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## Routine Laboratory Screening for Acute and Recent HIV Infection in Lima, Peru

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### Abstract

**Background**—Prior to implementing screening programs for acute HIV infection in developing countries, key issues including cost, feasibility, and public health impact must be determined. We compared fourth-generation enzyme immunoassay (EIA) with pooled HIV-1 RNA assays for the detection of acute and early HIV infection in counseling and testing populations in Lima, Peru.

**Methods**—Adults presenting for HIV testing at designated clinics in Lima-Callao, Peru were offered additional screening for acute HIV infection. All serum samples were tested with fourth-generation Ag/Ab EIA and confirmed by line immunoassay (LIA). Negative specimens were combined into 50-sample pools for HIV-1 RNA screening by PCR analysis in standard pooling algorithms. RNA-positive samples were re-tested with a third-generation EIA to evaluate the relative sensitivity of standard testing procedures.

**Results**—Between 2007 and 2008 we recruited 1,191 participants. The prevalence of HIV infection was 3.2% (38/1191; 2.2-4.2%) overall and 10.6% (25/237; CI=6.6-14.5%) among men who reported sex with men (MSM). The prevalence of acute or recent HIV infection was 0.2%

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**Authors' Contributions:** Study concept and design was developed by JLC under the guidance of CFC, JDK, and TJC. Data collection was coordinated by ERS with the participation of JLC, HJS, SRL, and JA. Laboratory analysis of specimens was completed by SRL, SM, and TK. Data analysis was completed by JLC. JLC took primary responsibility for drafting the manuscript with the contribution and final approval of all of the authors.

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**Competing Interest** None declared.

(CI=0-0.4%) overall and 0.8% (CI=0-2.0%) among MSM. Compared with third generation EIA testing, both fourth generation EIA and RNA PCR increased the rate of HIV case identification by 5.6% overall and by 8.0% within the subpopulation of MSM.

**Conclusions**—Screening for acute HIV infection within Peru's resource-limited public health system was acceptable and detected a high prevalence of acute and recent HIV infection among MSM. Additional efforts are needed to screen for and prevent transmission of HIV among MSM in Peru during the acute seroconversion stage.

## Keywords

Acute HIV Infection; Pooled RNA Testing; Peru: Men Who Have Sex with Men

## Introduction

Screening for acute and recent HIV infection provides new opportunities for HIV treatment and prevention. Nucleic acid analysis is the gold standard for diagnosing acute and recent HIV infection, though the technical requirements often exceed developing country resources.(1-5) Fourth-generation EIA (which adds a p24 antigen component to the third generation antibody assay) is also effective in detecting acute and recent HIV infection and adaptable to the existing infrastructure of many developing areas.(6) We report the results of a pilot program to screen for acute and recent HIV infection in Lima, Peru, comparing pooled HIV-1 RNA nucleic acid analysis and fourth generation EIA with the standard of care third generation EIA/LIA testing algorithm.

## Methods

Adults seeking HIV testing at the Barton Health Center in Lima, Peru between December, 2007 and May, 2008 were offered supplementary screening for acute and recent HIV infection. The protocol was approved by the Institutional Review Boards of the University of California, Los Angeles, Universidad Peruana Cayetano Heredia, and U.S. Naval Medical Research Center.

Specimens were screened with a fourth generation EIA to detect HIV-1/2 IgG and IgM antibodies and/or HIV-1 p24 antigen (Vironostika Uni-Form II). Positive samples were confirmed by HIV-1/2 LIA (Inno-Lia HIV I/II Score). Samples negative or indeterminate by fourth generation EIA or LIA were aggregated into pools of 50 and screened for HIV-1 RNA (Roche Taqman) using a 50:10:1 algorithm.(2,7) RNA-positive samples were subsequently tested with a third generation EIA capable of detecting HIV-1/2 IgG and IgM but not p24 antigen (Genetic Systems HIV-1/2 Plus O).

Samples positive by fourth generation EIA and/or RNA PCR, but negative by third generation EIA (p24 Ag+; IgG/IgM-), were defined as *acute* infection. Samples positive by third *and* fourth generation EIA (p24 Ag+; IgG/IgM+), but negative or indeterminate by LIA were classified as *recent* infection. Only samples positive by LIA, meeting Peruvian Ministry of Health criteria for the diagnosis of HIV infection, were diagnosed as *chronic* infection.

## Results

1,391 men and women requested HIV testing during the study period. 1,191 (85.6%) consented to an additional blood draw to screen for acute or recent HIV infection. (Figure 1)

The prevalence of HIV infection in the study population was 3.2% (38/1191; 95% Confidence Interval [CI] = 2.2-4.2%) and the prevalence of acute or recent infection was 0.2% (2/1191; CI = 0-0.4%). The prevalence of HIV among MSM was 10.6% (25/237; CI = 6.6-14.5%), including both cases of acute or recent infection (2/237; 0.8%, CI = 0-2.0%). Compared with the standard of care EIA/LIA algorithm, fourth generation EIA with RNA PCR confirmation increased the frequency of HIV diagnosis by 5.3% (CI = 0-12.3%) overall and by 8.0% (CI = 0-18.6%) among MSM.

## Discussion

In our evaluation of 1,191 men and women, we identified two cases of acute or recent HIV infection that would not have been diagnosed using Peru's standard of care screening and confirmation algorithm. While HIV-1 RNA and fourth generation EIA assays were both effective in screening for acute or recent infection, there were significant differences of cost and complexity associated with the different assays. Implementation of RNA-based testing requires substantial investments in training and infrastructure while the technical demands of fourth-generation EIA are similar to currently used assays. In contrast, while RNA PCR can screen for and confirm HIV infection, fourth generation EIA is not as specific and often requires an additional confirmatory assay for definitive diagnosis. The introduction of rapid fourth generation assays and EIAs that differentiate the presence of HIV p24 antigen from IgG/IgM antibody may contribute to use of fourth generation EIA as a single screening and confirmation assay.

The small number of participants in our pilot study limits any conclusions regarding the sensitivity or specificity of the different assays. Our inclusion of specimens positive by fourth generation EIA but negative or indeterminate by LIA in the pooled nucleic acid analysis algorithm may have increased costs compared with individual RNA testing of these specimens. Finally, recent modification of Peruvian HIV testing protocols to include rapid antibody testing could alter the context and utility of screening for acute and recent HIV infection in the country.

Our findings establish the acceptability and performance of fourth-generation EIA and pooled HIV-1 RNA PCR assays for the diagnosis of acute and recent HIV infections within a resource-limited public health system and identify a high prevalence of acute and recent HIV seroconversion among MSM in Peru. Future public health efforts should address cost-effective methods to increase detection of acute and recent infection among MSM in Latin America.

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**Figure 1.** Diagnostic algorithm and results of screening for acute and recent HIV infection; Lima, Peru 2007-08.