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Seventh Annual Conference on Human Retrovirus Testing

Jerome R. Cordts

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U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, Maryland 21702-5012

Conference held March 3-5, 1992, Chicago, Illinois

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SEVENTH ANNUAL CONFERENCE
ON
HUMAN RETROVIRUS TESTING

REPORT

March 3-5, 1992
Chicago, Illinois

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.
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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This conference was supported, in part, by a financial grant from the Department of the Army, U.S. Army Medical Research and Development Command.
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The importance of retrovirus testing in today's society demands that every effort be made to conduct testing in as rigid and uncompromising a manner as possible. As the complexity of retrovirus testing increases, the necessity for standardization also increases. The Seventh Annual Conference on Human Retrovirus Testing, sponsored by the Association of State and Territorial Public Health Directors (ASTPHLD) and held in Chicago, Illinois on March 3-5, 1992, recognized and addressed many of the issues relevant to retrovirus testing.

The conference serves as a forum for the exchange of ideas about retrovirus testing and provides an opportunity to make recommendations to ASTPHLD.

This year's conference was attended by nearly 350 participants from public health laboratories, industry, and the private sector. Eight plenary session speakers spoke on a diverse range of issues. The exhibit area accommodated 54 poster presentations and 15 exhibitors. Four panel sessions were conducted.

The CDC-proposed change in the criteria for AIDS case definition based upon the CD4+ lymphocyte count presented the conference attendees with an opportunity to present their views on this subject. The introduction of combination kits leads to the need for new testing algorithms and criteria for HIV-2 and HTLV-I/II. A special highlight of the conference was quantitation of CD4+ lymphocytes by flow cytometric methods. A supplemental flow cytometry lecture and workshops were presented with the collaboration of three vendors of flow cytometry equipment. Approximately sixty people attended these workshops.

Panel Session I - Testing: The Serologic Diagnosis of HIV - comprised HIV 1/2 combination kits, ASTPHLD/Centers for Disease Control Interpretive Criteria, proficiency testing and dried blood spot testing. The panel also proposed a testing algorithm for use with combination HIV-1/2 EIA kits.

Panel Session II - Testing: Lymphocyte Subset Quantitation - covered flow cytometric techniques and monitoring of antiviral therapy. It also addressed the issues associated with initiating a flow cytometry program.


Panel Session IV - Testing: Special Topics included topics such as urine testing, saliva testing, HTLV-I/II and HIV in newborns. The panel made recommendations concerning Western blot interpretation for HTLV-I/II.

In conjunction with the conference a survey was sent to all 54 ASTPHLD members. Forty seven members returned the survey which asked for Fiscal Year 91 data (July 1, 1990 to June 30, 1991). This report reflects information obtained from that survey. In contrast to last year, data from California local health departments and clinical laboratories are not included. Table 1 indicates the routine testing performed by the members of ASTPHLD who responded to the survey. Table 2 indicates the testing done for the CDC Family of Serosurveys. A total of 3,699,796 HIV-1 test specimen results were reported on the survey.
### Table 1
Survey Data

<table>
<thead>
<tr>
<th></th>
<th>HIV-1</th>
<th>HIV-2</th>
<th>HTLV-1/II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme ImmunoAssay</td>
<td>1,315,677</td>
<td>1,014</td>
<td>3,623</td>
<td>1,316,378</td>
</tr>
<tr>
<td>Western blot</td>
<td>44,991</td>
<td>12</td>
<td>3,623</td>
<td>48,626</td>
</tr>
<tr>
<td>Indirect Fluorescent Antibody</td>
<td>16,611</td>
<td>23</td>
<td>689</td>
<td>17,323</td>
</tr>
<tr>
<td>Polymerase Chain Reaction</td>
<td>1,281</td>
<td>2,873</td>
<td>4,154</td>
<td></td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>3,711</td>
<td></td>
<td>3,711</td>
<td></td>
</tr>
<tr>
<td>Culture</td>
<td>1,583</td>
<td></td>
<td>1,583</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,383,854</td>
<td>1,049</td>
<td>10,808</td>
<td>1,395,711</td>
</tr>
</tbody>
</table>

### Table 2
Family of Serosurvey Results

<table>
<thead>
<tr>
<th></th>
<th>Number Tested</th>
<th>Number Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child-bearing women</td>
<td>2,111,871</td>
<td>3,952</td>
</tr>
<tr>
<td>All others</td>
<td>204,071</td>
<td>6,452</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2,315,942</td>
<td>10,404</td>
</tr>
</tbody>
</table>

### Table 3
HIV-1 Retrovirus Testing

<table>
<thead>
<tr>
<th>Testing Method</th>
<th>Tested</th>
<th>Reactive</th>
<th>Non-reactive</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme ImmunoAssay</td>
<td>1,315,677</td>
<td>55,910</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western Blot</td>
<td>44,991</td>
<td>38,113</td>
<td>3,721</td>
<td>3,157</td>
</tr>
<tr>
<td>Indirect Fluorescent Antibody</td>
<td>16,611</td>
<td>12,113</td>
<td>4,131</td>
<td>367</td>
</tr>
<tr>
<td>Polymerase Chain Reaction</td>
<td>1,281</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>3,711</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue culture</td>
<td>1,583</td>
<td></td>
<td></td>
<td>306</td>
</tr>
</tbody>
</table>

2 Seventh Annual Conference on Human Retrovirus Testing
Testing for HIV-1 is the predominant retrovirus test and the principal screening test method is Enzyme ImmunoAssay (EIA); over 1.3 million specimens were tested for HIV-1 using EIA. Although HIV-1/2 combination kits are available, only eight states reported testing for HIV-2 and this accounted for only 1,014 specimens tested - of which 49 were reactive for HIV-2. Eight of the twelve Western blot tests performed for HIV-2 were reactive. Five states reported that testing for HIV-2 was done on all specimens which were EIA reactive and failed to confirm for HIV-1. Almost 45,000 Western blot tests were performed on the over 55,000 reactive EIA tests. (See Table 3.) The number of indeterminates resolved by Polymerase Chain Reaction (PCR) testing is not known. The overall reactivity rate for this year's data is 4.2% as compared to 3% last year (on the survey conducted for last year's conference). A major reason for this elevated rate may be the absence of the large amount of California data which were included in last year's survey.

ALTERNATE TESTING METHODS

<table>
<thead>
<tr>
<th>Method</th>
<th>States using method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>1</td>
</tr>
<tr>
<td>Beta-2 Microglobulin</td>
<td>3</td>
</tr>
<tr>
<td>Synthetic Peptide</td>
<td>2</td>
</tr>
<tr>
<td>Culture</td>
<td>6</td>
</tr>
<tr>
<td>PCR</td>
<td>7</td>
</tr>
<tr>
<td>Recombinant Antigen</td>
<td>7</td>
</tr>
<tr>
<td>p24 Antigen</td>
<td>8</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 1 The total number of these alternate testing methods has increased from 31 to 46 in the last year.

Retrovirus testing procedures are becoming more complex as the need for better control of the spread of infection and management of the disease in the patient increases. Figure 1 shows the number of responding states which utilize each of the procedures listed. The increase in the use of surrogate markers as a diagnostic tool has increased from eight states last year to 13 states this year. Last year the use of these procedures was listed a total of 31 times by the respondents, this year 46 instances were indicated by the respondents. Testing as a medical management tool is becoming increasingly important. Some of these methods, notably flow cytometry and polymerase chain reaction (PCR), are used primarily to monitor the course of infection in the infected individual. Culture methods, although reported by the same number of respondents as last year, may increase significantly with the development of micro methods.

Flow cytometry, a testing method of increasing interest, is used in eight states, one territory and New York City, and accounts for 3,711 specimens tested during FY91. This method, along with PCR testing, is used for confirmation of HIV-1 positivity and for clarification of questionable test results. A survey conducted in December, 1991 (See table 4.) indicated an additional four states were about to initiate flow cytometric analysis. The survey also indicated that, although most were following the manufacturer's recommended procedures, some were also following those of CDC and/or NCCLS. The survey results confirmed that laboratories using flow cytometry need to expend considerable time and effort to ensure that proficiency testing, training, etc. are adequately addressed. Eight of these laboratories indicated they are providing continuing education for their employees. The reasons indicated by survey respondents for NOT having flow cytometry included funding, laboratory space, and lack of trained personnel.

The CDC coordinates a very extensive survey for HIV-1 in various populations. The largest population tested, by far, is child-bearing women - 2,111,871 out of a total 2,315,942 tests for FY91. (REMEMBER: the data presented here are the results of this survey and may differ from those published by CDC.) These tests are performed on dried blood spots collected from the newborn, generally required by law for metabolic disease screening, and are an
indication of the presence of maternal antibodies. The positivity rate distribution for 30 states is shown in Figure 2. The positivity rate of this population is considered a reflection of the rate in the general population. Other patient populations tested include those from sexually transmitted disease clinics, drug treatment centers, TB clinics, women's health clinics and correctional institutions. These high-risk groups (8.8% of the tested population) contained a disproportionate number (62.0%) of confirmed positives. (See Table 2.) Thirty seven states indicated participation in this survey. Some states participate by contracting with other state laboratories to perform the testing. Six states participate in the Family of Serosurveys but do not test child-bearing women. It should be noted that the increase in the last few years of the incidence of Multiple-Drug Resistant Tuberculosis is believed by many to be tied to the increase of HIV-1 infection.

Figures 3 and 4 indicate the turnaround time for screening and confirmatory testing. As can be seen, 42 out of the 47 respondents had a turnaround time of three days or less for a negative testing. In contrast, when the specimen was EIA reactive and needed to be confirmed, it took up to seven days for 43 of the respondents to report a confirmed specimen.

This extended period of time is a constant source of concern between testing facilities and counseling programs in the various program authorities.
Tables 5 and 6 summarize survey data submitted by those states testing for HIV-2 and HTLV-I/II. As concerns about other retrovirus-induced infections increase, the testing resources of Public Health entities are being directed toward efforts to gather the needed information. Data in 1990 indicated 6 laboratories testing for HIV-2 and 11 laboratories testing for HTLV-I/II. The 1991 Survey data indicate this number has changed to 8 and 7 laboratories respectively.

There are several indications that this trend of increasing tests for retrovirus other than HIV-1 will continue into the immediate foreseeable future.

The CDC is coordinating a study to gather data on research HIV-2 Western blot kits. This study will provide states with a confirmatory HIV-2 testing procedure and will assist states which are using HIV-1/2 combination kits to develop an acceptable algorithm.
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6 Seventh Annual Conference on Human Retrovirus Testing
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PANEL SESSION I
TESTING: THE SEROLOGIC DIAGNOSIS OF HIV

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PANEL MEMBERS:
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PANEL RECOMMENDATIONS

1.01 Additional data presented at this conference support the ASTPHLD/CDC interpretative criteria for Western blot evaluation. Nevertheless, concern was expressed about positive Western blot patterns with env only and those with weakly reactive bands.

1.02 A small number of samples with env reactivity only, meeting ASTPHLD/CDC criteria for positive Western blot interpretations have been demonstrated to occur in apparently uninfected individuals. Weak or incomplete Western blot patterns resembling seroconversion samples can result from cross contamination. To assess the significance of such patterns, follow up specimens should be obtained whenever possible to establish infection status or progression. Individual laboratories should collect such information for submission to the ASTPHLD Retrovirus Committee for review.

1.03 It was re-emphasized that for all first time positive samples/patients a second follow-up dedicated sample be requested and tested to verify the result. The need for a follow-up sample should be indicated in the laboratory report.

1.04 There are concerns about basing laboratory proficiency testing on programs that use testing panel samples prepared from pooled materials which can cause technical variation. It is recommended that pooled materials not be used to prepare proficiency panels.

1.05 Each state public health laboratory must determine whether to use the combination HIV-1/2 EIA screening for HIV infection based on epidemiologic data pertinent to their region. If the combination assay is adopted, the following algorithm is recommended pending the development of a licensed combination Western blot and other confirmatory tests that can be incorporated in the algorithm. (See following page.)

1.06 It is suggested that the algorithm be modified upon development and approval of combination HIV-1/2 Western Blot and other supplemental assays.

1.07 The development of combination HIV-1/2 Western Blot and other supplemental assays for the confirmation for HIV-1/2 testing is encouraged.

1.08 It was recommended that the ASTPHLD Retrovirus Committee initiate a validation study of the newly licensed IFA test kit for HIV-1. The findings of such a study should be reported to the conference at the 1993 meeting.

1.09 The development and licensure of a confirmatory procedure for use with dried blood spots is encouraged.
* An immunofluorescence assay (IFA) for HIV-1 antibodies has recently been licensed by the FDA and can be used instead of Western blot (WB). Positive and negative IFA results should be handled in the same manner as similar results from WB. IFA INDETERMINATES should first be tested by HIV-1 WB and then the results should be treated as indicated by the WB results in the algorithm.
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Jack Phillips
Dr John V Parry
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Kathryn Anne Pittman
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Madonna Young
Suzanne Zanto, MT(ASCP)
Maan Zrein
PANEL SESSION II
TESTING: LYMPHOCYTE SUBSET QUANTITATION

PANEL CHAIR:
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PANEL RECOMMENDATIONS

Immunophenotyping of peripheral blood lymphocytes is the principal laboratory technique for monitoring the course of HIV infection. The following recommendations are provided to assist laboratories which are considering flow cytometry testing.

2.01 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.

(b) Clinical flow cytometry training courses specific for laboratory directors and supervisors should be developed.
(c) The feasibility of certification programs for laboratory technicians trained in flow cytometry should be investigated.
(d) The development and implementation of accreditation standards for all training courses and workshops in clinical flow cytometry is recommended.
(e) Information about clinical flow cytometry training courses and workshops by CDC and other organizations should be provided and updated as necessary.

2.02 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) Standardization of instrument optical alignment, spectral sensitivity, and fluorescence compensation must be performed daily.

2.03 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) In-house training at experienced laboratories may be an acceptable alternative.
(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.


2.05 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 NCCLS Tentative Standard (1984), H20-T, Leukocyte Differential Counting is endorsed. 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 Panel 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 (both HIV positive and HIV negative) for which hematologic results are comparable to fresh specimens.

(c) The laboratory performing the hematology should maintain a documented intra-laboratory coefficient of variation less than five percent for the white blood cell (WBC) count.

(d) Laboratories should evaluate and characterize intra-laboratory 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 less than 24 hours old.

(b) Recognizing that laboratories may receive suboptimal (old) flow cytometric specimens, it is recommended 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.

2.06 Whole blood lysis and two-color immunofluorescence are the methods of choice for flow cytometric immunophenotyping.

2.07 The following two-color monoclonal antibody panel for routine immunophenotyping is recommended:

See table next page
Monoclonal Antibody | Cell Type Enumerated
---|---
1. IgG1/IgG2 | Isotype controls
2. CD45/CD14 | % lymphocytes in gating region
3. CD3/CD4 | T-helper/inducer subset
4. CD3/CD8 | T-suppressor/cytotoxic subset
5. CD3/CD16+CD56 | Total T cells/Total NK cells
6. CD19 | Total B-Cells

(a) FITC/PE-labeled reagents
(b) Lymphocytes will be CD45+CD14+
(c) Indicated cell type will be positive for both antibodies
(d) Total T cells = all cells expressing CD3; Total NK cells = all cells which are CD3 negative but positive for CD16 and/or CD56.

2.08 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 lowest limit of acceptable lymphocyte representation in the lymphocyte gate.
(b) At least 95 percent of lymphocytes should be contained within the light scatter gate.

2.09 Lymphocyte subset percentage values from the flow cytometer should be corrected by dividing the observed percentage by the percentage of lymphocytes (CD45+CD14+) in the lymphocyte gating region.

2.10 For most specimens, the total of the corrected CD3+ (Total T), CD19+ (Total B), and CD5+ &/or CD16+ (Total NK) percentages should sum to between 95 and 105 percent.

2.11 Each laboratory must establish age- and population-appropriate reference ranges in accordance with validated statistical criteria. It should be noted that pediatric reference ranges differ substantially from the reference ranges for adult populations.

2.12 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

2.13 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+CD4+, T-suppressor/cytotoxic = CD3-CD4-).
(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.

2.14 The efforts by the Centers for Disease Control to continue a national lymphocyte immunophenotyping performance evaluation program including training and education programs is strongly supported.

2.15 The efforts of the NCCLS, the NIAID Flow Cytometry Advisory Committee, and the CDC in setting guidelines for clinical flow cytometric immunophenotyping is strongly supported.

2.16 The continued development of alternative (non-flow cytometric) methods for the enumeration of CD4+ lymphocytes through CDC, NIH and WHO is encouraged.

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PANEL SESSION III
TESTING: NON-SEROLOGIC TESTING METHODS

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PANEL RECOMMENDATIONS

3.01 For specimens obtained “on site”, with same day processing, whole blood specimens with EDTA, Heparin, or ACD are suitable for PCR testing. For specimens which are shipped from another site, ACD may be suitable for up to 5 days. For tubes with EDTA or heparin, the maximum transport time is 48 hours.

3.02 Every effort should be made to obtain whole blood samples 24-48 hrs after venipuncture. Samples should be transported at room temperature.

3.03 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.

3.04 Each laboratory should optimize critical components of its PCR protocols (e.g. magnesium concentration, annealing and extension conditions, etc.) for optimum sensitivity and specificity as determined by proficiency test results.

3.05 Solution hybridization with radioactive oligonucleotide probes is a highly sensitive and specific method and is recommended for detection of PCR products. However, a variety of non-radioactive detection methods are now available and those with comparable sensitivity and specificity are also recommended.

3.06 In order to obtain the authority to perform and report PCR results, the PCR sub-committee recommends 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.

3.07 Primers with documented sensitivity and specificity should be selected and used under optimum conditions.

3.08 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.

3.09 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.

3.10 The overall interpretation of PCR testing should be reported as HIV-1 DNA detected, HIV-1 DNA not detected, or Indeterminate.

3.11 Complete testing algorithms need not be reported.
3.12 Appropriate positive, negative, and reagent controls should be included with every PCR test. HLA controls may be used to validate specimen integrity.

3.13 All published guidelines for minimizing the contamination of specimens with amplified PCR product should be rigorously followed.

3.14 Biochemical sterilization of PCR products should be instituted in all PCR laboratories as soon as possible.

3.15 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.

3.16 The development of validation panels for distribution to PCR laboratories by the private sector, NIH, and CDC is recommended.

3.17 The development of standardized reagents and controls in the form of commercial kits is encouraged.

3.18 The immediate initiation of a proficiency testing program for PCR is recommended.

3.19 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 three months of life are needed.

3.20 The use of PCR for retroviral diagnosis in "high risk" seronegative adults and for the resolution of indeterminate serology in adults is not recommended as a routine procedure. PCR can be helpful as a supplemental test in these situations if used judiciously and in the full laboratory and clinical context.

3.21 PCR may be helpful in the diagnosis of rare individuals with defective antibody production.

3.22 PCR is useful for the differentiation of viral sub-types (e.g., HTLV-I/II or HIV-1 vs HIV-2).

3.23 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.

3.24 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.

3.25 Based on review of published and presented data, the consensus of the panel is that the detection of HIV-1 by PCR or culture in antibody-negative individuals is restricted to the seroconversion window which is less than six months (95% confidence interval). The Retrovirus Committee could play an important role in disseminating this information to the community to clarify a continuing misunderstanding.
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TESTING: SPECIAL TOPICS

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PANEL RECOMMENDATIONS

4.01 At the present time, based on several concerns, the use of urine or saliva for HIV-1 antibody testing is not recommended. These include the assurance of sample adequacy at the time of collection, the increased frequency of indeterminate Western blots and suitability of various HIV-1 EIA assays for antibody screening.

4.02 The nature of oral samples needs to be assessed as to subclasses of IgG detected and the amount of IgG present. Manufacturers need to include a marker or method of sample adequacy in their collection devices. Further testing needs to be done to assess the utility of oral samples for HIV-1 testing.

4.03 Interested investigators and manufacturers should continue to collect data supporting the use of saliva and urine for HIV-1 antibody testing and supply these data to appropriate agencies for approval.

4.04 It is recommended that the interpretative criteria for HTLV-III Western blot be changed due to the under-reporting of true positive results using the current criteria. A positive test should include at least one gag (p19,p24) band and one env band. Bands used for interpretation should be consistent with manufacturers recommendations.
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FLOW CYTOMETRY WORKSHOP

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Verna Willis
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3 Comparison of LAV and HIV-1 Viral Lysate EIA for Detection of HIV-1 Antibody • Robin Botchle, Jerry Kudlac, Garry L McKee

4 Comparative Evaluation of Six Rapid Serological Tests for HIV-1 Infection • J D Malong, E S Smith, J Sheffield, D Bigelow, K C Hyams, S G Beardsley, R S Lewis, C R Roberts

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6 Development of a Combination Assay for the Detection of HIV-1 and HIV-2 Antibodies • R Bauer, A Alonso, C Benoit, J Carten, J Connelly, J Johnson, S Lambert, Y Devash

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8 A Combination Screening EIA for the Simultaneous Detection of HIV-1 and HIV-2 Antibodies in Serum or Plasma • Miriam Stella, Teresa Chan, Annelie Wilde, Gary Mantha

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18 A Rapid Automated Immunoassay for the Detection of Antibodies to HIV-1 and HIV-2 Utilizing Recombinant Antigens • Charles Ginsburgh, G Hall-Steele, K Nordmark, J Pawlowski, R Stumpf, S Webber, S Stramer
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Use of and HIV Combination Assay to Screen STD Clinic Populations: Increase in Initial Reactive Samples Compared with an HIV-1 Assay for Antibody • Barbara G Werner, Lauri C Gibbons

Application of the Polymerase Chain Reaction to Guthrie Cards for Identification of HIV Infection at Birth • Anne Comeau, Ho-Wen Hsu, Mark Schwerzler, Galina Mushinsky, George Grady

Evaluation of a New Generation Combination Synthetic Peptide Assay Designed to Detect Antibodies to HIV-1, HIV-2, HTLV-I, and HTLV-II Simultaneously • Niel T Constantine, X Zhang, J Bansal, J Callahan

Accuracy and Precision of T-Lymphocyte Immunophenotyping: Results of a Blind Inter-laboratory Survey • K M Peddecord, R S Garfein, D P Francis, G D Cross, W O Schalla, A S Benenson, L K Hofherr

Shopping for a TL1 Reference Laboratory • Criteria for Selecting the Best • D P Francis, K M Peddecord, A F Back, R S Garfein, L K Hofherr, A S Benenson, G D Cross, R N Taylor

Evaluation of Three Manufacturers’ FDA-Licensed HIV EIA Kits on Indeterminate Serum Specimens • Adj S Virji, Warren L Kleinsasser, Robert S Shea, Betsy R Sears, Kimberlee Anderson

Human T-Lymphocyte Virus Antibody Screening of Paid Serum Donors • Barbara J Weiblen, Patricia E Garrett, Leanna Durgin, Richard T Schumacher

Testing for HTLV Antibody: Results of the Centers for Disease Control HTLV Survey Questionnaire • S O Blumer, W O Schalla, J S Hancock, A L Branch, R J Fehd

The Testing of Saliva for Presence of Antibodies to HIV-1 • Robert L Stout, Ph. D.: Mark Magee

Urine Testing • Robert L Stout, Ph. D.: John Bo岌; Mark Magee

Saliva for Antibodies to HIV Using Specimens Collected with the Omnisa Saliva collection Device • Dawn Fitzgibbons, BS, MT(ASCP): Eugene Seymore, M.D., M.P.H.; Sharon Adair, B.S., MT(ASCP)

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Comparison of Sensitivity of Three Anti-HIV-1/2 Kits: Whole Cell Viral Lysate (VL) - Genetic Systems; Synthetic Peptides (SP) - IAF BioChem; Recombinant Protein(RP) - Abbott Laboratories • K Gupta, P Gill, B K Buchner, M V O’Shaughnessy

Monospecific Polyclonal Reactivity to HIV-1 gp41 as the Cause of HIV-1 Western Blot False Positive Test • K Sayre, MS; G Tegtmeier, Ph.D.; W Bayer, M.D.; S Alexander, Ph.D.

Identification of HTLV-II Infection among Amerindians in Venezuela • Gloria Echeverria de Perez, M.D.; Nicolas Bianco, M.D.

HIV Antibody Testing on Saliva collected with the Omni-Sal Device • Carol Major, Stanley Read, Peggy Millson, Randall Coates, Dale Dematteo, Liviana Calzavara, Janet Rigby, Margaret Fearon, Michael O’Shaughnessy

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