND†
EIA‡	4,460	114	N/A
WB**	194	22	32
IFA††	17	0	1

\* Includes local laboratories in California.

† Indeterminant.

‡ Enzyme immunoassay.

\*\* Western blot.

†† Indirect fluorescent antibody.

to that seen for HIV-1. However, western blot confirmation rates were very different from HIV-1 in that only 11% of the EIA reactive tests were confirmed, while 16% were indeterminate. In using IFA methodologies, there were no reactivities and only one indeterminate. These data appear to indicate the need to refine testing conducted for HIV-2.

Table 4 demonstrates that there were approximately 500,000 tests conducted for human T-cell lymphotropic virus types I/II (HTLV-I/II). Of these,

less than 1% were reactive. Those reactive specimens, which were then confirmed by western blot, showed a 25% confirmatory rate, while 11% were indeterminate. In using the IFA methodology, 55% were confirmed, while 6% were indeterminate.

Figure 7 demonstrates the average testing turnaround time when the results of the EIA tests are negative. Most laboratories indicate that the results are reported within three calendar days. The testing turnaround time for an EIA positive specimen is longer and more inconsistent (Figure 8). It is clearly evident that extended periods of time are needed to confirm results; the largest number of laboratories (11) indicated that seven calendar days were needed in order to report a confirmed positive result. This extended turnaround time is a source of frequent discussion between the state public health laboratory and the acquired immunodeficiency syndrome (AIDS) program in each of the states and territories. Many requests have been made to consider various schemes to reduce the reporting turnaround time when positive EIA results are involved.

The principal manufacturers of EIA testing rea-

**TABLE 4**  
**HTLV-I/II RETROVIRUS TESTING\***

Test	Total	Reactive	IND†
EIA‡	523,524**	1,946	N/A
WB††	1,702	407	195
IFA‡‡	1,942	1,063	112

\* Includes the 37 local public health and 99 clinical laboratories in California.  
 † Indeterminant.  
 ‡ Enzyme immunoassay.  
 \*\* Of this total, 515,093 were performed by 35 clinical laboratories in California.  
 †† Western blot.  
 ‡‡ Indirect fluorescent antibody.

**TABLE 5**  
**SUPPLIERS OF EIA\* USED BY STATE PUBLIC HEALTH LABORATORIES**

Abbott  
 DuPont  
 Genetic Sytems  
 Organon Teknika  
 Ortho

\* Enzyme immunoassay.

**TABLE 6**  
**SUPPLIERS OF WB\* REAGENTS USED BY STATE PUBLIC HEALTH LABORATORIES**

Abbott  
 Bio-Rad  
 Cappell  
 DuPont  
 Epitope (Organon Teknika)  
 Genetic Systems  
 Ortho

\* Western blot.

gent for public health laboratories are listed in alphabetical order in Table 5. The leading suppliers of western blot testing systems are indicated in Table 6.

Survey data also contained the following information. Six state laboratories are performing flow cytometry, while 11 plan to initiate the procedure within the next 12 months. Of the six laboratories currently performing flow cytometry, two test all HIV-positive samples. Thirty-nine laboratories (75%) participate in the CDC family of surveys. Ninety-two percent of the responding laboratories follow the recommendation of ASTPHLD-CDC in performing and interpreting the western blot. Twenty-eight percent of the responding laboratories routinely request a second sample in

**TABLE 7**  
**US ARMY HIV-1 TESTING FOR FISCAL YEAR 1990**

Test	Total	Reactive	IND*
EIA†	1,304,224	6,012	N/A
WB‡	6,875	2,278	863
Culture	2,417	1,092	
PCR**	657		
Flow††	7,500		

\* Indeterminant.  
 † Enzyme immunoassay.  
 ‡ Western blot.  
 \*\* Polymerase chain reaction.  
 †† Flow cytometry.

**TABLE 8**  
**US ARMY HIV-2 TESTING FOR FISCAL YEAR 1990**

Test	Total	Reactive	IND*
EIA†	1,590	16	N/A
WB‡	16	0	1
PCR**	5		
Flow††	10		

\* Indeterminant.  
 † Enzyme immunoassay.  
 ‡ Western blot.  
 \*\* Polymerase chain reaction.  
 †† Flow cytometry.

**TABLE 9**  
**US ARMY CONFIRMATORY HTLV-I/II TESTS FOR FISCAL YEAR 1990**

Test	Total	Positive
Culture	15	8
PCR*	177	
Flow†	100	

\* Polymerase chain reaction.  
 † Flow cytometry.

order to confirm a positive test result. Only one state (Michigan) has laws regarding HIV testing of pregnant women.

Tables 7, 8, and 9 represent, respectively, the HIV-2, HIV-2, and HTLV-I/II testing done by the US Army. Although not a member of ASTPHLD, the US Army testing laboratories represent a considerable amount of testing performed on members of the armed forces. These data are significant in that this is an excellent cross-section of the young adult population of the United States. The Army data are included for comparison.



## TESTING (HIV-1)

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### TOPICS

Rapid testing methods; blots; testing of nontraditional samples; surrogate markers; recombinant/synthetic-based assays; simultaneous detection methods; western blot criteria; IFA; viral detection methods.

### RECOMMENDATIONS

- 1.01 Rapid tests for the detection of HIV-1 antibody not be a waived test under CLIA regulations. It is essential that a proficiency testing program be developed to ensure the quality of testing.
- 1.02 At the present time, we cannot recommend the use of urine or saliva specimens for HIV antibody testing. Available data, however, indicate that these specimens may be acceptable. We recommend that manufacturers and interested investigators publish in peer-reviewed journals and submit data to the FDA that would allow urine and saliva to be used for HIV antibody testing.

- 1.03 We encourage manufacturers to evaluate nontraditional markers, such as p17 antigen, and develop quantitative assays for staging.
- 1.04 FDA-licensed recombinant or synthetic peptide ELISA tests for the detection of HIV-1 antibody can be used in an HIV-1 algorithm. The following algorithm has been proposed as an alternative to the existing algorithm for the detection of HIV-1 antibody. This algorithm should be evaluated during the coming year and data submitted at the next meeting.
  - a. HIV-1 ELISA A
    1. Negative—Report
    2. Reactive—Go to b
  - b. HIV-1 ELISA B
    1. Negative—Report
    2. Reactive—Go to c or d
  - c. HIV-1 IFA
    1. Positive—Report
    2. Negative or nonspecific—To to WB and/or follow-up specimen
  - d. HIV-1 WB
    1. Positive—Report
    2. Indeterminate—Go to follow-up specimen
    3. Negative—Report or repeat
- 1.05 The ASTPHLD criteria for western blot interpretation should be re-evaluated in light of recent reports of uninfected individuals with envelope only or envelope and *gag* bands. The issue of contamination by another specimen may be a contributing factor and must be addressed.
- 1.06 Quantitative antigen assays for measuring virus burden are needed to monitor disease progression and efficacy of therapy. Further development of better quantitative assays should be encouraged. It is recommended that a national

reference standard for antigen quantitation be developed for use by manufacturers for standardizing their antigen detection kits.

- 1.07 IFA has a place in HIV diagnostic schemes as a supplemental test. Some of the currently available commercial kits show promise for use as confirmatory tests.
- 1.08 Laboratories with significant experience with HIV IFA may use their own IFA-based protocols for confirmation of screening test results.
- 1.09 Nonspecific IFA results should be clarified whenever possible. The use of absorption will resolve many of these problems, and manufacturers are encouraged to develop standard protocols for this and to make cells available for this purpose. Other supplemental tests should be used when absorption cannot resolve the antibody status of a specimen. A positive IFA can be reported as positive. However, a negative or a nonspecific result should be followed by an additional supplemental test.
- 1.10 The IFA should not be used as a screening procedure.
- 1.11 IFA kit manufacturers are encouraged to submit their products to the FDA for licensure. Public health and other laboratories are encouraged to actively participate in gathering data needed to support these applications.
- 1.12 The following references should be used for delta value calculations:
  1. Crofts N, et al. Evaluation of enzyme-linked immunosorbent assays: a method of data analysis. *J Virol Methods*. 1988;22:51-59.
  2. Maskill WJ, et al. An evaluation of competitive and second-generation ELISA. *J Virol Methods*. 1988;22:61-73.

## POLYMERASE CHAIN REACTION

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### TOPICS

Quality assurance; methodology; applications; interpretation/reporting; proficiency testing.

### RECOMMENDATIONS

- 2.01 For specimens obtained "on site," with same-day processing, EDTA, Heparin, or ACD are suitable. For specimens that are shipped from another site, samples in ACD may be suitable for up to five days. For specimens in EDTA or heparin, the maximum transport time is 48 hours.
- 2.02 Every effort should be made to obtain whole blood samples 24 hours to 48 hours after venipuncture. Samples should be transported at room temperature.
- 2.03 Dried blood spots appear to be suitable for PCR testing and may be stable for long periods of time. Further data are awaited regarding the sensitivity and specificity of PCR testing from dried blood spots.
- 2.04 Magnesium concentration must be optimized for each set of primers. Current primers: 1.5 mM to

2.5 mM.

- 2.05 Annealing and extension conditions must be optimized in each laboratory.
- 2.06 The number of cycles in the amplification process should be between 30 and 35 for HIV detection.
- 2.07 Liquid oligonucleotide hybridization (OH) is a highly sensitive and specific detection method and is recommended.
- 2.08 A variety of nonradioactive detection methods are now available and some have sensitivity and specificity comparable to OH detection. Further comparative testing is recommended.
- 2.09 In order to obtain the authority to perform and report PCR results, the PCR subcommittee recommends initiation and continuation of discussions with those who hold patents affecting the use of PCR for the purpose of ensuring continuation of public health investigations of the diagnosis, etiology, and pathogenesis of diseases of public health importance.
- 2.10 Laboratories performing PCR should employ a minimum of two primer pairs for initial testing and/or resolution of discrepant results.
- 2.11 Primers with documented sensitivity and specificity should be used.
- 2.12 A minimum of two separate PCR reactions (two primer pairs or duplicates of one primer pair) should be performed for each specimen. Splitting of the original specimen is recommended when possible.
- 2.13 Discrepant results should be resolved by additional analysis of the original specimen using the same or different primer pairs. If discrepant results cannot be resolved by repeat testing, the results should be reported as indeterminate and another specimen should be requested.
- 2.14 The overall interpretation of PCR testing should be reported as HIV DNA detected, HIV DNA not detected, or indeterminate.
- 2.15 Complete testing algorithms need not be reported.

- 2.16 Appropriate positive, negative, and reagent controls should be included with every PCR test.
- 2.17 All published guidelines for minimizing the contamination of specimens with amplified PCR product should be rigorously followed.
- 2.18 Biochemical sterilization of PCR products should be instituted in all PCR laboratories as soon as possible.
- 2.19 The committee recommends that each laboratory performing PCR conduct validation studies on an appropriate number of well-characterized test samples and maintain appropriate records of such test results.
- 2.20 The development of validation panels for distribution to PCR laboratories by the private sector, NIH, and CDC is recommended.
- 2.21 The development of standardized reagents and controls in the form of commercial kits is encouraged.
- 2.22 The immediate initiation of a proficiency testing program for PCR is recommended.
- 2.23 The application of PCR, in combination with other tests, for the diagnosis of infants born to seropositive mothers is recommended. Additional studies on the use of PCR in the first three months of life are needed.
- 2.24 The use of PCR for retroviral diagnosis in "high risk" seronegative adults is not recommended as a routine procedure.
- 2.25 The use of PCR for the resolution of indeterminate serology in adults is not recommended as a routine procedure.
- 2.26 PCR may be helpful in the diagnosis of rare individuals with defective antibody production.
- 2.27 PCR is useful for the differentiation of viral subtypes (e.g., HTVL-I/II or HIV-1 versus HIV-2).
- 2.28 The determination of viral burden by PCR may be helpful in the prognosis of HIV disease. However, quantitative PCR requires complex procedures that are not established in most laboratories.
- 2.29 The use of PCR to monitor the effects of antiviral therapy on viral burden is an important area of future study but has not been validated at this time.

## FLOW CYTOMETRY

### CHAIR

Jonathan M. Kagan, PhD, Chief, Clinical Sciences Section, Treatment Research Program, Division of Acquired Immunodeficiency Syndrome (AIDS), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

### MEMBERS

A. Russell Gerber, MD, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia; Janet K.A. Nicholson, PhD, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia (Rapporteur).

### TOPICS

Quality assurance; training; monitoring antiviral therapy; reporting criteria and interpretation; disease staging.

### RECOMMENDATIONS

- 3.01 Each public health laboratory should evaluate the need to establish flow cytometry capability based on an assessment of criteria including: prevalence of HIV infection and cumulative incidence of AIDS within the area served; ability to monitor and evaluate the quality of flow cytometry and pertinent hematology results; cost effectiveness; and the availability of other early HIV intervention services. Additional data are needed before specific recommendations can be made on nonretroviral public health and environmental applications of flow cytometry.
- 3.02 Flow cytometry training should be mandatory for all relevant personnel including instrument operators, laboratory supervisors and laboratory directors.
- a. All instrument operators should, in addition to flow cytometer manufacturer's training, receive supplementary training through additional courses or workshops offered by independent organizations. In-house training at

experienced laboratories may be an acceptable alternative.

- b. Clinical flow cytometry training courses for laboratory directors and supervisors should be developed.
  - c. The ASTPHLD recommends the development of certification programs for laboratory technicians trained in flow cytometry.
  - d. The ASTPHLD recommends the development and implementation of accreditation standards for all training courses and workshops in clinical flow cytometry.
- 3.03 The ASTPHLD should assemble and make available updated information on clinical flow cytometry training courses and workshops.
- 3.04 Before accepting specimens for clinical flow cytometric analysis, each laboratory must have in place a comprehensive quality assurance protocol that includes standardization, quality control procedures, and proficiency testing.
- a. Standardization of instrument optical alignment, spectral sensitivity, and fluorescence compensation must be performed daily.
  - b. Quality control includes daily monitoring and recording of instrument performance and cell preparation methodologies. Reagent stability should be assessed with lot changes and as otherwise needed.
  - c. Proficiency testing within a nationally recognized program on a quarterly basis is required as an integral component of comprehensive quality assurance.
- 3.05 The ASTPHLD endorses the National Committee for Clinical Laboratory Standards (NCCLS) Proposed Guideline, H42-P, Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes.
- 3.06 The determination of absolute counts for lymphocyte subsets requires both hematologic and flow cytometric measures. For hematologic measures:

- a. Determination of the absolute lymphocyte count requires both a white blood cell count (WBC) and a differential (including percent lymphocytes). The ASTPHLD endorses the NCCLS Tentative Standard (1984), H-20T, Leukocyte Differential Counting. The optimal specimen for these hematologic measures is EDTA-preserved whole blood (lavender top tube) less than six hours old.
- b. Recognizing that laboratories may receive hematologic specimens more than six hours old, the ASTPHLD recommends consideration of the following options:
  1. Hematologic analysis may be performed within six hours locally, and a second specimen (drawn simultaneously) for flow cytometry may be transported to the flow cytometry laboratory. It may be desirable to obtain a fresh smear for quality assurance.
  2. Laboratories can verify the maximum age of specimens for which hematologic results are comparable to fresh specimens.
- c. The laboratory performing the hematology should maintain a documented intralaboratory coefficient of variation of less than five percent for the WBC.
- d. Laboratories should evaluate and characterize intralaboratory bias and establish confidence intervals for the WBC and the differential lymphocyte counts.
- e. Automated differentials are strongly recommended. Manual differentials should count at least 400 cells.

For flow cytometric measures:

- a. The optimal specimen for lymphocyte immunophenotyping by flow cytometry is either an EDTA-preserved whole blood specimen less than six hours old or a heparinized whole blood specimen up to 24 hours old.
  - b. Recognizing that laboratories may receive suboptimal (old) flow cytometric specimens, ASTPHLD recommends that laboratories verify the maximum ages of specimens for which immunophenotyping results are comparable to fresh specimens.
  - c. Optimally, specimens should be maintained at room temperature (18°C to 22°C) until tested.
- 3.07 Whole blood lysis and two-color immunofluorescence are the methods of choice for flow cytometric immunophenotyping.
- 3.08 The ASTPHLD recommends the two-color monoclonal antibody panel specified in the Table

**TABLE**

**RECOMMENDED TWO-COLOR MONOCLONAL ANTIBODY PANEL FOR ROUTINE IMMUNOPHENOTYPING**

<b>Monoclonal Antibody Combination*</b>	<b>Cell Type Enumerated</b>
IgG <sub>1</sub> /IgG <sub>2</sub>	Isotype controls
CD45/CD14	Percent lymphocytes in gating region†
CD3/CD4	T-helper/inducer subset‡
CD3/CD8	T-suppressor/cytotoxic subset‡
CD3/CD16 + CD56	Total T cells/total NK cells**
CD197	Total B-cells

\* FITC/PE-labeled reagents.  
 † Lymphocytes will be CD45<sup>bright</sup>CD14<sup>negative</sup>.  
 ‡ Indicated cell type will be positive for both antibodies.  
 \*\*Total T cells = all cells expressing CD3; total NK cells = all cells that are CD3-negative but positive for CD16 and/or CD56.

for routine immunophenotyping.

- 3.09 Lymphocyte light scatter gates must be validated by anti-CD45 (pan-leukocyte) and anti-CD14 (monocyte) reactivity.
  - a. Optimally, nonlymphocyte contamination within the gate should not exceed 5%. Eighty-five percent is the lower limit of acceptable lymphocyte representation in the lymphocyte gate.
  - b. At least 95% of lymphocytes should be contained within the light scatter gate.
- 3.10 Lymphocyte subset percentage values from the flow cytometer should be corrected by dividing the observed percentage by the percentage of lymphocytes (CD45<sup>bright</sup> CD14<sup>negative</sup>) in the lymphocyte gating region.
- 3.11 For most specimens, the total of the corrected CD3<sup>positive</sup> (Total T), CD19<sup>positive</sup> (Total B), and CD3<sup>negative</sup> CD56<sup>positive</sup> and/or CD16<sup>positive</sup> (Total NK) percentages should sum to between 95% and 105%.
- 3.12 Each laboratory must establish age-and population-appropriate reference ranges in accordance with validated statistical criteria.
  - a. It should be noted that pediatric reference ranges may differ substantially from the reference ranges for adult populations.
- 3.13 The manufacturers of flow cytometry instrumentation and reagents are urged to cooperatively expedite the development of:
  - a. Improved lymphocyte gating reagents.
  - b. Automated sample preparation technology.
  - c. Flow cytometers capable of determining absolute numbers for lymphocyte subsets.
  - d. Improved laboratory quality control reagents.

- e. Anticoagulants and preservatives suitable for both hematologic and flow cytometric measurements.
- 3.14 Laboratory reports should optimally include lymphocyte subset percentages, absolute values, and laboratory reference ranges.
- a. Laboratory reports should specify the immunophenotype (CD designation) for all lymphocyte subsets reported therein (e.g., T-helper/inducer = CD3<sup>positive</sup>/CD4<sup>positive</sup>).
  - b. Values for lymphocyte subsets should be corrected for the lymphocyte representation in the gating region.
  - c. Absolute values for lymphocyte subsets should be reported unless hematologic results are suspect.
- 3.15 The ASTPHLD strongly supports efforts by the Centers for Disease Control to establish a national lymphocyte immunophenotyping performance evaluation program including training and education programs.
- 3.16 The ASTPHLD strongly supports the efforts of the NCCLS and the NIAID Flow Cytometry Advisory Committee in setting standards for clinical flow cytometric immunophenotyping.
- 3.17 The ASTPHLD encourages the development of alternative (nonflow cytometric) methods for the enumeration of CD4<sup>+</sup> lymphocytes.
- 3.18 The ASTPHLD endorses Universal Precautions and the flow cytometry safety guidelines outlined in NCCLS document H42-P, Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes.

## TESTING (HIV-2, HTLV-I/II)

### CHAIR

Chyang Fang, PhD, Director, National Reference Laboratory for Infectious Diseases, American Red Cross, Rockville, Maryland.

### MEMBERS

J. Richard George, PhD, Chief, Developmental Technology Section, Centers for Disease Control, Atlanta, Georgia; Jonathan Kaplan, MD, Retrovirus Diseases Branch, Centers for Disease Control, Atlanta, Georgia; Helen Lee, PhD, Director, Transfusion Biology Research and Development, Abbott Laboratories, North Chicago, Illinois (Rapporteur); Roy Stevens, PhD, Director of Laboratories for Diagnostic Immunology, New York State Department of Health, Albany, New York; Barbara Werner, PhD, Director, Clinical Investigation and Virology, State Laboratory Institute, Boston, Massachusetts.

### TOPICS

HTLV-I and HTLV-II differentiation; confirmation methodology and criteria for HIV-1/HIV-2 and HTLV-I/II; HIV-1/HIV-2 combination tests; rapid testing methods.

### RECOMMENDATIONS

- 4.01 We encourage manufacturers to develop second-generation confirmatory tests to replace the current WB-RIPA procedures with assays utilizing recombinant proteins and/or synthetic peptides in formats that allow objective reading.
- 4.02 The inclusion of p19+gp46/61 pattern as confirmatory for HTLV-I/II seropositivity should be further evaluated.
- 4.03 The utility of p21e in the current confirmation criteria needs to be validated; the specificity of recombinant p21e, in particular, should be further evaluated.
- 4.04 Since HTLV-I and HTLV-II infections have different disease manifestations, we recommend that public health laboratories carry out, if possible, routine differentiation of HTLV-I from HTLV-II in HTLV-I/II seropositive samples.
- 4.05 We encourage the development and evaluation of recombinant and/or synthetic peptide reagents for the differentiation of HTLV-I from HTLV-II antibodies.
- 4.06 We recognize that current HTLV-I screening assays will occasionally fail to detect HTLV-II antibodies and encourage systematic epidemiological and laboratory studies to determine the sensitivity of HTLV-I reagents for HTLV-II antibody detection. If a sizable percentage of HTLV-II antibodies are missed, the development of a more sensitive second-generation HTLV assay is recommended.
- 4.07 National surveillance for HIV-2 infections should be continued.
- 4.08 HIV-1 seronegative or indeterminate persons with AIDS-related symptoms or those at risk for HIV-2 infection should be considered as candidates for HIV-2 antibody testing.
- 4.09 Upon the availability of HIV-1/HIV-2 combination tests, the public health laboratories should consider the importance of adopting the combination assays based on epidemiological data pertinent to the local area.
- 4.10 The ASTPHLD Committee on Human Retrovirus Testing should appoint a committee composed of representatives from public health laboratories and manufacturers to determine the characteristics of standard HIV-2 confirmatory tests. For example, for western blot, these would include the virus strain, purification and processing of antigens, and interpretive criteria.



## DIAGNOSIS OF HIV INFECTION IN NEWBORNS

### CHAIR

Sara Beatrice, PhD, Director of Retrovirology and Immunology, New York City Department of Public Health, New York, New York.

### MEMBERS

Anne M. Comeau, PhD, Co-Principle Investigator, Newborn HIV Project, Theobald Smith Research Institute, Jamaica Plain, Massachusetts; Francis Lee, MD, Assistant Professor of Pediatrics, Pediatric Infectious Diseases, Emory University, Atlanta, Georgia; Barbara Weiblen, MS, Senior Scientist, Newborn HIV Project, Theobald Smith Research Institute, Jamaica Plain, Massachusetts.

### TOPICS

Flow cytometry; polymerase chain reaction and culture assays; ELISPOT and *in vitro* assays; IgA and other serologic assays.

### RECOMMENDATIONS

- 5.01 The committee recommends the continuation of prospective studies for evaluation of relevant tests for the early diagnosis of infection, especially to identify rapid progressors. Prospective studies should include comparison analysis of PCR, culture, ELISPOT, and HIV-specific IgA antibodies.
- 5.02 PCR appears to be the most sensitive test for diagnosis of HIV infection in the first three months of life.

- 5.03 Negative results obtained on specimens from infants less than one month of age must be regarded as tentative. Additional specimens should be tested from these infants at ages greater than one month. Positive results obtained within the first month of life must be confirmed with tests on an independent specimen as soon as possible (preferably in the first two months of life). All positive PCR results, regardless of age, should be confirmed with tests on an independent specimen.
- 5.04 Virus culture is an effective tool for diagnosis of HIV infection in young children. A negative result does not rule out infection; a positive result should be confirmed.
- 5.05 ELISPOT is a promising new test for the diagnosis of infection in infants in the first three months of life. It may complement PCR testing during the neonatal period. The committee recommends further testing on early specimens to evaluate its predictive value.
- 5.06 IgA HIV antibody tests are of limited diagnostic value in neonates but may be positive by three months of age. Because the IgA test is a simple modification of existing technology, it may be useful as a supplemental test in regional laboratories.

## STANDARDS OF PRACTICE

### CHAIR

Dale Lawrence, MD, Chief, Clinical Development Section, Vaccine Research and Development Branch, Basic Research and Development Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Rockville, Maryland.

### MEMBERS

Susan Mottice, PhD, Director, Microbiology, Utah State Health Department, Salt Lake City, Utah; Lt. Col. Chester Roberts, PhD, Chief, Diagnostic Retrovirology, Walter Reed Army Institute of Research, Rockville, Maryland; Judith Wethers, MS, Director, Testing Services, Retrovirology Laboratory, State of New York Department of Health Laboratories, Albany, New York; Keith Lawrence, Administrator, New York City Department of Health, New York, New York (Rapporteur).

### TOPICS

Reporting; testing of vaccinated individuals.

### RECOMMENDATIONS

- 6.01 This workshop reaffirms the reporting procedures recommended by the Fifth Consensus Conference, with the addition by reference to Section 9.02f, Item 2: "A narrative laboratory interpretation, including a reference to immune response elicited by vaccines."
- 6.02 We welcome the drafting by CAP of anti-HIV test reporting guidelines and encourage all ASTPHLD attendees to review and provide feedback to CAP.
- 6.03 Persons interpreting western blot tests should be aware that participation by initially seronegative volunteers in clinical trials of HIV/AIDS vaccines will likely result in the acquisition of detectable immune responses to HIV. These responses may include the development of a positive ELISA test and western blot bands reflecting the antigenic formulation and immunogenicity of the vaccine. Some vaccines may induce western blot bands which meet published criteria for a positive test.
- 6.04 During succeeding years, the testing of various types of vaccine formulations can be anticipated. To preclude misclassification of a vaccinated volunteer with a positive HIV antibody test result as infected, it will be necessary that interpretation take into account the past HIV vaccine immunization history. To assist in this, the following procedures should be implemented by HIV/AIDS vaccine investigators.
  - a. At the conclusion of a trial, volunteers should be provided with information on the vaccine formulation(s) administered along with, as a minimum, the results of their ELISA and western blot tests post-vaccination.
  - b. Participants should have long-term accessibility to this information with safeguards to protect confidentiality.
- 6.05 The NIH is supported in its effort to develop for publication, in concert with the CDC and other relevant agencies, an *MMWR* advisory acknowledging the unique circumstances surrounding HIV/AIDS vaccine study participation.

## POSTER PRESENTATIONS

1. **J.E. Johnson**. Comparison of Whole Cell Viral Lysate (VL), Synthetic Peptide (SP), and Recombinant Protein (RCP) EIAs for Detection of HIV-1 Antibody.
2. **J.P. Montana**, L. Gosting, C.A. Cole, N. Monji, P. Su, P.F. Coleman. Detection of Antibodies to HIV-1 and HIV-2 by a Single, Rapid Peptide-Based Immunoassay.
3. **C. Ferrera**, N. Dock, J. Huprikar, J. Phair, M. Krieger. Use of a Peptide-Based, Rapid Immunoassay for Antibody to HIV-1 to Clarify True Antibody Status of EIA Repeat Reactive, Western Blot Indeterminates.
4. **W. Link**. Effect of Nonspecific Glycoprotein Bands on Interpretation of HIV-1 Western Blots.
5. **B.P. Griffith**, T.M. Chacko. Comparative Performance of Peptide, Recombinant and Viral Lysate-Based Enzyme Immunosorbent Assays for the Detection of HIV-1 Antibody.
6. **E.W. Chan**, W. Schulze, M. Leather, K. Knigge. Monitoring IgG, IgA, and IgM Responses to HIV-1/HIV-2 Infections by Modified Abbott Matrix Assays.
7. **S. Pederson**, L.J. Croy, L. Adams, S. Cobel, C. Ferrera, M. Krieger, K. Shriver. Development of an Enzyme Immunoassay for Simultaneous Detection of Antibodies to HIV-1 and HIV-2.
8. **K. Richards**. Performance of the Microtrak HIV-1 (*env* and *gag*) Recombinant Antigen Enzyme Immunoassay—Summary of Field Trial Data.
9. **B.G. Werner**, C. Schable, M. Krieger. HIV-2 Infection in a Patient Screened HIV-1-Negative.
10. J. Gregg, **C. Ludvigsen**, **B. Roberts**. Detection of Antibodies Directed to HIV-1 in Oral Mucosal Transudate.
11. **C.K. Cossen**, S.J. Hagens, M. Ascher, P. Stewart, M. Krieger. Use of an HIV-1/HIV-2 Combination EIA Test with Dried Blood Spot Samples.
12. **C.K. Cossen**, S.J. Hagens, M. Ascher. A Comparison of Three HIV-1 Western Blot Methods.
13. **B. Forghani**, J. Hurst, C. Chan. Study of Nonspecific Immunofluorescence of Repeat EIA-HIV-1 Reactive Sera Using HIV-1 Persistently Infected HeLa T4<sup>+</sup> Cells.
14. C. Starkey, B. Yen-Lieberman, **M. Proffitt**. Evaluation of the Cambridge BioScience Recombigen HIV-1 EIA as a Supplementary Test for Detection of Antibodies to Human Immunodeficiency Virus-Type 1.
15. **M. Proffitt**, C. Starkey, B. Yen-Lieberman, D. Hatch, S. Schindler. Serum HIV P24 Antigen Testing of Individuals at Risk of Having Been Infected With HIV-1.
16. **M.T. Ramirez**, N.S. Swack, W.J. Hausler, Jr. Reactivity of Six Commercial HIV-1 EIA Test Procedures on Western Blot Indeterminate Sera.
17. **M.A. Redus**, S.I. Wasser, W.H. Hannon, J.R. George, M. Gwinn, G.F. Grady, L.M. Mofenson. Estimates from the National HIV Survey in Childbearing Women.
18. **J.S. Niedbalski**, S.J. Dahlen, B.P. Braun. Matrix HIV-1/HIV-2: A New Alternative to the Western Blot.
19. **J.H. Jean**, S. Dee, D. Phillips, K. Hurley, M.P. Verna, J.C. Ridderhof. Evaluation of Microtrak HIV-1 EIA (*env* and *gag*) Test With Selected Specimens.
20. **R.T. Schumacher**, J. Howard, L. Ayres, A. Pista, F. Avillez, P. Garrett. Cross-Reactivity of Anti-HIV-2-Positive Specimens in FDA Licensed Screening Tests for Anti-HIV-1.
21. **G.D. Cross**, W.O. Schalla, S.O. Blumer, J.S. Hancock, R.N. Taylor, A.R. Gerber, T.L. Hearn. Inter-Shipment Reproducibility of Laboratories Participating in a Performance Evaluation Program.
22. **S.L. Stramer**, C.J. Mitten, M.B. Mathieu, J.L. Shenaut, X. Alcade, J.P. Allain, C. Schable, R. Schumacher. A Combination Anti-HIV-1/HIV-2 EIA Using Both Viral and Recombinant Antigens.
23. J.A. Connell, J.V. Parry, **P.P. Mortimer**. IgG Antibody Capture ELISA A Diagnostic Test for Anti-HIV Applicable to All Body Fluids.
24. J.A. Connell, J.V. Parry, **P.P. Mortimer**. GACPAT: A Tool for Unlinked Testing of Urine Specimens.
25. **K.M. Peddecord**, A.S. Beneron, D.P. Francis, D.J. Hewitt, R.S. Garfein, L.K. Hofherr, A.R. Gerber, G.D. Cross, W.O. Schalla. What Do Human T-Lymphocyte Immunophenotyping Reports Contain? Results of a Case-Series.

*Boldface names indicate the presenter(s) of each session.*

*From the Sixth Conference on Human Retrovirus Testing, March 5-7, 1991, Kansas City, Missouri. Poster presentations. Infect Control Hosp Epidemiol. 1991;12:484-485.*

26. E. Oluoch, S. Pederson, J. Schwebke, K. Homes, J. Kleyn, D.A. Caslyn, A.J. Saxon, S. Whitaker, L. Olmsted, R. Masinovsky, K. Shriver. PCR Analysis of Intravenous Drug User Samples Reactive with HTLV-I and HTLV-II.
27. A.M. Comeau, B. Weiblen J.A. Harris, K. McIntosh, R. Hoff. Analysis of Early Infant Specimens by the Polymerase Chain Reaction (PCR) for the Detection of HIV-1 Proviral DNA.
28. A.M. Comeau, G. Mushinsky, K. Adler. Analysis of PCR Sensitivity Using DuPont HIV-1 *pol* Repliprimers and DNA Titerplate Hybridization System.
29. J. Ackerman, W. Link. Effect of Including gp21 in Interpretation Criteria for HTLV-I Western Blot.
30. H.F. Polesky, M. Hanson. Practice Guidelines for Reporting HIV Test Results.
31. M.E. Schwerzler, B. Weiblen, B. Werner, M. Krieger, G.F. Grady. HIV-2 Infection in Childbearing Women Detected by Newborn Screening.
32. M. Dobec, J.A. Wilhelm, R. Baumgartner, M. Jung. Differentiation of HTLV-I and II Infection Using IFA.
33. B. Weiblen, A. Comeau. Early Diagnosis of HIV Infection in Infants by Detection of IgA and IgM HIV Antibodies.
34. S.O. Blumer, W.O. Schalla, G.D. Cross, J.S. Hancock, R.N. Taylor, A.R. Gerber, T.L. Hearn. Evaluation of the Performance of Laboratories that Test for Human T-Lymphotropic Virus Antibodies.
35. A.R. Gerber, G.D. Cross, W.O. Schalla, J.S. Hancock, S.O. Blumer, T.L. Hearn. Evaluation of Laboratory Performance of T-Lymphocyte Immunophotyping in Support of Early HIV-1 Intervention and Prevention.
36. D. Gallo, P.J. Dailey, C.V. Hanson. Antigenicity of New HTLV-II-Infected Cell Lines Derived from California IVDU.
37. T.C. Granade, B. Parekh, S. Phillips, C-P. Pau, M. Redus, M. Gwinn, J.R. George. Characterization of the Western Blot Testing Methods for Detecting Antibodies to HIV-1 in Elutes of Dried Blood Collected on Filter Paper.
38. J.W.D. Kay, A. Goldstein (Detection in Urine), J.W.D. Kay, T.H. Grove (Detection in Saliva). Adaptation of a Serum/Plasma/Dried Blood Spot Licensed High Performance ELISA System for Detection of HIV-1 Antibodies in Saliva and Urine.