A New HIV Detection Method for Infants May Help Control Disease in Impoverished Countries

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HIV/AIDS remains one of the world's most significant health challenges, with 34 million people currently living with HIV worldwide. Although adults account for a large percentage of newly diagnosed HIV infections in the United States, children account for a substantial portion of new HIV cases on a global scale, particularly in sub-Saharan Africa and other resource-limited areas. The statistics are staggering: In 2010 an estimated 390,000 children were diagnosed with HIV, and nearly 50% of HIV positive babies die by two years of age if the infection is not detected and treated early in infancy. However, early infant diagnosis requires the detection of HIV itself, as opposed to the presence of HIV antibodies. Such tests are more cumbersome, and must be sent to centralized laboratories for analysis. A team led by Dr. Julie Overbaugh (Human Biology Division at the Hutchinson Center) set out to design a DNA-based HIV test that was temperature cycling-independent, and to determine its efficacy in detecting a range of HIV strains. The results are very promising.

Although many HIV diagnostic tests for early infants rely on PCR and the relatively complex and expensive instruments needed for PCR-based detection, novel isothermal techniques have also been devised to detect HIV. Boyle and colleagues hypothesized that a technique called RPA (recombinase polymerase amplification) would enable the rapid detection of HIV proviral DNA using a minimal amount of blood. RPA is a technique that amplifies DNA in less than 20 minutes at a constant temperature between 25 to 42C. RPA uses a recombinase to insert oligonucleotide primers into double-stranded DNA molecules, followed by a strand-displacing DNA polymerase, to synthesize a new complementary DNA strand. The RPA product is ultimately detected using a modifiable probe.

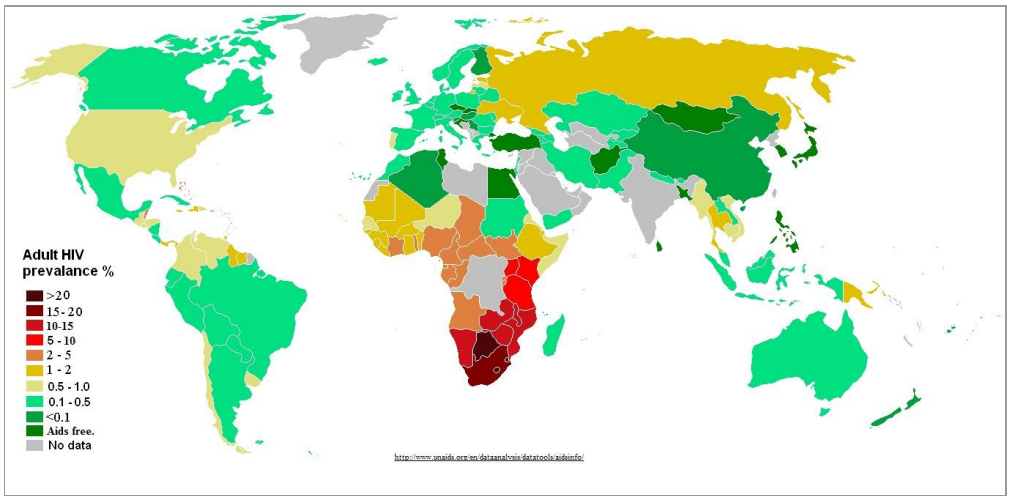
As a starting point, Boyle *et. al.* screened sixty-three primer/probe pairs to determine the optimal combinations for amplifying 40 copies of exact sequence-matched HIV-1 DNA template. Although they attempted to amplify HIV-1 *gag*, LTR, and *pol*regions, no *gag* amplification was observed. Fortunately, LTR and *pol* were successfully amplified with five and six primer pairs, respectively. Fewer copies of the HIV-1 DNA were used in the RPA reactions to determine if the primer sets could

detect less concentrated DNA. The ability to detect the presence of HIV-1 DNA in samples with low levels of HIV-1 DNA could correspond to smaller blood samples required in the clinic. Surprisingly, only ten copies were required to amplify LTR in ~90% of the reactions. Approximately 50% of the reactions showed amplification when three copies of HIV-1 DNA were added. These data indicate that RPA could successfully amplify the LTR and *pol* regions of HIV-1 DNA when it is present at low levels.

A variety of HIV-1 subtypes exist, with subtypes A, C, and D constituting the dominant strains in Africa. In order for the RPA-based HIV detection method to be used on a global scale, it would have to be able to amplify these HIV-1 subtypes. The authors used a panel of 16 genomic DNAs obtained from HIV-1 primary isolates and found that the *pol* primers amplified all 16 DNAs, some of which contained subtypes A, C, and D. Diverse HIV-1 sequences obtained from the NIH International Panel of HIV-1 viruses were also successfully amplified with the *pol* primers (55 of 56 viral variants tested). Also, the success of RPA in detecting the presence of HIV-1 DNA was independent of the probe used: ICS (intracellular cytokine staining) gave similar test results when compared to the more elaborate fluorescence-based probes.

Taken together, Boyle and colleagues have developed an RPA-based HIV-1 diagnostic that can be used with basic laboratory equipment and minimal user technique. This test can efficiently amplify less than 10 copies of proviral DNA, an amount that is well below the ~ 3,750 copies of HIV-1 DNA in a drop of blood in HIV-1-infected early infants. The application of RPA for early infant HIV-1 diagnosis in clinical settings remains to be explored. However, the initial results published by the authors suggest that RPA could be instrumental in detecting HIV-1 to reduce deaths from AIDS-related illness, particularly in early infants.

Boyle DS, Lehman DA, Lillis L, Peterson D, Singhal M, Armes N, Parker M, Piepenburg O, Overbaugh J. 2013. Rapid Detection of HIV-1 Proviral DNA for Early Infant Diagnosis Using Recombinase Polymerase Amplification. *MBio*. doi: 10.1128/mBio.00135-13.



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Although HIV is a worldwide epidemic, people living in Africa have been affected the most (data from 2011). A new HIV test devised by the authors will likely help detect HIV infection and accelerate treatment in impoverished regions.