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Challenges for Rapid Molecular HIV Diagnostics

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Abstract

The introduction of serological point-of-care (POC) assays 10 years ago dramatically changed the way HIV infections were identified and diagnosed. Testing at the POC has lead to a dramatic increase in the number of individuals who are screened and, most importantly, receive their HIV test result. As the AIDS epidemic continues to mature and scientific advances in prevention and treatment are evaluated and implemented, there is a need to identify acute (viremic pre-seroconversion) infections and to discriminate "window phase" infections from those that are serologically positive, especially in resource limited settings (RLS), where the majority of the world's vulnerable populations reside and where the HIV incidence is highest. Rapid testing methods are now at a crossroad. There is opportunity to implement and evaluate the incremental diagnostic utility of new test modalities that are based on sophisticated molecular diagnostic technologies and which can be performed in settings where laboratory infrastructure is minimal. The way forward requires sound scientific judgment, as well as an ability to further develop and implement these tests despite a variety of technical, social and operational hurdles to declare success.

Keywords

HIV; point-of-care; resource-limited settings

Despite the global effort to control the AIDS pandemic, HIV infections continue to spread relatively unabated in many parts of the world. Recent figures from the World Health Organization (WHO) suggest that close to 33 million people are living with HIV infection worldwide, with more than two thirds of these individuals living in developing countries

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with limited resources. While some countries have demonstrated remarkable success in reducing the burden of HIV/AIDS, others still struggle to identify infected individuals, reduce transmission rates through behavioral interventions, and implement effective treatment programs for infected individuals. Many high-incidence countries have limited resources for health care and rudimentary health care systems and hence are unable to implement health care plans to address complex issues such as HIV/AIDS prevention and care. Although several government initiatives and international organizations are making a difference through partnerships and capacity building, it is clear that the existing capacity of treatment programs will not be sufficient to handle the number of infected people who require treatment in the future[1].

Identifying those who are acutely infected is a priority both in terms of behavioral and biological factors that make them the catalyst of the epidemic. Based on mounting evidence from pathogenesis and clinical trial studies in RLS, risk behavior reduction counseling can control subsequent transmission events, and together with early HAART intervention, should theoretically reduce incidence rates. Yet, this task is particularly challenging as the trend in HIV-1 transmission is moving away from urban settings into rural settings with women being disproportionately affected by the epidemic. In fact, this shift is being observed worldwide regardless of the resources, as more vulnerable populations are being exposed to HIV-1 due to poverty, lack of education and gender inequality, resulting in the highest incidence in the most difficult to reach subpopulations.

Current state of the art in HIV detection

Rapid HIV-1/2 antibody-based tests performed at POC are becoming the global standard for HIV testing, particularly in the developing world. The efficacy and acceptability of rapid, POC HIV antibody tests have been confirmed by over a decade of studies which show that rapid HIV testing increases the number of people that truly know their infection status compared to non-rapid HIV testing strategies[2-5]. In addition, cost-analyses have shown that rapid POC testing is cheaper per test result delivered when compared to standard blood testing[6]. HIV diagnosis is typically accomplished through the sequential use of a two separate rapid HIV-1/2 antibody tests[7,8]. In resource-limited settings (RLS), concordant serological reactivity on two appropriately selected rapid tests is highly predictive of infection and frequently considered sufficient for presumptive diagnosis of HIV infection. In more developed countries, confirmation of antibody positive rapid samples are accomplished by use of an independent HIV test method, such as the detection of HIV nucleic acids or specific HIV antigen reactivity patterns using Western blots or line immuno-assays.

Despite these advantages, rapid HIV antibody, as well as non-rapid serologic, tests do have certain limitations. They can yield false-negative results for acute HIV infection (prior to, or early following seroconversion), and false-positive results in uninfected HIV vaccine participants and infants with passive maternal antibodies derived from HIV-seropositive mothers. In addition, the current rapid antibody testing strategies may yield false-positive interpretations unless relatively costly confirmation and/or follow-up testing are conducted.

Combination antigen-antibody tests have been introduced to make ‘near-to-patient’ testing possible[9]. These so-called 4th generation enzyme or chemiluminescent immunoassays (EIAs; ChIAs) are engineered for dual detection of p24 antigen and immunodominant HIV antibodies. These antigen-antibody tests are widely available in Europe, Australia, and recently in Latin America[10-12] and can detect a subset of acute infections without the need for specimen pooling, which is normally done to increase efficiency and reduce costs of (Nucleic Acid Amplification Tests) NAATs[13]. While somewhat more expensive than

antibody tests, these new 4th generation assays have the advantage of a single test that can be performed on a wide array of available equipment, making them adaptable to most '1st tier' laboratory settings in the developing world. However, some of these tests have formats that only work with a given dedicated instrument, and thus implementation might be more costly than traditional EIAs that do not require such equipment. Unfortunately, 4th generation HIV EIAs/ChIAs assays are not yet FDA-approved for use in the US, and only a few Ag/Ab assays are available in rapid POC formats outside the US. However, novel rapid-format 4th generation technologies are being developed to capture more window phase infections in areas with limited resources. These types of tests have shown encouraging early results in terms of detection and discrimination of acute and chronic HIV infections (Inverness Medical: Determine® HIV-1/2 Ag/Ab Combo)[14]. Nevertheless, the accuracy of this test will be compromised for HIV-vaccine participants and babies born to HIV-infected mothers because of vaccine-induced or maternal antibodies.

High-quality, sophisticated diagnostic tests targeting HIV-1 DNA or RNA, definitive markers of active HIV-1 infection, are widely available in the developed world. However, these types of tests are currently neither affordable nor likely accessible in RLS due to lack of financial resources, absence of trained personnel and minimal laboratory facilities. Furthermore, the majority of currently available molecular diagnostic assays require expensive equipment that is often dedicated to a specific manufacturer's platform. These assays are technologically complex and require physical resources such as clean water, air conditioning, cold storage, efficient transport under low temperature, and an uninterrupted electricity supply, all of which are often not available in RLS.

Gen-Probe's (APTIMA®) HIV-1 RNA qualitative assay[15] is the only molecular assay that is FDA approved for diagnosis of acute infections and as a confirmatory test for diagnosing HIV-1 in samples that test reactive for HIV-1 antibodies[16]. Other commercially available molecular assays were developed as quantitative viral load assays for therapeutic monitoring, and are currently only available in expensive, high-throughput formats (see Stevens in this supplement). The use of RNA testing of dried blood spots (DBS), often as a complement to rapid serological assays, has enabled these molecular technologies to reach more remote settings, especially for the detection of HIV-1 nucleic acid in infants[17-23]. However, DBS test results do not return to the point of initial collection for patient notification for weeks to months. Therefore, DBS molecular testing is neither efficient nor effective since it does not provide the benefits of same-day treatment decisions and fails to address the substantial proportion of tested individuals who are lost to follow up.

Meeting the diagnostic needs

To meet the diagnostic needs of vulnerable populations living in RLS, a new generation of POC NAATs must be developed. To be successful, these tests must be rapid, simple to run and to interpret in non-laboratory environments, and be stable under real-world environmental conditions. Developing high-quality and cost-effective rapid NAATs would improve the efficiency and lower the cost of care in many parts of the world and fill the gap created by the increasingly decentralized testing environment.

The term POC can actually encompass a wide variety of devices used in many different settings. It could be used to describe an automated instrument in a clinical lab that can run samples without significant technical input, to devices that can perform an assay at the bedside or at home. For the purposes of this supplement, we will define POC diagnostics as tests that are performed near the patient, have a short turn-around time, and do not require dedicated space in a clinical laboratory. This definition can encompass two scenarios, one being in a clinic without dedicated lab space and the other being at the bedside. In both of

these situations, the objective is to use advanced materials and technologies to make testing simpler and faster, yet retain the accuracy, precision, sensitivity and specificity for detecting HIV.

There are multiple needs for POC devices to detect HIV in RLS. One need is to accurately diagnose HIV-1 infections in persons with vaccine-induced seropositivity (VISP), i.e. to discriminate HIV-infected from HIV-uninfected individuals following participation in vaccine trials[24,25]. Many HIV vaccine strategies evaluated in the past decade have concentrated on the induction of immune responses to multiple viral antigens rather than envelope alone[26]. Antibody responses to multiple viral proteins are elicited by these vaccines and detectable by commercial HIV antibody tests[27]. This may result in unintentional unblinding of a participant while in-study if the individual decides to obtain an out-of-study test. Moreover, serological reactivity may persist for more than a decade and result in misidentification of the individual as HIV-infected, particularly in areas where HIV rapid tests are confirmed by a second rapid test that detects the same analyte. Vaccine efficacy trials are conducted in populations with a high incidence of HIV, in areas that often have limited resources available for advanced methods of HIV diagnosis and monitoring to distinguish true HIV-infection from VISP. Although an alternative antibody-based assay (SELECTest) has been proposed to circumvent this problem[28], the assay is still in the research and development phase, is not commercially available and has limited utility outside the HIV vaccine field.

Beyond the vaccine arena, the most urgent application for rapid technologies is to detect HIV in pregnant women, in newborn infants born to HIV-infected mothers and infants who are breastfed by HIV-infected lactating women[29]. Diagnosis in infants is complicated by the presence of maternal antibodies for up until 18 months post-delivery, which gives rise to false-positive results in the infants. The needs and challenges have been extensively reviewed elsewhere and the reader is referred to those publications for more details[30,31]. In addition, the therapeutic needs in RLS (see Stevens in this supplement) as well as public health implications of acute HIV infection (see Pilcher et al. in this supplement) are demanding more sensitive analytical tools to capture window-phase infections in high-risk groups. Moreover, the ability to quickly assess the HIV infection status in the context of medical procedures for acute trauma in remote or outlying areas (e.g. emergent pre-screening of potential blood donors in RLS) is of high concern.

Paving the way forward

One interesting paradox is that novel POC NAAT tests often use advanced technology to simplify complex molecular steps, which is counterintuitive since most technical advances require more sophisticated equipment to increase the sensitivity of the analytical parameters measured. Examples include the development and use of nucleic acid technologies to directly identify bacterial pathogens as opposed to the culture methods used in the past[32]. Although direct nucleic acid technology streamlined the process, the advances required a more sophisticated infrastructure in the form of technical expertise to provide superior results. Similarly, POC devices in RLS need to condense a lengthy complex multi-step procedure into a few simple steps, while retaining key performance specifications. What is gained by reducing costs and turn-around times cannot be at the expense of performance parameters such as sensitivity to detect low quantities of HIV or specificity[33]. Ultimately, what is clinically achievable is a moving target, and it is in part dictated by the resources available to support diagnostic and treatment options.

On July 12–13, 2007, the Division of AIDS, National Institute of Allergy and Infectious Diseases hosted a workshop to discuss "Novel Technologies in Rapid HIV-1 Viral

Detection". The lessons learned over the course of the workshop are presented in this supplement and are directed primarily at inventors, developers and manufacturers of HIV diagnostic assays with input from end users, purchasers and regulators of diagnostic devices. Several recurring challenges were highlighted in the meeting. Obviously the issue of cost is foremost in the minds of manufacturers and those paying for tests. Cost depends not only on market forces such as projected volume of sales, but also on the true cost, including sample acquisition, reagents and delivery of the test results to the healthcare professional and patient. Moreover, the calculation of the cost-benefit ratio should also be balanced against the cost to society of not having the test available. Further details on this important issue can be found in Purden et al. in this supplement. Lack of infrastructure impacts cost and should not be overlooked since transportation and courier services (with needed environmental and safety controls), an educated workforce, communication, electricity, equipment and maintenance are intricately woven into prerequisites for support of diagnostic testing. The additional component of operator training must be included early in the process to minimize error and maximize predictive value.

Although some newly emerging nucleic acid-based testing assays/platforms have the potential for true POC (<2 hours) screening for acute HIV infections, none of these technologies are as yet commercially available[34–37]. Some technologies appear to be sensitive and robust, as reported in this supplement (see Tang et al., Reiske et al., Lee et al.). However, most of these POC NAATs require an additional nonintegrated sample preparation step, and some also require separate amplification and detection steps. In addition, most of them have not yet been rigorously evaluated to establish analytical sensitivity and specificity, performance on pedigreed clinical sample sets, or performance in field trials to fully establish their real-world performance characteristics.

Truly revolutionary technological advances in materials science, flow-based physics, isothermal amplification, detection chemistries and nanotechnologies have fueled the POC revolution over the past several years[38,39]. In fact, the number of publications for POC diagnostics has grown exponentially over the past decade. The biggest technological gap in the POC NAAT exists in the area of specimen processing. Optimal specimen procurement and processing to yield target HIV RNA are two key steps for assuring high quality results[40]. Research is currently focused on how to reliably and cost-effectively extract the element(s) of interest from a biologically complex sample like blood or other body fluids, and perform this function without the use of laboratory equipment. This is proving to be an extraordinarily difficult challenge due to the presence of natural assay inhibitors within samples, specimen contamination and the increasing concern about the environmental disposal of chemical and biological waste.

Non-technical challenges

In addition to the technical challenges outlined in this supplement, several operational challenges need to be considered prior to rolling out POC testing for detection of HIV nucleic acids in RLS. First and foremost is whether the assay specifications meet the clinical needs of the patients and whether the device is field deployable (see Guillerm et al. in this supplement). Moreover, the device employed should be field tested in small pilot projects in strategic locations to obtain support for the required training and implementation with appropriate health ministries. The existing medical infrastructure will need to be assessed for providing various levels of communication to capture and resolve potential issues. In addition, developing a procurement strategy and establishing a supply chain for reagents along with maintenance contracts for instrument repair remains a significant challenge. These issues should be addressed beforehand with contingency plans for smooth field operation.

The challenge of sustainability must be considered for the long-term viability of quality diagnostic testing. This includes tapping into local economies for manufacturing, developing a supply chain and working to ensure that distribution channels meet the needs. Although these challenges are not directly related to assay performance from a test developer's point of view, considerable attention needs to be given to these issues to achieve success in implementing a POC HIV nucleic acid diagnostic device in RLS. Furthermore, it is important to realize that not all RLS have the same issues or challenges, and that a certain degree of flexibility needs to be factored in depending on the specific application. The one size fits all approach must be abandoned as the only viable business plan. Although this has negative implications for companies that are interested in streamlining production and gaining a large market share, it presents unique opportunities for smaller businesses to carve out a specialized and potentially lucrative niche.

Finally, some thought needs to be given to address these challenges at the local level and not impose solutions based on foreign experiences. Involving local government health agencies, collaborations with local investigators, and developing public/private partnerships in RLS to assist in addressing the issues will give a sense of local ownership to enhanced HIV diagnostic programs and lead to more sustainable advances in the future. The development of these collaborations is important in order to actively engage local health care professionals in assessing the utility of a given technology in specific geographic areas or diagnostic applications. Ensuring quality testing will be a concern and implementing appropriate QA/QC procedures to address specific validation parameters such as accuracy and precision will be needed. However, with proper planning, POC NAAT for early detection of HIV will enable identification of infections prior to seroconversion and confirmation of active viremia in seropositive individuals, providing critical data that can enhance counseling and treatment programs which are designed to prevent HIV transmission and reduce disease progression.

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