Boosted p24 Antigen Assay for Early Diagnosis of Perinatal HIV Infection

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Objective: *The authors evaluated the accuracy of in-house boosted-p24 antigen assay for diagnosis of perinatal HIV infection.*
Material and Method: *The author has retrospectively reviewed the medical records of infants born to HIV-positive mothers. The infants were tested for boosted-p24 antigen assay at the age of 1-2 months and 4-6 months. HIV infection was defined as positive anti-HIV at the age 18 months or older, or had positive HIV-PCR with clinical signs and symptoms compatible with HIV/AIDS.*
Results: *There were 168 infants included in this review and six were HIV-infected. The boosted-p24 antigen assay had the sensitivity, specificity, positive predictive value, and negative predictive value of 33.33%, 98.27%, 50%, and 95.8%, respectively at 1-2 month-old, and 100%, 98.27%, 71.43%, and 100%, respectively at 4-6 month-old.*
Conclusion: *Boosted-p24 antigen assay could be a cheaper alternative test to help diagnosis of perinatal HIV infection in infants. The test was very accurate when performed at 4-6 months.*

Keywords: *Boosted-p24 antigen, HIV-exposed infants, Diagnostic test*

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Early diagnosis of HIV infection in infants is crucial for early initiation of treatment with highly active antiretroviral therapy (HAART), which has been associated with substantial reduction in mortality[1], Because of transplacental transfer of maternal antibody, HIV antibody tests cannot be used for diagnosis of HIV infection in infants under 18 months of age. Therefore, virologic tests, mainly HIV DNA-polymerase chain reaction (PCR) or HIV RNA-PCR assays, have been recommended as standard methods for diagnosing perinatal HIV infection in infants[2].

In many resource-limited countries, PCR assays are expensive and not widely available. Therefore, cheaper diagnostic tests are needed. The regular p24 antigen assay is cheaper and can be performed in most basic labs, but has low sensitivity and specificity due to immune complex formation. Modification of the regular p24 antigen assay by using pre-heated immune complex dissociation, combined with a booster step to quantify HIV-1 p24 antigen in plasma, have shown to be correlated with HIV RNA levels and can accurately be used for diagnosis of perinatal infection in infants[3-5]. The present study was done to evaluate the accuracy of an in-house boosted p24 antigen assay as a diagnostic test for perinatal HIV infection in infants in routine care.

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Material and Method

Between January 2004 and July 2006, when HIV DNA or RNA-PCR were not provided free in Thailand, the authors offered the boosted p24 antigen assay as a diagnostic test for early detection of perinatal HIV infection in infants as part of routine service. One hundred sixty seven infants born to HIV-infected mothers were tested by boosted p24 antigen assay at

age 1-2 months and 4-6 months. HIV antibody testing was performed at the age of 12 months, and was repeated at 18 months if the result at 12 months was positive. The authors retrospectively reviewed the medical records of these infants. HIV infection can be definitively excluded in children with negative HIV antibody at 12 months of age or older. Any children at age 18 months or older with positive HIV antibody or had positive HIV-PCR with clinical signs and symptoms compatible with HIV/AIDS were diagnosed as HIV infection.

The Vironostika® HIV-1 p24 antigen assay (bioMerieux) was modified by heat-denatured immune complex dissociation and signal amplification of p24 antigen ELISA with biotinyl tyramide according to the manufacturer's instructions for the ELAST® ELISA amplification system. Eight negative controls and six positive controls were performed for each test. The cutoff value of the optical density (OD) at wavelength 450 nm was determined by using the mean of eight negative controls plus three standard deviations. Samples with an OD value equal to or greater than the cutoff value were defined as positive for HIV-1 p24 antigen and those with an OD value less than the cutoff value were defined as negative.

Results

Of the 167 infants born to HIV-positive mothers between January 2004 and July 2006, six (3.6%) met the authors's definition of HIV infection. Eleven infants who did not have two blood tests for boosted p24 antigen at age 1-2 months and 4-6 months (one child was HIV-infected) were excluded from the analysis, as well as 33 infants who were lost to follow-up before the definite status of HIV infection was determined. The final analysis included data from 123 infants, five of whom were HIV-infected. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) at 1-2 months and 4-6 months of age are shown in Table 1. There were two false positives and four false negatives at age 1-2 months of age, and two false positives and no false negatives at 4-6 months of age. When using the criteria of any positive boosted p24 antigen at age either 1-2 months or 4-6 months, the sensitivity, specificity, PPV, and NPV were 100%, 96.6%, 55.6%, and 100%, respectively.

Discussion

In resource-poor countries, access to nucleic acid assays is limited. Early diagnostic testing is important in the era of HAART, which can improve survival in infants if initiated early[1]. Alternative to nucleic acid assays, HIV-1 p24 antigen assay is attractive because it is cheaper and can be performed in most labs in resource-limited settings. To improve its sensitivity, the regular p24 antigen assay is modified by heat-mediated immune complexes dissociation and signal amplification. Studies found that the boosted p24 antigen assay correlated well with HIV RNA[3,6-8]. Previous studies in Switzerland and Tanzania[4,5] and the study from Siriraj Hospital[6], compared boosted p24 antigen assay with nucleic acid tests for diagnosis of perinatal HIV infection and found excellent sensitivity (98-100%) and perfect specificity (99-100%), equal to HIV RNA-PCR. The study in Switzerland, however, found that the sensitivity was low at 50% in infants less than 10 days old. All of these studies used biotinyl tyramide ELAST□ ELISA Amplification system tested in stored blood samples, presumably performed in batch. The present study was different in that it was performed in routine clinic service, and all the samples were tested in separate runs.

In the present study, the authors found that the presented in-house boosted p24 antigen assay has poor sensitivity at age 1-2 months, concur with the Swiss study, but the sensitivity increased to 100% at age 4-6 months. However, the test had high specificity. The findings implied that boosted p24 antigen assay did not perform well in young age, which could be from low antigenemia in early onset of infection in infants.

Table 1. Accuracy of boosted p24 antigen assays for diagnosis of HIV infection in 123 HIV-exposed infants

Age (mo.)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
1-2	1/5 (20) (3.6-62.4)	116/118 (98.3) (94.0-99.5)	1/3 (33.3) (6.1-79.2)	116/120 (96.7) (91.7-98.7)
4-6	5/5 (100) (56.6-100)	116/118 (98.3) (94.0-99.5)	5/7 (71.4) (35.9-91.8)	116/116 (100) (96.8-100)
One positive at any age	5/5 (100) (56.6-100)	114/118 (96.6) (91.6-98.7)	5/9 (55.6) (26.7-81.1)	114/114 (100) (96.7-100)

The present study was limited to small number of HIV-infected infants because of the efficiency of the prevention of mother-to-child transmission program. Many children were excluded because of missing data and lost to follow-up. However, even with the limited number of infants in this analysis, the authors were able to demonstrate that boosted p24 antigen has limited sensitivity at 1-2 months of age with some false positives, and therefore limited positive predictive value.

Boosted p24 antigen assay is a cheaper, simpler technology that is less time consuming than DNA PCR or RNA assays and can be performed with a large number of samples at a time. If it is to be used, it should be performed at and age older than 2 months, and a positive test will need to be confirmed with other tests such as nucleic acid assay. Because of its relatively high negative predictive value when testing at 4-6 months, this test may be most useful in ruling out infection among uninfected children. All the infants need to have their HIV status confirmation with serology at 12-18 months of age regardless of tests used in infancy.

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การตรวจ "บู๊สต์-พี24 แอนติเจน" (Boosted-p24 antigen assay) เพื่อการวินิจฉัย การติดเชื้อเอชไอวีในทารกที่คลอดจากมารดาที่ติดเชื้อเอชไอวี

อรศรี วิทวัสมงคล, นิรันดร์ วรรณประภา, พิมพ์พัณณดา เจียสกุล, วนัทปรียา พงษ์สามารถ, วาสนา ประสิทธิสืบสาย, รวงผึ้ง สุทเธนทร์, กุลกัญญา โชคไพบูลย์กิจ

วัตถุประสงค์: *เพื่อประเมินประสิทธิภาพของบู๊สต์-พี24 แอนติเจน ในการวินิจฉัยการติดเชื้อเอชไอวีของทารกจากมารดา*

วัสดุและวิธีการ: *เป็นการศึกษาแบบย้อนหลังในทารกที่คลอดจากมารดาที่ติดเชื้อเอชไอวี และได้ตรวจบู๊สต์-พี24 แอนติเจน เมื่ออายุ 1-2 เดือน และ 4-6 เดือน โดยจะวินิจฉัยยืนยันว่าทารกติดเชื้อเอชไอวีหากตรวจพบแอนติบอดีที่อายุมากกว่าเท่ากับ 18 เดือน หรือตรวจพบว่าพีซีอาร์เป็นบวก ร่วมกับมีอาการที่เข้าได้กับการติดเชื้อเอชไอวี หรือโรคเอดส์*

ผลการศึกษา: *ทารกที่ศึกษาทั้งหมด 168 คน มี 6 คน ติดเชื้อเอชไอวีพบว่า บู๊สต์-พี24 แอนติเจนมี sensitivity, specificity, positive predictive value และ negative predictive value ที่อายุ 1-2 เดือน เท่ากับ 20%, 98.3%, 33.3%, 96.7% และเท่ากับ 100%, 98.3%, 71.4%, 100% ที่อายุ 4-6 เดือน ตามลำดับ*

สรุป: *การตรวจบู๊สต์-พี24 แอนติเจน สามารถใช้เป็นการตรวจทางเลือกเพื่อช่วยในการวินิจฉัยการติดเชื้อเอชไอวีในทารกได้ และจะให้ความแม่นยำมากเมื่อตรวจที่อายุ 4-6 เดือน*