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POSTER PRESENTATIONS: Sharon Blumer

EVALUATION OF LABORATORY PERFORMANCE IN DETERMINING HIV-1 VIRAL RNA LEVELS PRESENT IN CDC PERFORMANCE EVALUATION SAMPLES. Sharon O. Blumer, M.S., William O. Schalla, M.S., Thomas L. Hearn, Ph.D., and Ronald J. Fehd, B.S., Division of Laboratory Systems, Public Health Practice Program Office, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia

One of the objectives of the CDC Model Performance Evaluation Program (MPEP) is to monitor the quality of HIV-1 ribonucleic acid (RNA) determinations as reported by laboratories testing RNA performance evaluation panels provided by the CDC MPEP.

Replicate panels of five uniquely coded plasma samples from individual HIV-infected or HIV-uninfected donors were mailed in June, 1997 and February, 1998 to approximately 150 laboratories enrolled in the CDC MPEP. Duplicate samples from an HIV-infected donor were included in each survey panel. Prior to shipment, the CDC tested plasma samples from each donor with HIV nucleic acid amplification kits manufactured by Chiron Diagnostics, Organon Teknika, and Roche Diagnostic Systems. Laboratories were requested to test these samples for HIV RNA as they would routinely test clinical specimens and report their results to CDC on forms designed to collect data on method used (manufactured kit or in-house procedure), lot numbers, controls used, test results for controls and for panel samples.

From the results reported for the June 1997 and February 1998 surveys, the overall analytic sensitivity was 97.8% and 99.6%, respectively. The overall analytic specificity determined from the results reported was 97.3% and 93.9% for the respective surveys. The errors within and between surveys were random and were not associated with any specific donor sample, laboratory type, or manufactured test kit. For each HIV-infected donor the median number of RNA copies/ml varied 4-fold to 5-fold depending on which manufactured kit was used. The median RNA copies/ml, grouped by kit, was reproducible within 0.5 log10 difference for the duplicated sample in each panel.

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ANALYSIS OF p24 ANTIGEN TESTING RESULTS REPORTED TO THE CDC MODEL PERFORMANCE EVALUATION PROGRAM BY LABORATORIES PARTICIPATING IN THE JUNE 1997 AND MARCH 1998 SURVEYS. Sharon O. Blumer, M.S., William O. Schalla, M.S., Thomas L. Hearn, Ph. D., Ronald J. Fehd, B.S., Division of Laboratory Systems, Public Health Practice Program Office, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia

In June 1997, the CDC Model Performance Evaluation Program (MPEP) implemented a project to evaluate the performance of laboratories testing for the presence of human immunodeficiency virus type 1 (HIV-1) p24 antigen.
Approximately 200 laboratories enrolled in this voluntary, non-regulatory program.
Plasma samples for the surveys were obtained from individual HIV-infected or HIV-uninfected donors and were neither diluted nor pooled. The p24 antigen reactivity of survey donor samples was determined first by CDC after testing with p24 antigen test kits manufactured by Abbott Diagnostics, Coulter Diagnostics, and Organon Teknika. Replicate panels of five uniquely coded samples were mailed to participating laboratories in June 1997 and March 1998. Duplicate samples from an HIV-infected donor were included in each survey panel. Laboratories were asked to test these samples for HIV-1 p24 antigen as they would routinely test clinical specimens or blood donor units and report their results to CDC on forms designed to collect data on test kits used, lot numbers, controls used, and test results for controls and for panel samples.

For p24 antigen qualitative tests, the overall analytic sensitivity and specificity, determined from laboratory results reported, exceeded 94% for each of the surveys. As determined from laboratory results reported, the overall analytic sensitivity and specificity of the p24 antigen confirmatory neutralization tests exceeded 98%.

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POSTER PRESENTATION: Barry M. Bredt

CMV DISEASE STATUS AND CMV-SPECIFIC LYMPHOCYTE RESPONSES MEASURED BY FLOW CYTOMETRY IN HIV-1-INFECTED SUBJECTS: EVIDENCE OF RESTORATION OF CMV-SPECIFIC CD4+ RESPONSES FOLLOWING THERAPY WITH GACICLOVIR AND HAART. Barry M. Bredt, M.A., Joseph M. McCune, M.D., Ph.D., Gladstone Institute of Virology & Immunology and San Francisco General Hospital, University of California, San Francisco, Krishna V. Komanduri, M.D., Mohan N. Viswanathan, B.S., Eric D. Wieder, Ph.D., Diane K. Schmidt, B.S., Gladstone Institute of Virology & Immunology, University of California, San Francisco, Mark A. Jacobson, M.D., San Francisco General Hospital, University of California, San Francisco

Recent studies of HIV-1-Infected subjects has produced conflicting results about the extent of reconstitution possible in the CD4+ lymphocyte repertoire after highly active antiretroviral therapy (HAART). Cytomegalovirus (CMV) infection is an important cause or morbidity and mortality in immunocompromised patients and is the leading cause of blindness in patients with AIDS. It is now possible to quantitate antigen-specific CD4+ lymphocyte responses by a flow-cytometric method. Using this method, we studied CMV-specific CD4+ lymphocyte responses in HIV-1-infected individuals with and without active CMV end organ disease (EOD), or with quiescent EOD.

After confirming the specificity of this approach by examining CMV-specific responses in a group of HIV-seronegative volunteers stratified according to CMV serostatus, we determined CMV-specific CD4+ lymphocyte frequencies in a total of 30 HIV-1-infected individuals. HIV-1-infected subjects without a history of CMV-associated-EOD have strong CMV-specific CD4+ lymphocyte responses. In contrast, individuals with CMV-associated EOD had significantly lower CMV-specific frequencies relative to those with no history of EOD. However, after treatment with HAART and ganciclovir therapy, those with quiescent retinitis demonstrated responses not significantly lower than those without EOD, though responses in these individuals were more variable.

Loss of CMV-specific immune responses by flow cytometry-based assessment correlates with active CMV EOD. The presence of strong CMV-

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POSTER PRESENTATION: Clive Loveday

THE DETERMINATION OF THE PREVALENCE OF HIV-1 NON-B SUBTYPES IN A UK HIV/AIDS CLINIC. C. Loveday, H Devereux, A Burke, L Dann, M Johnson. Department of Retrovirology and HIV/AIDS, Royal Free Hospital and Medical School, London, NW3 2PF.

Quantitative plasma HIV-1 RNA load (VL) determination has become the cornerstone for disease staging, and initiating and monitoring antiretroviral therapy in patients with HIV-1 infection. To date, only single or clusters of case of HIV-1 non-B subtypes (NB) have been reported in Europe and US. However, frequent divergent VL results using different commercial assays in our clinic suggested the presence of a significant number of patients carrying NB viruses. Therefore, we carried out a cross-sectional study to determine the prevalence of NB viruses in our patient populations and explored their variability in responses using 4 commercial VL assays. One hundred and fifty two consecutive clinic patients selected with epidemiological evidence of acquiring their infection outside the UK had their viruses characterized using a competitive peptide EIA and/or sequencing. VL's were measured using an RT-PCR (Roche v1.0:c/o 400c/ml), RT-PCRnb (Roche v1.0 and including add in primers SK145/151: c/o 400c/ml), NASBA (Organon Teknika:c/o 2000c/ml) and bDNA (Chiron Corp:c/o 500c/ml). In this cohort 133/152 were characterized using the EIA, of these 98 (74%) were NB and 35 (26%) were B. Using total clinic attendance over the study period as a denominator (n=1026) we calculated a crude clinic prevalence of 9.7% for our population. Detection of NB was significantly higher using RT-PCRnb than RT-PCR, NASBA, or bDNA with rates of 94%, 78%, 62% and 72%, respectively. Mean VL's were also highest for RT-PCRnb compared to RT-PCR (+0.58, +/- 1.01 log c/ml;$p<0.0001$) and maximum interassay difference of >1.0 log c/ml was seen in 49/98 (50%) of patients. Greater concordance was observed across assays for B viruses but maximum interassay difference of >1.0 log c/ml was still observed in 5/35 (14%) of patients. Proportion of NB patients would start therapy using RT-PCRnb. This is the first field study of its kind in the UK and defines a relatively high prevalence of NB (9.7%) in our clinic population. Variability in responses using different VL assays requires strategies for patient and

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POSTER PRESENTATION: Wendy Chen

PROPOSED ONE DAY ASSAY FORMAT FOR THE CURRENT LICENSED CAMBRIDGE BIOTECH HIV-1 WESTERN BLOT FOR DETECTION OF ANTIBODIES IN SERUM, PLASMA OR URINE. Wendy Chen, M.S., Richard Kowalski, Ph.D., Dana Villallas, Herbert Reyes, Anne Thornhill. Cambridge Biotech Corporation, Rockville, Maryland

A proposed five hour assay format for HIV-1 antibody detection in serum or plasma using Cambridge Biotech (CBC) licensed Western Blot was evaluated for its equivalency to the currently licensed overnight assay. The one day assay includes minor adjustments of the sample volume added (from 20 ul to 30 ul) and the concentrations of the conjugates (increased). Five hundred and eight samples including the CBER lot release panel, BBI mixed titer panel, seroconversion panel, known positive specimens, matched serum and plasma, in house QC and dilutional panel, and other clinically diagnosed diseased samples were tested among four different kit lots using both assay formats. The results showed a high degree of comparability in band presence and intensity between both assay formats. There were no discrepancies between the two assay formats in sample interpretation and meeting expected specifications. The data presented clearly showed that a procedural modification of the CBC HIV-1 Western Blot kit licensed by the FDA can provide the option to be used as either an overnight assay or one day assay for serum or plasma.
In addition, a proposed three hour assay format for HIV-1 antibody detection in urine specimens using the CBC recently licensed Western Blot kit for urine with a different substrate component was evaluated. Specimens from

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POSTER PRESENTATION: Yuqi Zhao

HIGHLY REPRODUCIBLE AND SENSITIVE HIV-1 DNA QUANTIFICATION USING TAQMAN TECHNOLOGY WITH A BROAD LINEAR RANGE OF DETECTION. Johann W. Miller[1], Mingzhong Chen[2], Eric Bremer[2], William Kabat[2], Ram Yogev[2-3] and Yuqi Zhao[1-4]. Center for Biotechnology, Northwestern University[1], Children's Memorial Institute of Education and Research[2], Departments of Pediatrics [3], Microbiology-Immunology[4], Northwestern University Medical School

Background: The use of highly active antiretroviral therapy (HAART) in HIV-infected patients yields dramatic reduction in the viral RNA load often to undetectable levels in plasma. This new strategy raises the hope for complete viral eradication. However, due to the inaccuracy of detecting the integrated proviral DNA in latently-infected CD4+ lymphocytes, it is difficult to evaluate how long the viruses can be completely cleared under HAART. Hence, a highly reproducible assay that can be used to detect low copy of proviral DNA is crucial to assess the efficacy and timing of viral eradication.
Methods: TaqMan technology (also known as real-time PCR, or 5' exonuclease assay) was used to quantify HIV-1 gag DNA using a quantitation standard (QS) and was performed on a Perkin-Elmer Thermocycler 7700.
Results: The HIV-1 gag gene was used as the HIV target for quantitation. A random DNA sequence, which has the same %GC as the HIV-1 gag gene and that can be amplified using the same pair of primers to yield a fragment of the same length, was cloned into a plasmid and used as the QS. Two fluorescent probes, one specific for the gag gene and the other for the QS sequence, were used in parallel detection of the amplified products. An dynamic linear range of more than 9 logs (10 to 1.0 x 1010 copies of HIV-1 gag DNA) was detected from the gag DNA amplified from in vitro infected cells. Both inter- and intra-assay variations are within 2.5-fold over the entire dynamic range. Testing of detection limit showed that 10 copies of HIV-1 gag DNA could be detected 92% of the time (45/49, n=49). 83% (15/18), 44% (8/18) and 33% (6/18) of the samples that contained 5, 2.5 and 1.25 input copies of gag gene were detected as actual 5.2+4.1, 1.9+3.4 and 1.7+3.5 copies, respectively. No gag DNA was

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POSTER PRESENTATIONS: Catherine W. Patrick

ROUTINE SCREENING FOR MYELODYSPLASIA IN LONG-TERM HIV+ INDIVIDUALS. Catherine W. Patrick, Ph.D. and Robert H. Keller, MD, FACP – Immune Balance Technologies, Hollywood, Florida.

As survival times for HIV positive individuals are increasingly prolonged due to advances in therapeutics, the effects of long-term therapy on the hematopoietic network continues to reveal itself.

Alterations to the erythroid line resulting in macrocytic anemias with megaloblastic changes and nuclear cytoplasmic asynchrony are common in those individuals receiving AZT, DDI and other drugs known to be associated with hematopoietic injury. Less commonly appreciated until survival duration was extended, was the existence of similar damage to the myeloid lineage, particularly the neutrophilic series. The emergence of neutrophilic subclones displaying macropolycytes, hypersegmentation, atypical nuclear chromatin patterns, Dohle bodies and granule anomalies, is observed with increasing frequency.

The Bayer H*3 RTX automated hematology system is particularly valuable in detecting these myeloid changes, as its technology is based on the measurement of cell volume, peroxidase content, nuclear volume and chromatin density. In the cytograms of these patients, the co-existence of 'normal' and dysplastic neutrophil populations is clearly defined. Longitudinal studies reveal the waxing and waning of populations, involvement of other cell lines (such as monocytes and eosinophils) and

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MEASUREMENT OF GLUTATHIONE [GSH] AS AN INDICATOR OF IMMUNE STATUS. Catherine W. Patrick, Ph.D., Miguel De Leon, Amled M. Leon, B.S., Robert H. Keller, MD, FACP – Immune Balance Technologies, Hollywood, Florida.

It has been previously demonstrated that HIV infection is associated with reduced levels of GSH, which favors viral replication and disease progression. Longitudinal assays of GSH levels in peripheral blood mononuclear cells [PBMC] can reveal trends that may be reversed through administration of supplements that restore intracellular GSH levels.

Heparinized peripheral blood specimens are immediately placed on ice and held until PBMC Ficoll-hypaque [FH] enrichment is performed. Intracellular GSH concentrations are volume dependent. Using a Bayer H*3 RTX automated hematology system, PBMC populations are quantitated and characterized as to cell type and volume. Using metaphosphoric acid, a cryoprecipitate is prepared from $5X10^6$ PBMC/ml. At this step, specimens may be held indefinitely at –85° C, which allows for batch analysis. Duplicate specimen aliquots of 50 µL are assayed in a 96 well microtiter plate using glutathione oxidoreductase and NADPH. Color development is monitored every two (2) minutes for six (6) minutes using Bio-Tek EL808 Ultra microplate reader. Patient specimens are compared to a working calibrator curve. Low, normal and elevated GSH patient pools are employed as controls. Results are reported in nm X 10^6 / L of PBMC [reference range: 1000-1500 nm X 10^6 / L PBMC]. Lineage and subset specific GSH levels may be obtained in the same fashion by inserting a magnetic negative selection step after the FH PBMC enrichment and prior to the cryoprecipitation. CVs on duplicate in assays should be in the range of

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POSTER PRESENTATION: G. David Cross

COMPARISON OF ABSOLUTE CD4 AND CD8 T-CELL RESULTS MEASURED BY MULTI-PLATFORM AND SINGLE-PLATFORM METHODS G. D. Cross*, J.H. Handsfield, W.O. Schalla. Centers for Disease Control and Prevention (CDC)

Absolute CD4 and CD8 T-cell counts can be measured by multi-platform methods (flow cytometer in conjunction with a hematology instrument) and single-platform methods (single instrument or procedure). Single-platform methods include flow cytometers in which absolute counts are directly determined and machines using spatial capillary cytometry to determine absolute counts. Interlaboratory CD4 and CD8 T-cell results reported by participants in CDC's Model Performance Evaluation Program (MPEP)and determined by multi- and single-platform methods were compared. The results were from mailed whole blood specimens collected from both HIV-infected and non-infected donors. For the analysis, donor specific test results were combined from three shipments (April 1997, September 1997, March 1998). In the study, we assumed that any observed difference in absolute T-cell count values were method rather than laboratory associated, i.e., all laboratories were equally capable of performing testing.

Conclusions: Although, the difference between the mean absolute CD4 and CD8 T-cell count values obtained with multi-platform methods values were significantly different than the values obtained with single-platform methods (α=.05), the differences were small and may not have biologic significance. As measured by observed standard deviations from the mean, absolute CD4 and CD8 T-cell counts were more reproducible (α=.05) across laboratories using single-platform methods than across laboratories using multi-platform methods.

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POSTER PRESENTATION: Gary Murphy

A MODIFIED HIV 1 PEPTIDE SEROTYPING ASSAY WITH INCREASED EFFICIENCY. Gary Murphy BSc, Kim M. Lewis, Angus G. Nicoll MD and John V. Parry PhD. Central Public Health Laboratory, Colindale, London, U.K.

HIV 1 subtype specific serotyping was undertaken on 100 specimens of known genotype. Specimens were tested using a published method and subsequently by a modified method that included a 6M urea wash step following sample incubation. This was expected to remove low affinity cross-reactive antibodies and thereby increase the serotyping assay's efficiency.
Washing with urea increased the sensitivity of the assay, especially for subtype B infections: without urea 65% of subtype B infections were correctly identified; with urea 74% were. Additional effects were a reduction in the number of serum dilutions that had to be assayed to maximize the number of monotypic responses and a small increase in the specificity of the assay for HIV 1-B detection from 95.7% to 96.2%.
After testing at various dilutions, 77 specimens were assigned a monotypic subtype without a urea wash, and 79 following a urea wash. The number of assays performed to obtain a conclusive result were 203 and 154, respectively.
Significant accuracy and cost gains may be obtained by employing the urea wash. In populations where subtype B infections predominate serotyping can be used as a screening method, with genotyping reserved to identify the non-B subtypes. The improved accuracy of the urea modified serotyping assay further reduces the proportion of samples for genotyping.

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POSTER PRESENTATION: Gloria Echeverria de Perez

HIV-1 IMMUNOFLUORESCENCE (IF): VALIDATION OF THE TEST AND COST- EFFECTIVENESS IN HIV DIAGNOSIS. Jacqueline Perez Bio, Matilde Leon-Ponte MSc, Gloria Echeverria de Perez MD, MSc. Institute of Immunology, Central University of Venezuela.

Objectives: to evaluate sensitivity (S) and specificity (Sp) of IF as supplementary-confirmatory test as compared to Western blot (WB); and to calculate cost-efficiency of IF vs. WB algorithms (A).

Material and methods: 820 previously HIV characterized specimens were studied by EIA, WB and IF, 460 were negative and 360 positive. Commercial reagents were used for EIA (Abbott, OTC) and WB (Sanofi, Epitope); for IF dried spots of a 1:3 mixture of HTLV-III infected : H9 uninfected cells were done in our lab. For cost efficiency, years 1996 and 97 were analyzed, giving an average of 1500 specimens/year, 70% of which were EIA negative on first run and reported as such, while 30% required second EIA and supplementary testing. Cost of our routine algorithm (A1), where all EIA double reactive or discordant specimens are tests by WB, was compared to the cost of performing IF as first line confirmatory test (A2).

Results: results of IF were as expected for 819 sera, one positive sample was read as inconclusive (0-1+). Considering this last sample as a false negative, the S, Sp and PPV of IF was 99.7%. To calculate the cost of IF algorithm (A2), we assume as IF truly positive only those specimens with fluorescence of (2+; therefore, 4% of samples tested would have required WB to resolve. The cost of 1500 specimens, including 500 EIA reactive (30%) by A1 was $23,000, as compared to $8,950 for A2.

Conclusions: the inclusion of IF as the first line confirmatory test reduces the costs of HIV serological diagnosis in more than 60%. IF has been extensively validated in several centers including our Reference Laboratory; besides the need for trained personnel and facilities for cell lines expansion, it is a very simple procedure and most valuable for developing countries with limited resources and steady increase of HIV prevalence.

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POSTER PRESENTATION: Jerilyn Sturge

REDUCED RATE OF URINE COMPARED TO SERUM INDETERMINATE HIV-1 WESTERN BLOT RESULTS. Jerrilyn C. Sturge, M.A.[1], Toby D. Gottfried, Ph.D.[1], Roy W. Stevens, Ph.D.[2], Howard B. Urnovitz, Ph.D.[1] [1]Calypte Biomedical, Berkeley, CA and [2]Biomedical Resource, Albany, NY.

The number of urine Western blot indeterminates was far fewer than the number of serum Western blot indeterminates in clinical studies testing for HIV-1 antibodies by the Cambridge Biotech HIV-1 Western Blot kit using paired specimens. Urine and serum bands were compared to determine if there was any pattern or frequency differences.
Specimens from low and high risk groups, HIV infected individuals including AIDS patients, and subjects with other non-HIV medical conditions were tested regardless of EIA result by Western blot. In total, 2,159 urine specimens were tested, including 109 paired to serum blot indeterminates and 114 known urine EIA false positives.
Results show that 99.77% (2,154/2,159) of the urine blots resulted in the correct interpretation relative to known clinical status or serum result. Urine indeterminate rates were 0% in the low risk, HIV positive and AIDS groups. In the high risk group, the indeterminate rate was 0.5% (2/391) compared with 33.2% (123/371) for serum. In the other medical conditions group, the indeterminate rates were 3.9% (11/281) and 36.7% (73/199) for urine and serum respectively. Tests of 109 pre-selected EIA non-reactive blot-indeterminate sera, showed only 4 (3.7%) matched urine specimens were indeterminate and 105 (96.3%) were negative. Tests of 114 EIA false positive urines showed only 5 (4.4%) indeterminates, 109 (95.6%) negatives and no positives, compared with the paired serum findings of 22 (19.3%) indeterminates, 92 (80.7%) negatives and no positives.
The indeterminate serum band patterns showed p24 predominately, followed by p51 and p17. For the relatively fewer urine indeterminates, the predominate band also was p24.
Analysis of urine by EIA and blot may provide valuable information for resolving HIV status and for determining patient care when a serum blot is

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POSTER PRESENTATIONS: Diane P. Francis

RESULTS OF A SURVEY EXAMINING HIV VIRAL RNA REPORT CONTENT. Diane P. Francis, MT(ASCP)[1], K. Michael Peddecord, Dr.P.H.[1], Abram S. Benenson, M.D.[1], Louise K. Hofherr, Ph.D.[1], Carrie Rigdon[1], John R. Astles, Ph.D.[2], William Schalla, M.S.[2], [1]Laboratory Assurance Program, Graduate School of Public Health, San Diego State University, San Diego, CA. [2]Division of Laboratory Systems, Public Health Practice Program Office, Centers for Disease Control and Prevention (CDC), Atlanta, GA.

The Laboratory Assurance Program, Graduate School of Public Health, San Diego State University, conducted a national telephone survey of laboratories to collect data on practices for viral load testing, and the content and format of viral load reports.
A telephone survey of laboratories believed most likely to perform viral load testing was conducted. Laboratories were randomly selected from three source groups: medical schools, nationally advertised commercial laboratories, and laboratories participating in the CDC Model Performance Evaluation Program. Respondents were also asked to fax or mail a copy of a negative and positive viral RNA report without patient identifiers.
A total of 212 telephone surveys were conducted. Of these, 42 respondents furnished HIV-1 viral RNA reports representing 40 different laboratories of the following types: 47.5% hospital, 17.5% health departments, 27.5% independent and 7.5% other. All laboratories reported results as copies/ml, with 10% also reporting the results designated as $\log_{10}$ copies/ml. The primers used, the optical density, and the dilution factor were, in each case, reported by only one laboratory (2.5%). The test kit used was reported by nine (22.5%) laboratories. Of the eight laboratories using methods not yet FDA licensed, five (62.5%) included a "research only" phrase. Twenty-three (57.5%) laboratories reported a test lower limit of detection or lower linearity limit and 13 (32.5%) reported the upper limit of detection or linearity. In three reports (7.5%) the results were handwritten. The degree of change from baseline necessary to predict disease progression or be useful in monitoring anti-viral therapy was given in seven (17.5%) reports. Four reports (10%) showed previous patient report values.
The laboratory's report of test results is an integral part of laboratory testing

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RESULTS FROM A TELEPHONE SURVEY OF LABORATORY PRACTICES FOR VIRAL LOAD TESTING. Diane P. Francis, MT(ASCP)[1], Louise K. Hofherr, Ph.D.[1], K. Michael Peddecord, Dr.P.H.[1], Abram S. Benenson, M.D.[1], Carrie Rigdon[1], John R. Astles, Ph.D.[2], William Schalla, M.S.[2], Laboratory Assurance Program, Graduate School of Public Health, San Diego State University, San Diego, CA. [2]Division of Laboratory Systems, Public Health Practice Program Office, Centers for Disease Control and Prevention (CDC), Atlanta, GA.

The laboratory's role in viral load testing assumes new importance with the use of combined anti-retroviral therapy in the treatment of the HIV-1-infected patient. A national telephone survey was conducted to investigate laboratory practices and determine the availability of viral load testing.
A telephone survey was conducted of laboratories expected to be performing HIV RNA or p24 antigen (Ag) testing. Laboratories were randomly selected from three source groups (medical schools, nationally advertised commercial laboratories, and laboratories participating in the CDC Model Performance Evaluation Program). A person designated by each laboratory to be knowledgeable about HIV RNA and p24 Ag testing was interviewed.
A survey response rate of 76% was achieved with the completion of 212 surveys out of a total of 279 attempted. Respondent laboratories consisted of 37 blood banks (8% perform HIV RNA; 91.4% perform p24 Ag), 122 hospitals (56.6% perform HIV RNA; 34.4% perform p24 Ag), 13 health departments (84.6% perform HIV RNA; 38.5% perform p24 Ag) 32 independent laboratories (75.0% perform HIV RNA; 56.3% perform p24 Ag), and 8 "other" type laboratories (62.5% perform HIV RNA; 50% perform p24 Ag). One hundred twelve (52.8%) respondents performed HIV RNA testing. The most frequently used HIV viral RNA test kit was the Roche Amplicor HIV-1 Monitor. Patient test volume for users of this kit ranged from 15 to 8,000 tests per month. Fifty-two (46.4%) of all HIV RNA respondents used quality control material other than that provided by the kit manufacturer. The laboratory charge for an HIV RNA test ranged from $65 to $300. One hundred three (48.6%) respondents reported their laboratory performed p24 Ag testing. The patient samples tested ranged from 1 to greater than 40,000 per month. Sixty-two (60.2%) of those performing the p24 Ag test used quality control material other

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POSTER PRESENTATION: Douglas J. Haney

A REVIEW OF TRADITIONAL QUALITY CONTROL TOOLS AND THEIR UTILITY IN CD45/SSC-BASED FLOW CYTOMETRIC IMMUNOPHENOTYPING OF LYMPHOCYTE SUBSETS

Douglas J Haney, Ph.D., Lupe R Miller, BS, Betty A Eastham, MT(ASCP), Meg A Reilly, MT(ASCP) Becton Dickinson Immunocytometry Systems, San Jose, CA

This poster contains a set of arguments that supports the replacement of the traditional 2-color quality control tools with a new set of tools for laboratories making the transition to 3- and 4-color immunophenotyping. The driving force behind the evolution of the old two-color quality control tools was the shrinking of the health care dollar. Cost efficiency considerations fueled the need for smaller immunophenotyping panels with minimal loss of diagnostic information. Since the traditional quality tools identified acceptable/unacceptable samples by assessing consistency across multiple tubes, usage of fewer tubes requires tools that are fundamentally different. In particular, the new tools are based not upon tube-to-tube agreement but upon recognition of dot-plot patterns that differ from the norm. Aberrant patterns that are known to be associated with old blood or improper sample mixing served as a test-bed for this pattern recognition approach.

Existing traditional tools

- CD3 consistency system
- T-sum
- Lymphosum
- Purity and Recovery
- Isotype controls
- Visual Inspection

New Tools

- Expert Gate/QC
- Visual Inspection

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POSTER PRESENTATION: Fusun Erler

THE EVALUATION OF INTERLABORATORY HIV QUALITY CONTROL PROGRAM IN ANAKARA/TURKIYE.

Fusun Erler, M.D., Ugur Ciftci, Ph.D., Mine Tunaoglu, M.S., Yahya Laleli, M.D. Duzen Laboratory, Ankara, Turkiye

In 1995 The Ankara AIDS Prevention Association planned to start a Quality Control Program among volunteer laboratories in Ankara to improve the quality of HIV testing, to identify and correct the problems occurring during HIV detection procedures.
Duzen Laboratory organizes the HIV Quality Control Program for The Ankara AIDS Prevention Association. Samples are sent to participants 3-4 times each year, tested by the procedures routinely used by them and the reported results were evaluated. Each set includes 3 specimens: HIV Ab positive/weak-positive or negative sera. HIV Ab positive samples are obtained from HIV positive patients, tested by EIA technique and confirmed by Western Blot analysis. HIV Ab weak-positive patients are prepared by serial dilutions of HIV Ab positive serum with human sera. The frequency of the intended response regarding the results evaluated each year from 1995 to 1998 were found 88.8%, 98.5%, 98.9%, and 100% for HIV Ab positive samples; 76.5%, 61.2%, 90.5%, and 100% for HIV Ab weak-positive samples; 100%, 100%, 93.8%, and 100% for HIV Ab negative samples, respectively. The lower frequency rate of intended response among HIV Ab weak-positive reported results indicate that there are significant differences in the sensitivity of the various techniques and kits for screening HIV Antibody supplied by commercial manufacturers. This program helps both the improvement of the reported quality control results and the standardization of HIV Ab detection procedures among participants.

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POSTER PRESENTATION: John E. Kim

LABORATORY COMPARISON OF 2 HIV ANTIBODY RAPID TEST KITS.

John E. Kim, Ph.D., Susan C. Macpherson, MLT, Debbie G. Lepine, B.Sc.
National Laboratory for HIV Reference Services, Bureau of HIV/AIDS, STD and TB, Laboratory Center for Disease Control, Health Canada, Ottawa, Ontario, Canada.

The National Laboratory for HIV Reference Services (NLHRS), Health Canada compared the performance of 2 rapid test kits for the detection of antibodies to both HIV1 and HIV2. The Merlin Immediate® HIV 1 & 2 Diagnostic Test (Merlin Biomedical & Laboratories, Inc., Nova Scotia, Canada) were tested against 2 performance panels purchased from Boston Biomedica Inc. (West Bridgewater, MA). An Anti-HIV 1 / 2 Combo Performance Panel (PRZ203) and an Anti HIV 1 Low Titer Performance Panel (PRB106) each composed of 15 serum or plasma samples were used to assess the performance of the two kits. In addition 30 in-house samples were included as part of the evaluation. All samples were tested by ELISA and Western blot.

The kits were evaluated for ease of use, volume of sample required, ability to interpret test results, sensitivity, specificity and reproducibility. Both tests were very simple to run requiring no complex instrumentation or extensive technical training. The Immediate HIV 1 & 2 Diagnostic Test had fewer steps in the procedure than the MedMira Rapid HIV Test Kit. Although technically both kits were very easy to use the interpretation of test results was not as simple. Difficulties with reading weakly reactive specimens with the Immediate device and conversely reading non-reactive specimens with the MedMira test were encountered. The sensitivity and specificity for both kits was influenced by the experience of the person reading the results. These assays may be less reliable than some currently licensed tests for detecting early seroconvertors or low HIV antibody titer samples.

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POSTER PRESENTATION: Janet L. Lathey

MACROPHAGE REPLICATION KINETICS OF SI AND NSI CLINICAL ISOLATES USING DIVERSE CO-RECEPTORS.

Janet L. Lathey, Ph.D., Donald J. Brambilla, Ph.D., Mostafa Nokta, Ph.D., Suraiya Rasheed, Ph.D., Edward Siwak, Ph.D., Maureen M. Goodenow, Ph.D., James W. Bremer, Ph.D., Elizabeth A. Ladd, M.A., Ronald G. Collman, M.D., and Patricia S. Reichelderfer, Ph.D. Pediatric AIDS Clinical Trials Group, NIAID, Rockville, MD.

A monocyte-derived-macrophage (MDM) culture assay has been developed which defines the growth kinetics of HIV isolates. Peripheral blood mononuclear cells from HIV- donors were isolated and cultured in RPMI plus 10% FBS. On day 7 nonadherent cells were washed away. On day 10 the MDM's were infected with HIV. Supernatants were collected and assayed for HIV p24 on days 3, 7, 10, and 14 post-infection (PI). In this assay, SF162 (macrophage tropic, NSI) produced increasing amounts of HIV p24 antigen with increasing time in culture. Conversely, LAI (nonmacrophage tropic, SI) infection resulted in low levels of HIV p24 antigen with no increase in production during the culture period. A panel of 12 clinical isolates was evaluated. All isolates produced detectable levels of HIV p24 antigen in MDM's. However, the NSI viruses had significantly higher log10 p24 antigen values at all times PI ($p<0.01$). In addition mean log10HIV p24 antigen production increased more in NSI than SI viruses between days 7 and 14 (0.9 vs 0.5, $p=0.01$). Co-receptor usage was determined for all 12 isolates (8 NSI and 4 SI). All SI isolates used CXCR4 for entry; 2 used CXCR4 only, 1 used CXCR4 and CCR5, and 1 used CXCR4, CCR5, and CCR3. None of the NSI viruses used CXCR4 for entry. All used CCR5 as their predominant co-receptor. Of the 8 NSI isolates, 3 used CCR5 only, 2 used CCR5 and CCR2b, 1 used CCR5 and CCR3, and 1 used CCR5, CCR3, and CCR2b. Log10HIV p24 antigen production for viruses that used CCR5 was greater than for viruses that did not use CCR5, regardless of SI phenotype. In addition, viruses that used CCR5 and CCR3 produced higher levels of HIV p24 antigen than viruses that used CCR5 alone or in combination with co-receptors other than CCR3. Thus, in these primary isolates macrophage tropism and replication kinetics were closely linked to CCR5 utilization, while SI capacity was

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POSTER PRESENTATION: Spencer H. S. Lee

EVALUATION OF THE MEDMIRA RAPID SCREEN TESTS FOR ANTIBODY TO HIV-1/-2, HBcAG AND HCV. Spencer H.S. Lee, Ph.D., Donna Zinck, RT., Kevin Forward, M.D., Michelle Currie, B.Sc., RT., Walter Schlech, M.D. Divisions of Microbiology and Infectious Diseases, QEII Health Sciences Centre, Halifax, Nova Scotia, CANADA

We evaluated a rapid membrane-based immuno-dot binding system (MedMira Laboratories) designed as a quick and simple on-site screening test for antibody to HIV-1/-2, HBcAg and HCV respectively in serum or whole blood. Reference tests employed to define sero-negative (s-neg) and sero-positive (s-pos) sera for the respective pathogens included the Abbott bead-EIA for anti-HBc, Abbott AxSYM for HIV-1/-2, HBsAg and HCV, Ortho-Cambridge Biotech HIV-1 Western Blot, Roche Amplicor HCV RNA-PCR and Ortho-Chiron HCV RIBA-3. For 640 HIV-1/-2 s-neg and 191 HIV-1 s-pos sera, the Health Canada-approved MedMira Rapid HIV Screen had a 100% sens and spec respectively. Likewise, the MedMira Rapid Anti-HBc Screen exhibited a 100% sen and spec for 40 HBsAg-pos and 80 anti-HBc-neg sera. For HCV, the sens, spec, PPV and NPV of the MedMira Rapid HCV Screen were 98.9% (86/87), 98.2% (164/167), 96.6% (86/89) and 99.4% (164/165) respectively. The correlation of results of the three MedMira Rapid Screens with those of the Abbott AxSYM was 100% with 110 s-neg sera submitted for the testing of HIV-1/-2, HBV (HBsAg) and HCV concurrently. The MedMira Rapid HIV Screen for whole blood also gave pos results for 154 EDTA blood collected for routine follow-up investigation from HIV-1-infected patients at our AIDS Clinic.

We concluded that the MedMira Rapid Screen system provides highly sensitive and specific results. It has potential as a rapid and simple on-site screening test for these blood-borne pathogens.

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POSTER PRESENTATION: Tina Charbonneau

CLINICAL AND LABORATORY ANALYSIS OF A SUSPECTED CASE OF TRANSIENT HIV-1 INFECTION IN A NEONATE. Tina Charbonneau, B.A., Timothy Sullivan, B.S., Stephanie Heverly, B.S., Colleen Flood, M.S. New York State Department of Health, Albany, New York, Susan Schuval, M.D. and Steven Weiss, M.D., Ph.D., Schneider Childrens Hospital, New Hyde Park, New York.

Transient HIV infection has been defined by a positive HIV culture or PCR on only one or a few occasions. Documented cases are considered rare and have been attributed to laboratory error. Since April, 1995, New York State has maintained the Pediatric HIV PCR Testing Service for the diagnosis of perinatal HIV-1 infection. The qualitative assay used measures HIV proviral DNA and has a sensitivity of 100% for infants 4 weeks of age or older. Presented is the investigation of a probable case of transient HIV-1 infection in a neonate.

EDTA whole blood specimens were submitted at 2, 4, 8, 42, 46 and 52 weeks of age. In the standard assay 1µg of mononuclear cell DNA was amplified with gag region primers SK101/145 and end product detection was performed with a P32 labeled SK102 probe. HIV1/2 EIA and WB was performed on plasma aliquots as well as quantitative HIV-1 RNA PCR (Roche Amplicor).

EIA results were positive at all time points. WB remained positive through 42 weeks of age but was indeterminant at 46 and 52 weeks. HIV DNA PCR was positive at 2,4 and 8 weeks and negative at 42 and 46 weeks by the standard assay. A modified assay, using 10µg of mononuclear cell DNA or 3µg of CD4 specific DNA gave positive reactions at 52 weeks. RNA PCR was positive at 2, 4 and 8 weeks; 1430172, 5213 and 1123 copies/ml, respectfully but remained below the limit of detection at 42, 46 and 52 weeks. DNA typing (Amplitype PM+DQA1 PCRkit, Perkin Elmer)confirmed, with a 99.95% probability , that all specimens were from the same infant. Zidovudine treatment was initiated at 2 weeks with lamivudine and ritonavir added to the regimen at 8 weeks of age. The infant remains clinically well but exhibits mild signs of developmental delays.

The initial laboratory findings suggest a transient rather than a persistent

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POSTER PRESENTATIONS :Carol Major

Towards Real-time HIV Surveillance Using HIV Testing Data
Major,C[1]., Remis,R[2]., Galli, R.[1], Wu, K[1]., Degazio, T[1]., Fearon,M.[1], 1.Ontario Ministry of Health, HIV Laboratory, Toronto. 2. University of Toronto.

Objective: To carry out real-time HIV surveillance using laboratory testing data in a province where all HIV diagnostic testing is carried out at the Public Health Laboratory (PHL) System.
Methods: An Informix-based laboratory information system (LAByrinth, CoCam Pty, Australia) was implemented at the Ontario central and regional PHLs. Existing testing data (1.6 million records) was converted from a specimen-centered to a patient-centered database. New records were collected directly through on-line data entry, testing and reporting. Data quality assurance using data enhancement, data integrity and duplicate checking procedures is an integral component of the system. Using SAS, automated regular data extraction created data sets and tables and maps reporting on: new cases, positivity rates (eliminating duplicate positive and negative tests), incidence density among repeat testers and testing patterns, by age, sex, exposure category and geographical region.
Results: From 1992 to end 1997, 1,548,638 tests were done on 1,486,117 persons. Testing patterns appeared stable except for an increase in transfusion related testing in 1993-94. Overall positivity rates steadily declined among MSM (5.8% in 1993, 3.0% in 1997), were relatively stable in IDUs (1.5 % in 1993, 1.3% in 1997) and those from HIV endemic areas (2.6% in 1993; 2.4% in 1997). However, we observed regional variations in testing patterns and repeat testers; positivity rates in some geographic regions were higher than provincial means (MSM: Toronto 8.3%, Niagara 5.0%; IDU: Ottawa 3.7% Thunder Bay 3.4%, Sudbury 3.0%; HIV endemic: Toronto 5.5%). Incidence density (per 100 person-years) in 1996 was 3.2 (CL 2.3-4.1) for MSM and 0.8 (CL 0.2-1.3) for IDU.
Conclusions: The complex and dynamic nature of the HIV epidemic can be tracked with a real-time surveillance system using laboratory testing data. This data is important for identifying potential "hotspots" or outbreaks. Surveillance data is critical in resource allocation and health care and intervention planning.

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A First Look at the Performance Characteristics of Quantiplex HIV-1 3.0 Viral Load in a Clinical Laboratory Setting.
Carol Major, Rick Galli, George Strunc, Tanya DeGazio, Margaret Fearon. HIV Laboratory, Central Public Health Laboratory, Ontario Ministry of Health

Introduction: In response to demand for HIV viral load (VL) quantitation below 500 copies RNA/ml(c/ml), Chiron has modified the Quantiplex bDNA procedure to increase sensitivity (to 50 c/ml) and reduce non-specific hybridization. These modifications include: changes in the signal amplification system [increased numbers of capture extenders, label extenders and pre-amplifier binding sites, a reduction in amplifier binding sites, and incorporation of non-natural nucleosides into amplification sequences]; and the incorporation of 6 levels of HIV SF2 virus as quantitative standards.
Objective: To evaluate the performance of Chiron Quantiplex HIV-1 RNA 3.0 assay (Q3.0) compared to Quantiplex HIV-1 RNA 2.0 (Q2.0) on clinical plasma specimens in a high volume laboratory setting.
Methods: Plasma specimens were collected from HIV + patients seeking viral load testing. Specimens were selected to have sufficient volume for multiple tests and a history of VL results <500 c/ml (n=58), or, VL between 500 and 800,000 c/ml (n=65). Multiple aliquots of plasma were prepared within 8 hours of specimen collection and stored at -70 ° C until tested. All specimens were tested on Q2.0 as the test of record. A total of 328 Q3.0 tests were performed including multiple replicates (2-4) of each specimen. CV's were calculated using replicate and standards results. Mean VL values (Q3.0) were compared to Q2.0 VL to establish correlation.
Results: For specimens with values <500 on Q2.0 (n=58), 64% were <50 on Q3.0, 17% were >50<500, and 19% produced values between 501 and 1760 c/ml. For specimens with VL > 500 on Q2.0 and Q3.0, the Spearman correlation coefficient was 0.977; however, VL results with Q3.0 were consistently higher with an average ratio of 3.2 (range 1.2 - 6.5). Overall reproducibility for Q3.0 yielded a mean CV of 10.1%. For specimens >50<500, CV=19.9%; 501-10,000 CV=8.6%; >10,001 - 100,000 CV 7.5%; >100,000 CV 7.9% The overall CV of the raw data (log of relative chemiluminescence units) for the 6 standards on 4 different assay runs was 15% (range 4 - 28%).
Conclusions: Reproducibility of results with Q3.0 is excellent across the dynamic range, including raw values generated for the 6 standards on different results. VL results for Q2.0 and Q3.0 are well

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POSTER PRESENTATION: Robert A. Myers

DETECTION OF HIGHER HIV-1 VIRAL LOAD LEVELS (>1.1 log) USING A ULTRA SENSITIVE BRANCHE D CHAIN (bDNA) ASSAY IN FIVE NON-CLADE B HIV-1 INFECTED PATIENTS WITH LOW HIV-1 VIRAL LOADS (<500 COPIES/ML) IN THE STANDARD RT/PCR BASED ASSAY.

Robert A. Myers, Willa F. Szuch, Jagdish D. Patel and J. Mehsen Joseph.
Maryland Department of Health Laboratories Baltimore, MD.

The emergence of non-clade B HIV-1 infections in North America presents a challenge to laboratories performing HIV-1 viral load assays to provide accurate quantification of these genetically diverse subtypes of HIV-1. These non-B subtypes have been found within our testing population. To evaluate the performance of commercial assays to quantify HIV-1 viral loads in patients attending public health clinics in Maryland, a comparison of the Roche Amplicor HIV-1 Monitor(RT/PCR) assay versus the ultra sensitive Chiron Quantiplex HIV RNA v.3.0 (bDNA)assay was conducted. Part of this comparison utilized 61 frozen specimens with HIV-1 RNA levels of <500 copies/ml as determined by the standard RT/PCR assay. These specimens were blindly re-tested in the bDNA test system. Five specimens demonstrated significantly higher (>1.1 log) HIV-1 RNA levels in the bDNA assay (range: 5,466-19,105 copies/ml). The discordant samples were then re-evaluated in the ultra sensitive modification of the Roche RT/PCR assay. Low HIV-1 RNA copy numbers were again obtained (range: 72 -460 copies/ml). A review of available demographic data indicated that these specimens were from five patients who are African expatriates attending HIV clinics in the Maryland suburbs of Washington DC. Serological and sequencing HIV subtyping assays were performed on samples from these patients. The results demonstrated that they were all infected with non-clade B subtypes of HIV-1 and identified one individual who could also be co-infected with HIV-2.

The ultra sensitive bDNA assay produced considerably higher HIV-1 viral load results in comparison to results from the standard and ultra sensitive RT/PCR assays when testing this group of non-clade B HIV-1 infected patients. The bDNA results will allow clinicians to make more informed

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POSTER PRESENTATION: Ronald B. Moss

HIV-1 RECOGNITION

Ronald B. Moss, M.D.*, Mark R. Wallace, M.D.**, Wieslawa K. Giermakowska*, Erin Webb*, Jay Savary*, Carolyn Chamberlin-Brandt, R.N.**, Georgia Theofan, Ph.D.*, Roy Musil, Ph.D.*, S.P. Richieri, R.Ph.*. F.C. Jensen, D.V.M.*, D.J. Carlo, Ph.D.*

*Immune Response Corporation, Carlsbad, California.
**U.S. Naval Medical Center - San Diego, California

Objective: We hypothesized that anergy could be reversed in chronic HIV-1 infection with the proper immune stimulation.
Methods: In this study we examined HIV-1-specific lymphocyte proliferation before and after treatment with an inactivated HIV-1 immunogen (REMUNE) in 8 HIV-1 seropositive subjects with chronic HIV-1 infection by a novel flow cytometric assay. Results: Recognition of HIV-1, measured by the lymphocyte proliferation assay, increased approximately 10-fold to purified native p24 antigen ($p<0.01$) after treatment with this HIV-specific immune based therapy. Interestingly, by an HIV-1 antigen-specific flow cytometric method, T helper CD4 lymphocytes ($p<0.01$), CD8 lymphocytes ($p<0.01$) and NK (natural killer) cells ($p<0.01$) were the predominant cell types proliferating in vitro post treatment. The flow cytometric method highly correlated with the standard thymidine incorporation lymphocyte proliferation assay. Additional phenotyping of proliferating cells revealed predominantly CD4 memory ($p<0.01$) and CD8 memory ($p<0.01$) phenotypes.
Conclusions: This study supports the concept that in vitro lymphocyte proliferation to HIV-1 antigens, augmented after treatment with an inactivated HIV-1 immunogen, involves primarily CD4 and CD8 memory immune responses. Such findings suggest that HIV-1 anergy can be reversed with the proper immune stimulation in chronic HIV-1 infection and provides further rationale for combining HAART with HIV-1-specific immune based therapies.

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POSTER PRESENTATION:Charlotte A. Romain

COMPARATIVE EVALUATION OF THREE EIA SCREENING TESTS AND TESTING ALGORITHMS FOR THE DETECTION OF HIV-1 ANTIBODY IN SERUM FROM MILITARY PERSONNEL. Charlotte A. Romain, MT(ASCP), Barbara M. Moore, MT(ASCP), Gina Trebilcock, Ph.D., Matthew J. Bankowski, Ph.D., ABMM. ViroMed Laboratories, Inc., Minneapolis, Minnesota. Edith S. H. Smith, M.S., B.S., MT(ASCP), Jacqueline M. Sheffield, B.S. MT(ASCP), Shannon D. Woods, MLT. Navy Central HIV Program, Bethesda, Maryland. Hassan Zahwa, M.S., B.S. MT(ASCP). Walter Reed Army Institute of Research, Rockville, Maryland.

We evaluated three EIA-based test kits (LAV EIA [Genetic Systems™, LAV], rLAV EIA [Genetic Systems™, rLAV] and Vironostika® HIV-1 Microelisa System [Organon Teknika, OTC]) using five test algorithms for the detection of HIV-1 antibody in serum samples from USA navy personnel. Our goal was to find the most accurate and reliable HIV-1 screening algorithm for HIV-1 screening of military personnel. In total, four hundred and thirteen samples were tested using two evaluation panels (Panel 1: 200 negative, 100 positive. Panel 2: 25 reactive, 25 weak reactive and a mixture of gray zone and other sample types). The algorithms and results are as follows:

Initial Test	Repeat Test	Sensitivity	Specificity	PPV	NPV
1. OTC	RLAV	100	97	94	100
2. OTC	OTC	100	95	91	100
3. rLAV	RLAV	100	100	100	100
4. rLAV	OTC	100	100	100	100
5. LAV	OTC	100	100	100	100

Summarizing all five algorithms based on initial testing: #2 showed the

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POSTER PRESENTATION:Clive Loveday

GENOME SEQUENCING AND CLINICAL CARE OF HIV-1 INFECTION: APPLICATIONS IN DRUG NAIVE AND EXPERIENCED PATIENTS.
C. Loveday, H Devereux, L Huckett, M Johnson. Department of Retrovirology and HIV/AIDS, Royal Free Hospital and Medical School, London, NW3 2PF.

High rates of HIV-1 turnover with error prone replication drive an increase in viral genetic diversity, and in the presence of suboptimal antiretroviral therapy new quasispecies with mutations associated with drug resistance evolve. We have been funded by our Trust to carry out an ongoing study to determine the prevalence of genotypic changes in naive and treated patients and to devise strategies that may be applied to support treatment changes and salvage therapy. We present the first 120 patients (drug naive=16, drug experienced=104, including an ongoing 5 drug salvage therapy audit=46). Genotypic change were determined by sequencing in RT and protease (P) regions of the pol gene using plasma-derived-HIV-1 RNA. Plasma HIV-1 RNA load (VL) was measured using an RT-PCR (Roche Amplicor Monitor 1.5) with a cut-off of either 400 or 50 c/ml. The relative merits of automated sequencing with ABI377 and/or OpenGene (Visisble Genetics, Inc.) Have been compared. Drug naive patients had lower VL, fewer total polymorphisms (RP) compared to experience patients (VL 4.6 v 5.3 log c/ml; TP:11, range 4-20 v 18, range 6-34; RP:2 range 1-3 v 6, range 1-12). The experienced patients had higher frequencies of common mutations in RT: M41V (58%), M184V (66%), L210W (65%), T215Y (50%), and in P: L10I (40%), M36I (30%), I54V (38%), L63P (72%), V82A (40%) and L90M (40%) reflects the degree of prior drug usage. Patients on salvage therapy (Rit, Ind, ddl, HU, DMP 266) had higher baseloine demographics (VL 5.58 log c/ml; TP:24, range 9-36; RP: 9, range 3-16) but to date are showing 2 to 3 log declines in VL with 60% below detectability. Comparisons of automated sequencing technologies have shown distinct qualitative and quantitative benefits for the OpenGene system to date. Real time virological support with molecular technologies is an essential part of optimum clinical care of patients with HIV-1 infection.

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POSTER PRESENTATION: Ranga V. Srinvas

IDENTIFICATION OF DRUG RESISTANT HIV BY DIRECT ANALYSIS OF VIRION ASSOCIATED REVERSE TRANSCRIPTASE ACTIVITY. Ranga V. Srinivas, Wally Bitar, Arnold Fridland and Patricia Flynn. Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee.

Non-radioactive, 96-well microtiter-plate format reverse transcriptase (RT) assays using a poly-A:oligo-dT (for studies with thymidine analogs AZT, D4T) or heteropolymeric MS2 RNA were developed. RT assay using poly-A: oligo-dT template was more sensitive than the assay using heteropolymeric templates, although both assays were sufficiently sensitive to detect HIV in culture supernatants concentrated by PEG-precipitation or ultra-centrifugation. The in vitro enzymatic activities of purified HIV-1 RT and wild-type HIV-1 IIIB was efficiently inhibited by AZTTP (IC_{50}~0.017 uM). Virion associated RT from AZT-resistant mutants that carry the Q151M (and associated mutations) were ~1- to 30-fold less sensitive to inhibition by AZTTP. By contract, RT from AZT-resistant mutants with mutations at codons 40, 67, 70, 215, or 219 was not inhibited by AZTTP this assay. We also investigated whether RT activity (and resistance) could be measured in patient plasma. HIV-associated RT activity was detected in plasma from 10/15 of patients at one or more time points. However, none of these samples was resistant to AZTTP. PCR analysis of AZT-resistance mutations revealed only 40, 67, 70, 215 or 219, but no Q151M or associated mutations in these samples. DDI and PMEA-resistant HIV mutants could be identified by RT assay using heteropolymeric MS2 RNA templates. The scope and limitations of these procedures in clinical laboratory practice are discussed.

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POSTER PRESENTATION: Spencer B. Bennett

RAPID HIV TESTING vs. TRADITIONAL TESTING: IN A PUBLIC HEALTH OUTREACH SETTING. S. B. Bennett[1]; S. Fordan[1]; E. E. Buff[1]; E. C. Hartwig[1]; R. Caldwell[2]; L. Hill[2]; D. Williams[2]: [1]Retrovirology Unit, Florida Department of Health, Bureau of Laboratories, Jacksonville, Florida, USA. [2]River Region Human Services, Inc., Jacksonville, Florida, USA.

Objective: To compare the performance of a fingerstick-based HIV rapid test against a traditional serum-based EIA screening assay. This assessment is to target a high volume, high seroprevalent public health outreach setting. We are evaluating this rapid test on the basis of its potential benefit to identify and assess those individuals of unknown HIV serostatus. These individuals most often avoid traditional public health clinic settings.
Methods: A total of 277 paired specimens of fingersticks and venous blood were obtained from clients requesting an HIV test at River Region Human Services, Inc. (RRHS), a substance abuse treatment and prevention center. The fingerstick samples were tested by a rapid test (Quix HIV-1-2-O, Universal HealthWatch,Inc.) designed to detect HIV-1/2 and Group O antibodies. This rapid test was primarily performed in a non-laboratory setting by RRHS personnel trained by the assay manufacturer. Sera derived from the venous bloods were tested for HIV-1/2 antibodies by an FDA approved synthetic peptide EIA (Sanofi Diagnostic Pasteur, Inc.) at the Florida Bureau of Laboratories (FBL), by licensed laboratory technologists. Repeatedly reactive samples were further tested by Western Blot and PCR when indicated.
Results: Of the 277 patients, the rapid test correctly identified 20 of 20 HIV-1 seropositive individuals yielding a sensitivity of 100%. The assay also correctly identified 254 of 257 seronegative for a specificity of 98.9%. The PPV and NPV of the rapid assay is 87% and 100% respectively. The three (3) false positives encountered by the rapid assay were weak HIV-1 reactives as determined by RRHS and FBL personnel. All three patients were negative for HIV-1 by NASBA, a qualitative HIV-1 RNA amplification procedure.
Conclusion: HIV prevalence in this outreach population was 7.2%, approximately twice the state average. This high seroprevalence in conjunction with the rapid test sensitivity level tends to make it favorable for reaching a population of unknown HIV serostatus, for partner notification and for patient care management. However, the specificity of the rapid test is less than traditional laboratory based testing. This increased false positivity will require more supplemental testing to reach a true

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POSTER PRESENTATION: Jennifer Tosswill

QUALITY ASSURANCE (QA) OF THE UK ANONYMOUS HIV SURVEILLANCE OF DRIED BLOOD SPOTS COLLECTED FROM NEONATES. Jennifer H.C.Tosswill, M.Sc., Julie A. Newham, F.I.B.M.S., John V. Parry, Ph.D., Philip P. Mortimer, M.D. Hepatitis & Retrovirus Laboratory, Central Public Health Laboratory, London NW9 5HT, UK.

Since 1992 there has been a surveillance of anti HIV in neonatal blood spot specimens in UK. This now embraces 12 centres and involves anonymised tests, for surveillance purposes only, on approximately 400,000 infants per year. Up until 1998, QA operated to check the sensitivity and specificity of this testing as follows: eight performance assessment panels were distributed to 8 - 12 centres at roughly nine monthly intervals. The panels comprised approximately 50 HIV positive and 50 negative coded blood spot samples, and participants were required to use their preferred screening method to examine them and report their results within 4 weeks. Various methods based on HIV coated gelatin particle agglutination and IgG capture ELISA were used. For all 8 panels combined, sensitivity ranged from 98.6 to 100%. The small number of anti-HIV positive samples that were undetected in some laboratories were low-titre. Results obtained from the 391 negative specimens included in the eight panels showed an overall specificity of 97.1 to 100%. Specificity was also monitored through the referral of eluates of reactive specimens from the screening programme. Of 1,254 such eluates referred to CPHL, 490 (39%) were confirmed to contain anti HIV 1. The estimated denominator population of 2,000,000 suggests an overall specificity for the screening methods of > 99.99%.

QA shows that only rare, very weak, anti HIV positive blood spots would not be detected by the participating centres, and that the specificity of all the methods employed is at least as high as for other serological tests used in HIV screening.

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POSTER PRESENTATION: Neil T. Constantine

QUALITATIVE DIFFERENCES IN HIV-1 WESTERN BLOT PROFILES PREDICT GROUP O INFECTION. Niel T. Constantine, Ph.D., Rebecca D. Saville, M.S., Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland.

Twenty-three samples from individuals infected with HIV-1 Group O were tested to determine if Group O infection could be predicted from HIV-1 Western blot (WB) profiles. The samples were tested using an HIV-1 Group M WB (BioRad), and profiles were compared to those of 135 individuals with HIV-1 Group M infection. All Group O infected samples had been confirmed using specific Group O Immunoblot and/or synthetic peptide tests. Reactions to viral specific antigenic components by WB were scored from +/- to 4 + (quantitative intensity). Qualitative differences were scored as "typical" or "atypical" reactivity for each of the antigens. Based on visual interpretations, in a blinded fashion, samples were classified as "likely" or "unlikely" to be from persons with HIV-1 Group O infection. Two of the 23 samples were interpreted as WB indeterminate while the remaining 21 were classified as HIV-1 positive by the HIV-1 Group M WB. In 95% (20/21) of the Group O samples, several characteristics could be used to differentiate them from Group M. Of these 20 samples, 2 samples had no reactivity in the gp120 region while 18 (90%) produced atypical reactions in this region as compared to Group M samples. Reactions at gp41 were also atypical in 11/20 samples, and in 5 of these 11, the atypical reactivity in gp41was useful in differentiating Group O from Group M when reactivity in the gp120 region was uncertain. The remaining sample of the 21 HIV-1 positive samples exhibited a typical Group M profile. No other distinguishing features were of value for differentiation. Of the 2 samples that produced indeterminate results by WB, both reacted to p24 and p32, and neither showed reactivity to the envelope antigens.
In conclusion, 95% (20/21) of the Group O positive samples produced uncharacteristic WB profiles (when using HIV-1 Group M Western blots) that classified them as likely to be HIV-1 Group O infections. The primary differentiating variable was an absence of reactivity or the presence of atypical reactivity in the gp120 region, although atypical reactivity in the

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POSTER PRESENTATION: Judith Wilber

LABORATORY VALIDATION OF A QUANTITATIVE HIV-1 bDNA ASSAY WITH A REPORTING THRESHOLD OF 50 COPIES/ML. Judith C. Wilber, Ph.D., Jennifer E. Booth, B.A., Amalia B. Ocampo, B.S., Kimmy M. Leung, B.Sc., Dolores G. Duey, B.S., Maria D. Canuel, B.S., Marie Joyce M.P. Yap, M.S. and Lynette S.W. Sawyer, D.P.H., Chiron Diagnostics, Emeryville, CA

A bDNA assay with increased sensitivity for quantifying HIV-1 RNA has been evaluated in the Chiron Reference Testing Laboratory. The limit of detection in the HIV-1 RNA 3.0 assay was 50 copies/mL. With this reporting threshold the assay demonstrated a specificity of 99.5% and a dynamic range of 4 logs. Linearity between 50 and 500,000 copies/mL was established using a serial dilution panel made from tissue culture virus diluted in HIV-negative plasma and was confirmed using serial dilution of HIV-positive plasma specimens. Equivalent quantification of HIV-1 subtypes A-F was also demonstrated. Precision between 50 and 500,000 copies/mL was £0.21 logSD. The assay was accurate within ± 10% over the reportable range. The data generated in this validation were analyzed both as single wells and as duplicate wells and the variation was found to be similar over the range of the assay. High levels of hemoglobin (1 mg/mL), bilirubin (20 mg/dL) and triglyceride (>300 mg/dL) did not interfere with specificity. Nor did hemoglobin or triglyceride significantly affect quantification of positive samples. There was a small, but statistically significant decrease in quantification of HIV-1 RNA in the presence of high levels of bilirubin.
The HIV-1 RNA 3.0 assay was compared to the previous HIV-1 RNA 2.0 assay. Approximately 80% of assays with results of <500 copies/mL in the earlier version also had <500 copies/mL in the new version. For specimens quantifying in the HIV-1 RNA 2.0 assay, results averaged approximately 1.5 to 2.5 fold higher when tested in the HIV-1 RNA 3.0 assay. A set of 25 specimens generated very similar results when run in quadruplicate by HIV-1 RNA bDNA 3.0 (in the CRTL) and by RT-PCR (Roche Amplicor Monitor at a commercial reference laboratory). We conclude that HIV-1 RNA can be quantified with precision, accuracy and at very low concentrations with this assay.

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POSTER PRESENTATION: Joseph Adelsberger

METHOD TO DETERMINE CELL TURNOVER RATES OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES USING 5-BROMO-2' DEOXYURIDINE (BrdU). Joseph W. Adelsberger, B.S., Richard A. Lempicki, Ph.D., Randy A. Stevens, B.A., Bradley E. Foltz, B.S., Catherine M. Watkins, B.S., Laurie A. Lambert, B.S., Michael W. Baseler, Ph.D., SAIC Frederick, NCI-
FCRDC, Julia A. Metcalf, B.A., Joseph A. Kovacs, M.D., H. Clifford Lane, M.D., National Institute of Allergy and Infectious Diseases, NIH.

HIV-1 infection is associated with a failure in T cell homeostasis resulting in a gradual decline in CD4+ T cell numbers. Both destruction of productively infected CD4+ cells and interference with the regenerative capacity of CD4+ T cells have
been postulated to be important mechanisms underlying this loss. To better understand lymphocyte turnover rates in healthy controls and patients with HIV-1 infection, a sensitive method to determine cell turnover in whole blood was needed. We examined three methods of measuring cell turnover rates: a proliferation assay
using spontaneous 3H-thymidine uptake, cell cycle analysis using propidium iodide (PI) incorporation in conjunction with cell surface phenotypic analysis, and incorporation of the thymidine analog 5-Bromo-2'-Deoxyuridine (BrdU) in conjunction with cell surface phenotypic analysis.

The BrdU method was found to be superior to the PI cell cycle and 3H-thymidine methods. The sensitivity and specificity of the BrdU method were found to be excellent. BrdU+ cells were detected at 0.01% of peripheral blood leukocytes. The intra-assay and inter-assay variabilities of the BrdU method were 3.3% and 17.6%, respectively. The BrdU method had the following advantages: the capability for simultaneous detection of cell turnover and cell surface phenotype, and the ability to measure cell turnover in whole blood immediately after venipuncture. To date, the BrdU method has been used to successfully measure cell turnover rates in over 250 patients and healthy controls.