

# **TRANSFER OF HIV MONITORING TECHNOLOGIES INTO RESOURCE-POOR SETTINGS: MOVING THE FIELD FORWARD**

---

REPORT OF A FORUM FOR COLLABORATIVE HIV RESEARCH  
WORKSHOP  
FEBRUARY 26, 2005; WASHINGTON DC

---

FORUM FOR COLLABORATIVE HIV RESEARCH

---

DEPARTMENT OF PREVENTION AND COMMUNITY HEALTH  
THE GEORGE WASHINGTON UNIVERSITY  
SCHOOL OF PUBLIC HEALTH AND HEALTH SERVICES

## **HIV TREATMENT MONITORING TECHNOLOGIES FOR RESOURCE LIMITED SETTINGS: RECENT ADVANCES**

The hallmark of HIV treatment in the developed world is the individualized approach, made possible by the availability of over 20 drugs from four drug classes which are given in various combinations, adapting to individual needs according to toxicity and tolerability, previous treatment history, and compatibility with individual life styles or schedules and by the careful monitoring of treatment response. Treatment monitoring requires complex technologies to assess levels of circulating HIV-RNA (viral load), CD4 cell counts and HIV drug resistance.

The treatment options for the developing world are much more limited, and monitoring of treatment is frequently not available at all. Not only are the laboratory assays for monitoring treatment expensive, they are also complex and require a high level of investment in expertise, equipment purchase and maintenance as well as quality assurance and quality control. Whereas international grants frequently provide the necessary infrastructure in the clinical research setting, patients on treatment outside this setting rely on their own funds to pay for treatment monitoring, and these costs frequently exceed the cost of antiretroviral medications.

The need for inexpensive and simple to implement laboratory assays has stimulated some interest in new technology development. The Forum for Collaborative HIV Research has worked with an international network of industry, government agencies and research institutions to promote collaboration and support the clinical validation of new technologies as they become available. The latest developments in this area were presented and discussed at the Forum workshop on February 26, 2005 following the 12<sup>th</sup> Conference on Retroviruses and Opportunistic Infections in Boston, MA, held in collaboration with the Doris Duke Charitable Foundation. Close to 100 interested individuals stayed in Boston for this half-day workshop. Suzanne Crowe, from The

Macfarlane Burnet Institute of Melbourne, Australia reviewed new developments in CD4 technologies. Considerable advances in alternatives to the golden standard of flow-cytometry have been made since the development of manual, microscopic based CD4 cell counting technologies such as Dynabeads (Dynal) and CytoSpheres (Beckman Coulter), which remain useful in low-volume non-centralized laboratory settings. Newer automated technologies include the pan-leukogating (PLG, Beckman Coulter), the Guava EasyCD4 assay, the Partec CyFlow system, and the PointCARE system, ranging from \$3 to \$10/test, with a wide range in required capital costs. More basic point-of-care technologies, such as the LabNow microchip based method, are in the pipeline. Although some assays have been licensed in the USA (PLG, PointCARE), all these methods continue to undergo in-country analysis. Rigorous independent evaluation is absolutely essential as is participation in internal and external QA/QC programs.

Assay	Parameters Measured	Advantages/Disadvantages	Capital Cost	Cost/test (excludes costs of service, labor)
Dynal Manual Dynabeads assay	CD4 absolute (or CD8 absolute)	<ul style="list-style-type: none"> <li>• Simple</li> <li>• no instrument needed other than microscope with 40x objective and counting chamber (0.1 mm deep)</li> <li>• can be performed in remote sites without controlled utilities</li> <li>• can be used with light or fluorescence microscopy</li> <li>• No CD4 percentage capability, important for monitoring infants and children</li> <li>• Low throughput</li> <li>• Labor intensive</li> <li>• Specific magnet and rotating mixer needed</li> <li>• Less accurate at higher</li> </ul>	USD 2,000-10,000	USD 4-5 (WHO distributes at 30% discount)

		CD4 counts (>500)		
Beckman Coulter Cytosphere kit	CD4 absolute count	<ul style="list-style-type: none"> <li>• Simple</li> <li>• No instrument needed other than microscope with 40x objective and counting chamber (0.1 mm deep)</li> <li>• Can be performed in remote sites without controlled utilities</li> <li>• IVD cleared by FDA</li> <li>• No CD4 percentage capability, important for monitoring infants and children</li> <li>• Low throughput</li> <li>• Labor intensive</li> <li>• Less accurate at higher CD4 counts (&gt;500)</li> </ul>	USD 2,000	USD 8 (cost varies by region)
Guava EasyCD4	<ul style="list-style-type: none"> <li>• CD4 absolute</li> <li>• Can measure CD8 absolute</li> <li>• Can measure percentage CD4 as %CD3+ T cells</li> </ul>	<ul style="list-style-type: none"> <li>• Quick (&lt;1 hour)</li> <li>• Minimal waste disposal requirements</li> <li>• Easy to use</li> <li>• Existing QA panels not compatible with this technology</li> <li>• Still undergoing evaluation</li> </ul>	USD 45,000 (includes laptop)	Reagent cost ~ USD 3/test
PointCare System	<ul style="list-style-type: none"> <li>• CD4 absolute</li> <li>• CD4 percentage</li> <li>• WBC Lymphocyte % and count</li> </ul>	<ul style="list-style-type: none"> <li>• Mobile</li> <li>• Battery backup</li> <li>• Room temperature reagent storage and operation</li> <li>• Closed tube operation reducing potential for biohazard</li> <li>• Automated patient results</li> <li>FDA approved</li> </ul>	USD 15,000 – 20,000	< USD 10/test*

\*includes reagents and disposables, operator time, CD4, CD4%, WBC, lymphocyte count and % and service

---

Susan Fiscus, from the University of North Carolina at Chapel Hill reviewed new developments in viral load assays. The gold standards of HIV-1 RNA PCR (Roche Monitor 1.5), NASBA (bioMerieux NucliSens) and bDNA (Bayer Versant) may be used in reference centers. These assays have been validated for most HIV-1 subtypes, and work with dried blood spot samples, but the disadvantages include the expense of the equipment and reagents, the complexity of the technology and the need for equipment maintenance. Real-time PCR provides an alternative using less expensive reagents while improving sensitivity and specificity; another advantage is the potential to develop assays for other pathogens. Whereas the licensed viral load assays come with manufacturer's QA reagents, most real time PCR methods are "home brew", so the problem of reagent variability and lack of manufacturer's QA reagents must be dealt with.

Another approach has been to assay the levels of the p24 protein using a heat-denatured antigen assay. This assay is particularly well suited for infant diagnosis, an area in urgent need of technology development, and may be useful in diagnosis of acute infection in adults. Studies evaluating its usefulness in treatment monitoring have yielded mixed results.

The Cavidix ExaVir assay is based on reverse transcriptase enzyme activity. Presumably, it will work on all subtypes; the equipment is inexpensive and it is less prone to contamination problems than viral RNA amplification technologies. However, the assay duration is long, and the per-assay costs are relatively high.

Technological advances have also been made in shipping procedures, including dried blood spots and the Sample Tanker developed by Research Think Tank, Inc.

Tom Denny, from the University of Medicine and Dentistry of New Jersey and the NIAID Immunology Quality Assessment Program addressed specific issues in monitoring treatment in pediatric populations. Absolute CD4 values vary greatly and

---

undergo profound changes early in life; therefore, basing initiation of antiretroviral therapy and prophylaxis for opportunistic infections on CD4 cell counts is difficult. Thus technologies that allow both absolute and percent CD4 assessment are better suited for the pediatric setting. Diagnosis of HIV infection in children is also more problematic. A first negative test after birth does not reliably indicate HIV-free status; multiple tests may be required, the number dependent on factors such as additional potential exposure to HIV through breast-feeding.

Several recommendations were highlighted in the ensuing discussion:

- The importance of internal and external QA/QC programs and procedures cannot be overemphasized. QA/QC programs, together with mechanisms to ensure *sustainability* of technology, need to be integrated into research programs. Research sponsors need to realize that sustainability of equipment and preventive maintenance costs must be written into the research plans. Another issue is the need for compatibility between platforms and commonly available QA reference samples.
- An independent process of assay evaluation is absolutely essential. Leadership in this area, including the development of guidelines to country programs with regards to the necessary assay qualifications, needs to be developed.
- The development of treatment monitoring technologies needs to happen in the context of the treatment expertise. Whether absolute numbers or ranges of values are most useful in supporting treatment initiation or treatment switch decisions cannot be determined by the technology branch; the leaders in HIV treatment need to work hand-in-hand with the technology experts to develop guidelines for the specification of instruments and assays that will provide the most benefit.
- We need to differentiate what is going on in the clinical research setting vs. treatment programs. Central labs supported by research funds and foundation

---

programs may be able to provide (near) state-of-the-art technology. The need for the development of real “point-of-care” technology for patients not able to access centralized programs remains very urgent.

- A working group to address the specific needs of pediatric diagnosis and treatment monitoring should be established

These discussions set the stage for an afternoon workshop organized to discuss issues with the Doris Duke Charitable Foundation 2003 *Innovation in Clinical Research* grantees. The workshop was chaired by James Gita Hakim, from the University of Zimbabwe, who has had extensive direct experience in setting up both research and treatment programs in Zimbabwe, and Veronica Miller of the Forum for Collaborative HIV Research. The grantees and selected experts met to discuss the progress in each of their own areas, learn more about technology development and about adequate and appropriate plans for intellectual property management. Academia is not normally a place where development planning and intellectual property management are learned. The process of technology development can be daunting and overwhelming to groups that are primarily academia based. Thus this workshop provided an opportunity to consult with experts and consider different planning strategies.

Mickey Urdea (Halteres Associates) delved into his extensive experience with two different biotech companies to advise the grantees regarding assay development, including product specification, market analysis, manufacturing and user requirements. Mickey also discussed issues to consider to determine the path to commercialization for assay development, which includes the clinical questions to be answered and regulatory hurdles, which can be very different for different countries. Mickey stressed the importance of knowing what the user requirements are; including sample collection issues, storage temperature, needs for water and electricity and system performance in developing the product design specification.

---

Gregg Galloway (Falco-Archer) complemented this advice with a discussion of intellectual property (IP). Management of IP issues can be complex and overwhelming, but the short answer is that, whether dealing with academic, research or commercial organizations, IP issues cannot be avoided: IP management needs to be imbedded into the development and funding plan from the very beginning. IP can help generate benefits and offset significant risks and it is important for academics and their partners to have their IP issues in order.

A third expert, Rosanna Peeling, from the WHO Tropical Disease Research unit, described the process that she and her colleagues have used to partner with industry in the development of new diagnostic tools for various diseases. The support system that this group has developed includes specimen banks for rapid evaluations, multiple GLP and GCP compliant sites around the globe for testing of new diagnostics, testing for ease-of-use by field workers and modeling cost-effectiveness to facilitate national program decisions. Additional services include evaluation and prequalification guidelines for diagnostics, which translates into guidance of what to buy and how to integrate into treatment programs. This model benefits both the WHO programs and the companies. The companies retain the IP and they may use the WHO generated data for regulatory approvals. However, they are requested to provide test kits for evaluation and to negotiate pricing in order to be included in the WHO procurement program.

Of the eight grantee groups attending, three are developing CD4 cell assays, and five are developing viral load assays, ranging from purely academic to academic/private industry collaborations. Each grantee group was able to present their development achievements and these were discussed in the context of the development and IP management consultation. The CD4 assays in development include a microchip based assay (Bill Rodriguez, Harvard University), a program to optimize the Dynabead assay, and a novel manual low-cost CD4 test using reverse flow technology. The viral load technologies included a nanoparticle based approach to detect HIV p24 antigen in plasma, a microchip



---

based method to detect HIV DNA or RNA, and optimizations of the NASBA assay, the Cavid ExaVir assay and the heat-denatured p24 antigen assay.

Grantees were encouraged to find ways and means to support IP and development planning. For example, the NIH has a lot of experience helping academic investigators with IP. One mechanism is the Small Business Innovation Research (SBIR) grants. Elaine Gallin and Sylvie Le Blancq of the Doris Duke Charitable Foundation reiterated their willingness to support, at least in part, protection of IP. Other foundations, such as the Bill & Melinda Gates Foundations are other sources for continued funding to develop new technologies. It is hoped, that by concerted action and dedication on the part of all players, new technologies benefiting patients around the globe will be put into the hands of healthcare workers within a few years. The contribution that the Doris Duke Charitable Foundation has made by supporting this research will support this goal.