Summary of the 2010 HIV Diagnostics Conference

The 2010 HIV Diagnostics Conference was attended by approximately 250 individuals. The professional breakdown of attendees was as follows: 43% were employees of private companies that manufacture diagnostic tests, 19% were public health professionals (state and local public health programs and laboratories), 14% were federal employees (Centers for Disease Control and Prevention (CDC), Department of Defense and the Food and Drug Administration (FDA); the remaining 24% were members of academic institutions or employees of hospital or private clinical laboratories. The Conference included three topics of focus: point-of-care testing, laboratory based testing, and new technologies. In the Conference's final session, co-organizer and opening speaker, Dr. Bernie Branson of the CDC proposed a possible new algorithm for laboratory testing that would take advantage of the capabilities of current HIV testing technologies. Below is a summary of the Conference, organized by general topic.

Opening Session

In the first presentation of the meeting, Dr. Bernard Branson provided an overview of the evolution of HIV testing (and the HIV Diagnostics Conference). Dr. Branson pointed out that the last major recommendations for HIV testing algorithms came out in 1989. Those recommendations included interpretive criteria for using Western blot for confirmation of a positive screening test. Dr. Branson made the point that the vast majority of current commercially available HIV testing methods are more sensitive than the Western blot. Moreover, the turnaround times associated with centralized processing of specimens reduced the "effective sensitivity" of testing because of the number of persons who never received their test results. As such, it was noted that the need for novel algorithms (not dependent Western blot) has been recognized for some time, and has been a major topic during the preceding two diagnostics conferences. Dr. Barbara Werner then presented the results from the 2009 HIV Testing Practices Survey which had been performed by the Association of Public Health Laboratories (APHL).Testing volumes have diminished at many public health laboratories: total specimens decreased 22%, and oral fluid submissions 54%, likely as a result of increased point-of-care screening with rapid tests. Most public health laboratories are currently screening with "3rd Generation" EIAs that also detect HIV-2 antibodies, whereas no FDA approved confirmation test exists to confirm HIV-2 infection. After Dr. Werner provided an overview of laboratory testing practices, Steven Ethridge presented a point-of-care (POC) testing perspective by reviewing data from the Model Performance Evaluation Program (MPEP) Rapid Testing Survey. This program, in which a wide variety of POC sites participate, provides free semi-annual external quality assessment to participants. About two-thirds of participating POC sites used rapid tests sensitive for HIV-1 and HIV-2. Among 482 that participated, overall accuracy was 99.0% with the positive challenge specimens and 98.8% with the negative challenge specimens. The perspectives provided by Dr. Branson, Dr. Werner and Mr. Ethridge introduced a theme for the meeting: that we had come a

long way technologically, but that new guidance and recommendations should now follow.

Point-of-Care Testing with Rapid Tests

The remainder of the opening day of the meeting and part of day 2 consisted of presentations on various aspects of POC testing. Four groups had investigated the use of multiple rapid tests in combination for screening and confirmation (described as rapid test algorithms, RTA). Data from public health departments in New Jersey, New York, San Francisco and Los Angeles showed that between 35 and 50% of people who received preliminary rapid test results fail to return to get their laboratory confirmed, final HIV results. It was predicted that an RTA of two or more tests would provide onsite corroboration for reactive rapid tests that might enhance the linkage to care for positive individuals.

Thomas Knoble from the San Francisco Department of Public Health (SFDPH) provided real life anecdotes illustrating the benefits of an RTA. Algorithms with two rapid tests were less unwieldy than those that included three, primarily because of demands to maintain quality assurance for tests used only rarely. After a detailed description of the QA procedures that SFDPH used, Knoble offered a "wish list" indicating that his organization would prefer an RTA that included two tests from two different manufacturers. After the presentation the concept of "orthogonality" was discussed. Orthogonality, when applied to lab tests, is a concept whereby, for use in an algorithm, different tests that perform the HIV antibody detection must accomplish this by using either different antigens or different principles (e.g., immunochromatography (lateral flow) vs. immunoconcentration (flow-through) devices. This concept came up repeatedly during the remainder of the meeting in reference to rapid test algorithms.

The New York State Department of Health (NYSDOH) also implemented a two-test algorithm for approximately 15 months in 2008 and 2009. This study, presented by April Richardson-Moore, included more than four times the participants as the San Francisco RTA study and involved three rapid tests: Uni-gold, Clearview COMPLETE, and OraQuick. The NYSDOH quantified improvements in the proportion of persons who received results (85% vs. 75%), switch from anonymous to confidential status(99% vs, 70%) and improved linkage to care among those who received their confirmed test results (94% vs. 86%) with the RTA, compared with the time period when clients received a single reactive rapid test result.

Also from New York State (the state public health laboratory) was a study investigating the sensitivities of the various rapid tests used for screening in the RTA described above. Their findings, presented by Dr. Linda Styer, showed that, in a laboratory setting, the Uni-Gold rapid test was the most sensitive of the rapid tests used and could be used first in a multi rapid test algorithm. However, performance data from POC sites showed that Clearview and Uni-Gold had the same sensitivity in the field. Dr. Styer described some of the factors that could account for the difference in test performance between POC and

laboratory settings including the operator, the type of specimens tested (whole blood vs. plasma) and specimen handling and processing (fresh vs. stored). The conclusion made was that laboratory performance is not wholly representative of real life test performance at the point of care.

Similar experiences with multi-rapid test algorithms were presented by the State of New Jersey (Dr. Eugene Martin) and Los Angeles County (Jacqueline Rurangirwa). Both sites agreed with San Francisco that the use of 3 rapid tests in an RTA was unwieldy from the cost and QA points of view. In New Jersey, Dr. Martin found that verifying primary rapid screening test results with secondary rapid testing increased the linkage to care, with 75% of clients receiving a physician appointment on the same day.

The discussion session after the RTA presentations was directed not only against the orthogonality issue, described above, but on the order in which rapid tests are run as well. That is to say that data which shows certain rapid tests to be more sensitive for recent HIV infection should be taken into account when designing an RTA. Moreover, it was questioned whether the cost associated with running a second test was worth the benefit, and whether the benefit to patients could be clearly measured. The counselors who utilized these RTAs very much appreciated having a second test upon which they could rely; but might the primary benefit of the second test be to the counselor rather than the patient? Overall however, those who ran the RTA felt that patients benefitted tremendously.

Other studies involving POC testing were presented, including the experience of the Chicago Department of Public Health and Mt. Sinai Hospital in the expansion of rapid HIV testing in four urban hospital-based emergency departments. Nancy Glick and Karen Reitan presented a summary indicating that implementation of this program was feasible and resulted in the identification of a large number of HIV infected persons who would have been missed if such testing had not been made available. The presenters highlighted the benefit of hiring health educators to facilitate counseling and linkage to care. Indeed, this program created opportunities for HIV prevention education and awareness, and 69% of the clients testing positive were linked to care.

In the final session of point of care testing, both New York State (Mara San Antonio-Gaddy) and the San Francisco Department of Public Health (Teri Dowling) gave presentations describing the quality assurance programs associated with each entity's rapid testing program. Both presentations focused on the importance of a thorough training program, continuous monitoring (including site visits and proficiency testing), and strong collaboration with the laboratory. Kristen Mahle (CDC) presented an analysis of the impact of alternative HIV testing algorithm on case surveillance. CDC convened a workgroup to explore how new algorithms may affect the surveillance case definition and to assess what changes may be required. Much would depend on the algorithm recommended (and which tests are required for diagnosis), but a change to the surveillance case definition could necessitate changes to eHARS, state reporting laws and POC site training programs (to include reporting), and could even affect the HIV Incidence Surveillance program. Also of interest was a presentation by Silvina Masciotra

which showed that two rapid tests (the Stat-Pak and the Complete, both manufactured by Inverness) were both at least as sensitive and as specific as a Western blot when used as confirmation tests for repeat reactive EIA. The presented data contributed to the ongoing discussion of the role of the Western blot as a confirmation test and provided a transition to the next meeting topic: laboratory based testing.

Laboratory Based Testing

The second day of the Conference provided a focus on laboratory based testing, including both serologic testing and direct virus detection. The day opened with four presentations on miscellaneous topics, prior to a focus on the following three topics: recency testing, antigen-antibody combination tests ("4th Generation" tests) and lastly nucleic acid-based testing.

Kevin Delaney of CDC presented an analysis of immunoassay signal-to-cutoff data from multiple sites to evaluate the utility these numbers might provide to diagnostic algorithms. The data showed that the S/CO values from two different immunoassays can provide a very strong predictor of false positivity and therefore would be very useful within a testing algorithm. Although presented during the final scientific session, Dr. Michael Loeffelholz presented similar data from a study evaluating the use of S/CO values to guide patient care during pregnancy and delivery. His data showed that S/CO ratios on Ortho Vitros HIV1/2 were predictive of Western blot result – values greater than 30 were likely to confirm with Western blot while those under 10 were likely to be HIV-negative. Dr. Loeffelholz proposed using this data clinically, primarily for patients with unknown HIV status, to help determine whether intrapartum zidovudine should be administered. Laura Wesolowski, also from CDC, presented the findings of an enormous study (~2 million test results) looking at false-positivity on peptide-based EIA with a focus on the testing of pregnant women. Interestingly, pregnant women were shown to be no more likely than others to test falsely positive on a peptide-based EIA (false-positive rate of 0.14% vs 0.21%). However when pregnant women did test repeat reactive on an EIA, they were more likely to be negative or indeterminate on a Western blot. Dr. Michael Busch of Blood Systems showed the results of another large study (greater than 3.6 million tests) comparing the utility of the Immunofluorescence assay (IFA) to that of the Western blot. His data showed that the use of the IFA reduced the number of indeterminate samples thirteen-fold relative to the Western blot and eliminated the occurrence of unreadable results.

Dr. Robert Coombs of the University of Washington presented a study that investigated the use of the Multispot HIV-1/HIV-2 Rapid test (MS) as a confirmation test for EIA reactive specimens. His study showed that the use of an algorithm that included MS as a confirmation test resulted in faster turnaround time by a median of two days and the detection of two HIV-2 infections (1% of the HIV-1 WB "positives") that would have otherwise been missed. These data would prove to be very relevant in regards to the new testing recommendations that were proposed at the end of the meeting, whereby a test like the MS would be used a confirmation test.

Recency testing

Epidemiologic studies involving HIV incidence have relied upon modified antibody tests that can classify infection as either long-standing or recent. Few choices of such modified antibody tests are currently available. Perhaps in an effort to fill that void, three groups presented data demonstrating the new methods for recency testing. After a review of the topic of recency testing by Dr. Michael Busch, Sheila Keating, a member of his research group at Blood Systems presented the details of their construction of a modified version of the Vitros Anti-HIV-1+2 assay. They showed that by either a dilution strategy, or by an avidity alteration strategy, the Vitros test could be modified to be an effective test of categorizing the length of HIV infection. Also utilizing an avidity method, Silvina Masciotra of the CDC presented a modification of the Genetic Systems TM ½+O EIA that allows for discrimination of recent infection from long-term. Due to the widespread use of this assay as a screening test in public health laboratories, this work may have great utility. Of particular interest was a presentation by Kelly Curtis at the CDC showing that IgG_3 could function as a biomarker for distinguishing recent from established infection. Using a bead-based assay, the team was able to demonstrate IgG_3 sensitivity to multiple HIV antigens during early infection including p24, p66 and gp41. The reliability of the marker might make it amenable to actual clinical use if developed and characterized further.

Antigen-antibody (4th generation) testing

Immunoassays that detect IgG, IgM and virus directly have been in use outside of the U.S. for several years. Only recently have efforts been made to garner FDA approval for the use of such tests domestically. The ability of 4th generation tests to detect HIV infection during the antibody "window period" renders them very useful, particularly to certain localities where high HIV prevalence has demanded the use of some kind of virus detection for screening. The session opened with a presentation by Dr. Christiane Claessens (Institut national de santé publique du Québec, Canada) on their experiences using the AxSym Ag/Ab HIV Combo assay. Since implementing this 4th generation assay in their testing algorithm, INSPQ saw an increase in the number of acutely infected individuals identified without an increase in false-positive results. Next, three corporate entities presented data indicating the performance of their 4th generation products. All three products (Ortho-Clinical, Bio-Rad and Abbott Diagnostics) were analyzed using seroconversion panels and were shown to reduce the window period of infection by approximately one week relative to 3rd generation tests (which detect only antibody). A presentation by Dr. Mark Pandori of the San Francisco Department of Public Health Laboratory showed the performance of the Abbott 4th generation test on a panel of specimens from recently infected individuals. Their data showed that the antigen-antibody test could detect infection in 80% of specimens that were otherwise only detectable by an RNA test. The same group used the same panel to evaluate a rapid antigen-antibody test (the Determine, to be marketed by Inverness). They found that the sensitivity of the test was quite weak for antigen, relative to a lab based 4th generation test. However the test was capable of detecting antigen only in some specimens, and it

performed better as an antibody test than even a lab-based 3rd generation test. Questions following this session included how best shall we confirm positive results from antigen-antibody IA? Also, inquiries regarding the availability of such testing in the U.S. market were raised.

Applications of Nucleic Acid Testing Technologies

The final session on laboratory testing included three presentations looking at different aspects of RNA-based testing. Dr. Julie Nelson of the University of North Carolina, Chapel Hill presented useful data showing that the Abbott m2000 RealTime HIV-1 Viral Load assay could be used with many alternative specimen types, including urine, CSF, breastmilk, dried blood spots and genital secretions. In fact, Dr. Nelson showed that CSF and urine could be accurately tested using the standard assay protocol without sample pre-treatment. Carolyn Dawson of the CDC presented data from an investigation into the use of an HIV-1 DNA PCR assay as a confirmation test for infection. The data indicated that such a test has very high sensitivity (99.9%) and specificity (99.8%); however sensitivity was highly dependent upon cell counts (PBMC) in the assay input. Dr. James Bremer of Rush University presented a highly practical and valuable data set showing a detailed comparison of the two real-time PCR tests on the market as HIV-1 viral load assays (Roche and Abbott Molecular).

New Technologies

The 2010 Conference ended in a fashion similar to the 2007 Conference: with an eye toward the future. The final session focused upon new technologies for HIV diagnostics. Many of the methods currently in development are directed toward point-of-care testing, as is a general trend in medical diagnostics. Although presented during the rapid test session earlier, Dr. Michael Lochhead of mBio Diagnostics did show preliminary data of a new technology being produced by his company. The presentation described a point-of-care device that utilized disposable test cartridges to assay for HIV and syphilis antibody simultaneously. Since the tests are read by a portable fluorescence meter, testing appears simple, sensitive and accurate. Dr. Lochhead also highlighted that the assay's design allows for easy expansion of the testing panel. mBio is currently developing the technology to include HIV-2 and Hepatitis C serology, Hepatitis B Surface Antigen, and HIV-1 p24 direct antigen detection. Dr. Marco Schito presented three technologies that were developed by way of a Division of AIDS (NIH/NIAID) funded program. All three technologies were point-of-care devices that were capable of detecting HIV RNA. These POC assays could allow for the rapid detection of acutely infected patients as well as infection in infants and vaccinated individuals. They could also have applications for disease management – especially in resource-limited settings. Tim Granade of the CDC showed a potentially exciting technology based on magnetic immuno-chromatography that can detect antibodies to HIV and p24 (HIV particles) simultaneously. The test, in its current state of development was rapid and only slightly less sensitive than lab-based p24 tests. Another developmental p24 assay was presented

by Dr. Shixing Tang of the FDA. The assay detects p24 in a Europium nanoparticle-based immunoassay format and has a sensitivity approximately 25 fold higher than that of current p24 immunoassays. This may provide for antigen detection in a rapid and cheap format, heretofore not seen, that would be useful for both viral load and diagnostic purposes. The final research presentation, by Dr. John Kim of the National Lab for HIV Reference Services (in Ottawa, Canada), showed work involving the creation of lentiviral "pseudovirions" that would work well as standards in HIV-2 viral load assays. This would fill what is currently a large void in the assessment of HIV-2 infections by current methods.

Closing Session

Closing remarks of the meeting were given by Dr. Bernard Branson, whereupon he proposed a potential recommendation for laboratory-based HIV diagnostics. The proposed recommendation would state that specimens should be screened by a 3rd or 4th generation immunoassay.[i] Specimens that are positive by an initial IA screen would be confirmed by an antibody test that can discriminate between HIV-1 and HIV-2 antibodies. Specimens that do not confirm would be subjected to a nucleic acid amplification test, to assess for possible acute HIV infection. Such an algorithm would have the following advantages:

1. It will be capable of detecting window-period HIV infection if fronted by an antigen-antibody IA
2. It will have a more rapid turnaround time than an algorithm including a Western blot (as shown by Dr. Robert Coombs presentation)
3. It will detect HIV-2 infection, and will be able to differentiate HIV-2 from HIV-1 upon confirmation
4. It would eliminate the need for laboratories to use Western blot, which frequently generated indeterminate results.

Reception of this proposal was mostly positive, with only a few who offered concerns. One concern was that some doctors utilize the Western blot clinically and would like to see that it remains available. Another concern was whether the worry of HIV-2 infection was warranted. This was answered by pointing out that certain communities have greater need for concern for HIV-2 than others. A figure, depicting this novel proposed algorithm is shown in Figure 1.

The topics covered at the 2010 HIV Diagnostics Conference were highly relevant to the current state of HIV testing in the United States. The synthesis of data and ideas, along with the initiative shown in furtherance of new recommendations made this meeting a productive event for both the audience and its participants. It will be exciting to attend the next Conference in two years in order to see how HIV Diagnostics has changed, and whether any of the new methods or technologies presented at this meeting have become rooted into practice.

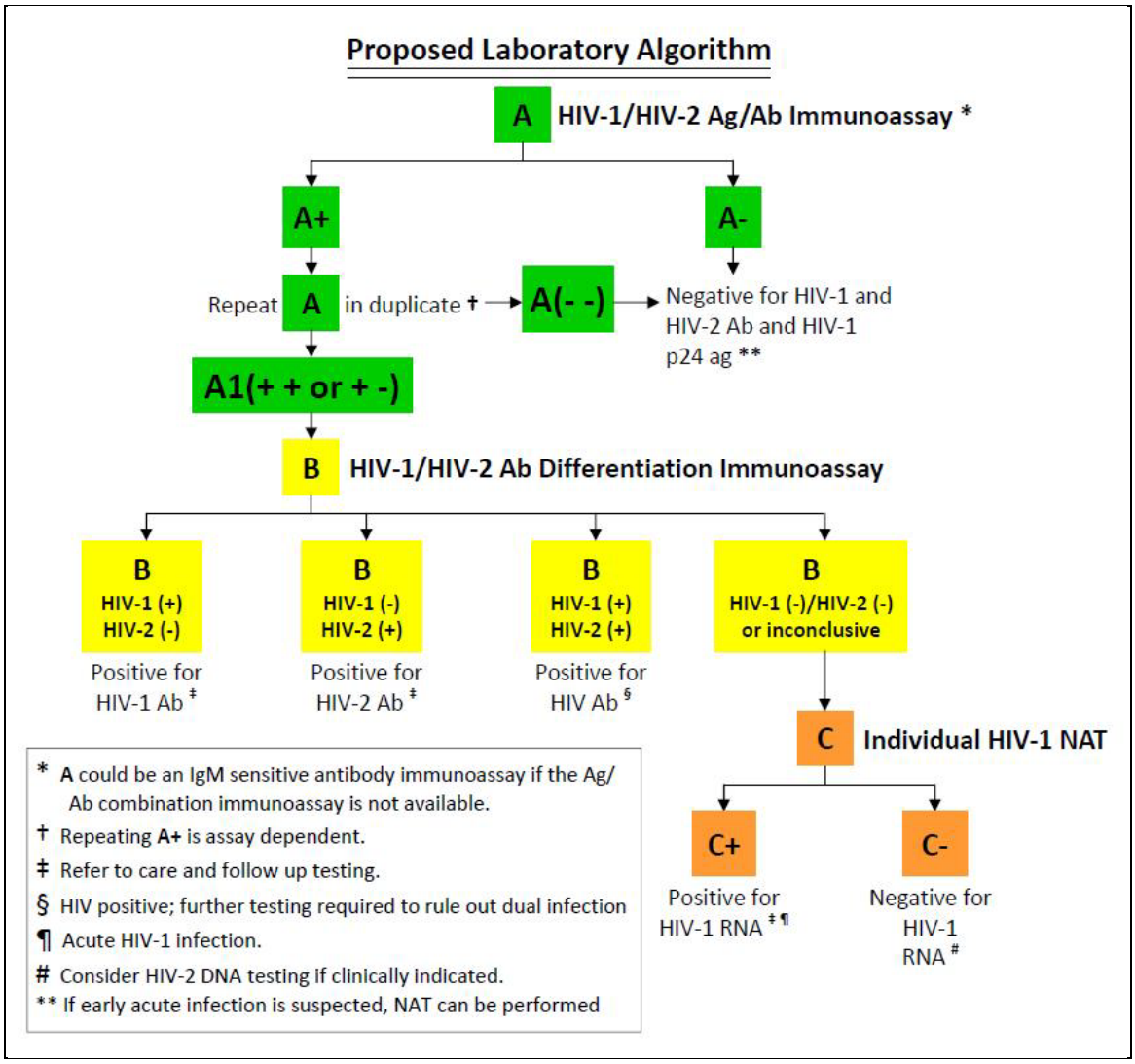

Figure 1. A new, proposed algorithm

[i] The first "4th Generation" HIV immunoassay was approved by FDA on June 18, 2010.