

An In-House HIV DNA PCR Assay for Early Diagnosis of HIV Infection in Children in Thailand†

THANYAWEE PUTHANAKIT, MD*,
CHATCHAWAN APICHARTPIYAKUL, PhD**,
VIRAT SIRISANTHANA, MD*

Abstract

Eighty-five venous blood specimens were collected at 4, 6 or 9 months of age from asymptomatic human immunodeficiency virus (HIV)-exposed infants and from symptomatic HIV-infected infants on admission to the hospital. The specimens were tested by in-house HIV deoxyribonucleic acid (DNA) nested polymerase chain reaction (PCR) and the commercial Amplicor HIV-1 DNA test. In order to determine the accuracy of the tests, the results were compared with the HIV infection status of the children. In-house HIV DNA PCR and the commercial Amplicor HIV-1 DNA test had overall sensitivity of 95.2 per cent and 100 per cent and an overall specificity of 100 per cent and 98.4 per cent, respectively. In the analysis of 62 HIV-exposed infants who received perinatal HIV prevention intervention, in-house HIV DNA PCR yielded 100 per cent sensitivity, specificity, positive predictive value and negative predictive value. The authors concluded that in-house HIV DNA PCR has comparable sensitivity and specificity to the Amplicor HIV-1 DNA test in detecting the HIV infection status of children born to HIV-infected mothers. The in-house HIV DNA PCR, which costs US \$10 per test, should be considered in developing countries for the early diagnosis of HIV-1 infection in children.

Key word : HIV DNA PCR, HIV Diagnostic Test, Children, HIV Infection

PUTHANAKIT T,
APICHARTPIYAKUL C, SIRISANTHANA V
J Med Assoc Thai 2003; 86: 758-765

* Department of Pediatrics,

** Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

† Presented at the 50th Thai Congress of Pediatrics, Pattaya, Chon Buri, Thailand, April 26-28, 2000.

Mother-to-child transmission is the leading cause of human immunodeficiency virus (HIV) infection in childhood. In Thailand, approximately 15,000 children are born at risk for mother-to-child HIV transmission each year⁽¹⁾. The perinatal HIV transmission rate in Thailand after using simplified zidovudine (ZDV) prophylaxis regimens and the avoidance of breast-feeding is approximately 10 per cent^(2,3).

The current practice of diagnosing HIV infection in HIV-exposed infants in Thailand is performed by HIV antibody testing when they are 18 months of age or older. However, HIV-infected children need earlier diagnosis to permit timely initiation of anti-retroviral therapy and prophylaxis against opportunistic infections in order to reduce morbidity and mortality⁽⁴⁾. The uninfected children, who represent the larger fraction of infants born to HIV-positive pregnant women, also need early diagnosis in order to avoid the stigma of the "HIV-infected" label. The importance of a diagnostic test that could determine whether an individual child is infected in the early months of life has become more substantial after the Ministry of Public Health of Thailand implement a national campaign of the mother-infant HIV prevention program^(5,6). The early diagnostic test can be a useful tool to evaluate an effectiveness of the program and also to identify HIV-infected infants in order to provide appropriate care for them.

HIV DNA polymerase chain reaction (PCR) is the standard test to diagnose HIV-exposed infants in developed countries⁽⁷⁾. The commercially available HIV DNA PCR test has a sensitivity of 98 per cent and specificity of 100 per cent in the diagnosis of HIV infection in infants older than 1 month of age⁽⁸⁾. However, the obstacle to a wider use of the commercially available HIV DNA PCR test in Thailand is its high cost, US \$50 per test, compared to US \$100 drug cost per case (using a Thai-manufactured generic) for simplified zidovudine regimen for the prevention of mother-to-child HIV transmission⁽⁹⁾.

An alternative to commercial PCR-based tests is in-house HIV DNA PCR. The in-house HIV DNA PCR performed in the present study is nested PCR specific to *pol* gene, using JA17/JA20 and JA18/JA19 primer sequences and gel electrophoresis as the detection method^(10,11). It was reported to have a high sensitivity (100%) and specificity (94.4%) in diagnosing HIV infection within 6 months after birth⁽¹⁰⁾. Most importantly, the cost is only US \$10 per test.

The authors conducted a prospective study to directly compare the diagnostic value of in-house HIV DNA nested PCR to the commercial HIV-1 test (Amplicor HIV-1 DNA test) for HIV infection in children in Thailand.

MATERIAL AND METHOD

Study population

The study population included two groups of children 1) 70 children who were born to HIV-infected mothers at Chiang Mai University Hospital, and 2) 15 children, aged less than 18 months, who were admitted to the hospital due to HIV-related illness.

The first group represented the target population to implement the in-house HIV DNA PCR as a diagnostic tool. They were children 9 months of age or younger who were born to HIV-infected mothers and were followed-up at the perinatal HIV-clinic at Chiang Mai University Hospital. They were enrolled from May to October 1998 and were followed-up to the age of 18 months for HIV antibody testing. The HIV-infected pregnant women received ZDV prophylaxis orally during pregnancy as 100 mg five-times-daily from 28 weeks of gestation until delivery and followed by 300 mg every 3 hours at intrapartum. The infants received 2 mg/kg/dose of ZDV syrup four-time-daily, within 6 hours of life for six weeks. The authors counseled the mothers regarding the risk of HIV transmission by breastfeeding and provided them with infant formula.

The second group represented HIV-infected children who were enrolled to determine the sensitivity of the test. They were children 18 months of age or younger who were born to HIV-infected mothers and were hospitalized for HIV-related illness from May to October 1998.

The study was performed under the institutional review board approval.

HIV DNA PCR and HIV antibody testing

In the first group of children, HIV DNA PCR was assayed by both commercial, Amplicor HIV-1 DNA and in-house PCR, once during the follow-up period of either at 4, 6 or 9 months of age. HIV antibody testing was performed by two methods 1) enzyme-linked immunosorbant assay (Cobas[®] Core anti-HIV EIA DAGS, Roche Diagnostics, Basel, Switzerland and Enzygnost[®] anti-HIV, Dade Behring, Marburg, Germany) and 2) Particle agglutination test (Serodia[®]-HIV, Fujirebio Inc, Tokyo, Japan). These tests were performed according to the manufacturer's instruc-

tions. HIV antibody tests were performed at least twice between 12 to 18 months of age.

In the second group of children, the HIV DNA PCR tests by both methods were done on a single occasion at the time of admission. The clinical diagnosis was categorized according to the 1994 CDC revised classification system⁽¹²⁾.

All laboratory testing was performed at the Department of Microbiology, Chiang Mai University Hospital, Chiang Mai, Thailand by personnel blinded as to the source of the specimens.

Definition of HIV infection status

A child was considered to be HIV-infected if the HIV antibody test remained positive at 18 months of age or if the child had clinical conditions that met case definition for AIDS⁽¹²⁾.

A child was considered not to be HIV-infected if at least two HIV antibody results were negative between the ages of 12 and 18 months and there was no clinical evidence of HIV infection.

Amplicor HIV-1 DNA test

The Amplicor HIV-1 DNA test (Roche Diagnostics Systems, Inc., Somerville, NJ, USA) was performed according to the manufacturer's instructions. This test consisted of four major steps: a) whole blood sample preparation, b) PCR target amplification, c) hybridization of the amplified products to a specific probe, and d) detection of the amplified product by color formation. In addition, the assay used the uracil-N-glycosylase carryover protection system to prevent false-positive PCR results caused by contamination with previously amplified HIV DNA.

Sample preparation A 100 μ l sample of EDTA-treated whole blood was treated with 1 ml of wash solution (sodium phosphate buffer containing < 0.4% detergent) to lyse erythrocytes. This solution was then centrifuged at 15,000 rpm for 3 minutes and the obtained leukocyte pellet was washed twice, then frozen and stored at -70°C until tested.

Amplification The DNA was extracted by adding 200 μ l of extraction buffer (Tris-HCl buffer containing 1% detergent, 7.5 mM MgCl₂, and 0.01% proteinase K) to each pellet, mixing in a vortex, and incubating at 60°C for 30 minutes and then at 100°C for an additional 30 minutes. For amplification, 25 μ l of prepared sample was combined with 25 μ l of DPEC water and 50 μ l of working Amplicor Master Mix (consisting of dATP, dCTP, dGTP, dUTP, AmpErase, AmpliTaq and biotinylated primers). The sequences

of the biotinylated primers (SK431/462) targeted a conserved region of the HIV *gag* gene.

Amplification was performed in a GeneAmp PCR system 9600 thermal cycler with the following amplification parameters: 50°C for 2 minutes; 5 cycles (denaturation: 95°C, 10 seconds; annealing: 55°C, 10 seconds; extension: 72°C, 10 seconds); and 30 cycles (denaturation: 90°C, 10 seconds; annealing: 60°C, 10 seconds; extension: 72°C, 10 seconds). After amplification, samples were held at 72°C until denatured.

Hybridization and detection of the DNA product upon completion of the PCR amplification, 100 μ l of denaturation solution (EDTA in 1.6% sodium hydroxide) was pipetted into each reaction tube and incubated for 10 minutes at room temperature. The 25 μ l of denatured amplification sample was mixed with 100 μ l hybridization solution (sodium phosphate buffer containing 0.2% solubilizer and < 25% chaotrope) in a microwell detection plate coated with the capture probe SK102 and then incubated for 1 hour at 37°C. The plate was washed with wash concentrate, reacted with avidin-horseradish peroxidase conjugate at 37°C for 15 minutes, and washed again; color was then developed by the addition of a working substrate reagent. After incubation for 10 minutes in the dark at room temperature, the colorimetric reaction was stopped by the addition of 100 μ l stop reagent (4.9% sulfuric acid). The plate was then read at 450 nm in a model EL311 microwell plate reader (Bio Tek Inc., Winooski, Vt.); the cut off point for PCR positive was an optical density (OD) of 0.35. The positive control should be above 3.0 OD and the negative control should be less than 2.5 OD.

The in-house HIV DNA PCR

The in-house HIV DNA PCR had four major steps similar to the Amplicor HIV-1 DNA. The major differences between the two tests are summarized in Table 1.

Sample preparation A 1 ml sample of EDTA-treated whole blood was treated with 6 ml of lysis buffer (0.32M sucrose, 10 mM Tris HCl pH 7.5, 5 mM MgCl₂ and 1% Triton X-100) to lyse erythrocytes, then centrifuged at 2,500 rpm for 10 minutes. This process was repeated three times and the resulting leukocyte pellet was washed with 1 ml of lysis buffer and transferred to epend-off tube centrifuged at 2,500 rpm for 7 minutes. The DNA was extracted by adding 50 μ l of Proteinase K buffer, which contained Proteinase K 100 μ g/ml and incubated at 56°C for 4 hours and then 95°C for an additional 15 minutes.

Table 1. Comparison of two PCR methods for HIV-1 detection: Amplicor HIV-1 DNA and in-house HIV DNA PCR.

	Amplicor HIV-1 DNA	In-house HIV DNA PCR
Blood specimen (ml)	0.1	1.0
Primer set	<i>gag</i> gene SK432/462	<i>pol</i> gene JA17/JA20, JA18/JA19
Detection method	Hybridization and color formation	Gel electrophoresis and band formation under UV illumination
Time (h)	7	11
Cost per test (US \$)	50	10

Table 2. The primers used in the in-house HIV DNA PCR.

Primer	Sequences (5'-3')	Location	Usage
JA17	TAC-AGG-AGC-AGG-TGA-TAC-AG	2431-2450	Outer primer
JA20	CCT-GGC-TTT-AAT-TTT-ACT-GG	2678-2697	Outer primer
JA18	GGA-AAC-CAA-AAA-TGA-TAG-GG	2481-2500	Inner primer
JA19	ATT-ATG-TTG-ACG-GGT-GTA-GG	2591-2610	Inner primer

The pellet was then frozen and stored at -20°C until tested.

Amplification The reaction was performed in a final volume of 50 μl . The first round PCR was performed over 35 cycles in a reacting mixture containing 1X PCR buffer, 3.5 mM MgCl_2 , 100 μM of each dNTP, 0.2 oligonucleotide outer primer set (JA17/JA20)⁽¹¹⁾ (Table 2), 2.5 units of Taq DNA polymerase and 10 μl of extracted sample. Each cycle consisting of 94°C , 1 minute; 50°C , 1 minute; 72°C , 1 minute for 35 cycles. The second round PCR was carried out using the same program as the first round PCR condition except MgCl_2 was decreased to 1.5 mM, 5 μl of the first round PCR product and inner primer set (JA18/19)⁽¹¹⁾ (Table 2).

Gel electrophoresis for detection of the DNA product upon completion of the PCR assay, the PCR products were electrophoresed in a 2 per cent agarose containing ethidium bromide. The amplified product was shown as DNA band with 162 bp in length under UV illumination.

Statistical analysis

Statistical analyses were performed with the use of Stata statistical package (Version 6.0; College station, TX). The sensitivity and specificity of both HIV DNA PCR tests were determined by comparison of the PCR results with the children's HIV infection status. In the subgroup analysis for the perinatal HIV

transmission rate, the positive predictive value and negative predictive value of both HIV DNA PCR tests were performed using the data from only the children born to HIV-infected mothers who received ZDV prophylaxis. The exact binomial method was used to calculate the 95 per cent confidence intervals for the estimates of sensitivity, specificity, positive predictive value and negative predictive value.

RESULTS

A total of 85 children born to HIV-infected mothers were enrolled from May to October 1998. They composed 15 symptomatic HIV-infected children hospitalized at Chiang Mai University Hospital and 70 HIV-exposed infants (Fig. 1).

The result of HIV DNA PCR tests performed by the in-house method and Amplicor HIV-1 DNA method stratified by age at time of blood sampling are shown in Table 3. The overall sensitivity and specificity of the in-house HIV DNA PCR were 95.2 per cent (95% CI 76.2 to 99.9%) and 100 per cent (95% CI 94.4 to 100%), respectively. There was one false negative sample, which was collected from a 13 month old, HIV-infected child who was in clinical category B who was hospitalized with bacterial pneumonia.

The overall sensitivity and specificity of the Amplicor HIV-1 DNA test were 100 per cent (95% CI 83.9 to 100%) and 98.4 per cent (95% CI 91.6 to

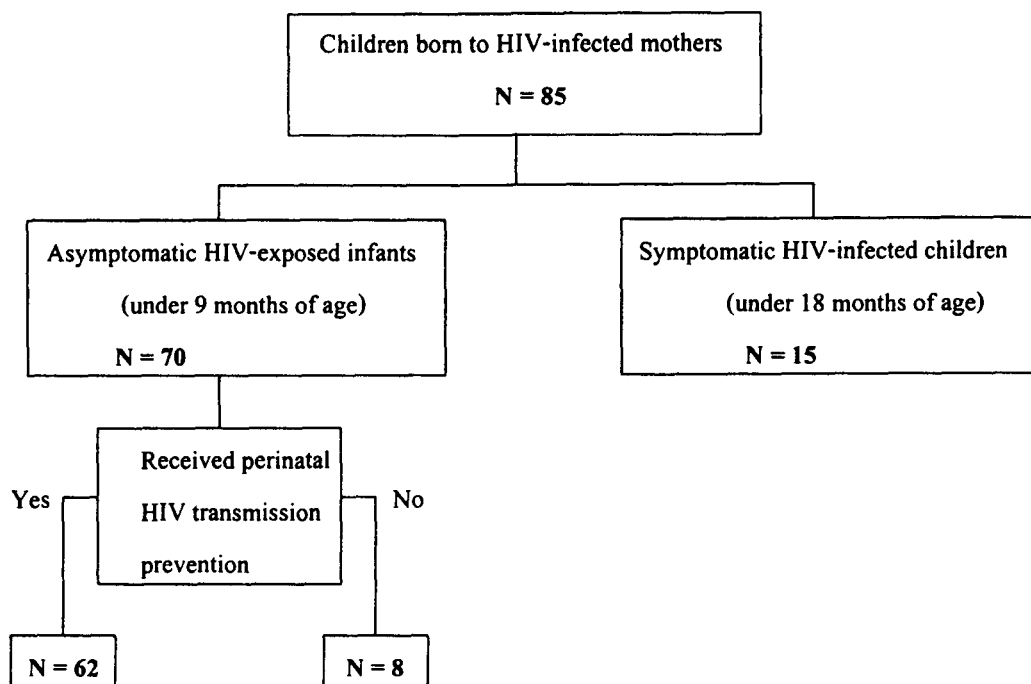


Fig. 1. Diagram of clinical information of 85 children born to HIV-infected mothers enrolled from May to October 1998.

100%), respectively. There was one false positive. The specimen was collected from a 4 month old HIV-exposed child. The Amplicor HIV-1 DNA test had an OD value of 2.21, in comparison to the 0.35 OD cut-off point for positive test.

From 70 HIV-exposed infants, only 62 (89%) infants actually received ZDV prophylaxis and were fed exclusively on an infant formula (Fig. 1). Therefore, the authors separately analyzed data from this group for the purpose of generalizing information to the HIV-exposed infants who received perinatal HIV transmission intervention, which is currently practiced in Thailand. The HIV DNA PCR tests were performed on samples from 29, 11 and 22 children at 4, 6 and 9 months of age, respectively. The perinatal HIV transmission rate in this population was 8.2 per cent (5/61, with twins counted as one), with 95 per cent CI of 2.7 to 18.1 per cent.

In-house HIV DNA PCR had 100 per cent sensitivity and specificity when compared to the results of HIV antibody tests. The Amplicor HIV-1 DNA had 100 per cent sensitivity and 98 per cent

specificity when compared to the HIV antibody test results (Table 4). The positive predictive value and negative predictive value of in-house HIV DNA PCR were 100 per cent (95% CI 54 to 100%) and 100 per cent (95% CI 94 to 100%), respectively. The positive predictive value and negative predictive value of the Amplicor HIV-1 DNA test were 86 per cent (95% CI 42 to 100%) and 100 per cent (95% CI 94 to 100%), respectively (Table 4).

DISCUSSION

In the present study, the authors evaluated an HIV DNA PCR based test for the diagnosis of HIV infection in children under 18 months of age who were born to HIV-infected mothers. The overall sensitivity and specificity of in-house HIV PCR were 95.2 per cent and 100 per cent, respectively when compared to HIV infection status. Thus, the diagnostic value of the in-house HIV DNA PCR was comparable to the commercial Amplicor HIV-1 DNA, but had a lower cost of US \$10 per test compared to US \$50 per test. The presented data, therefore, suggest that this

Table 3. Detection of HIV DNA by in-house HIV DNA PCR and Amplicor HIV-1 DNA tests.

Age	HIV-infection status*	Sample	In-house HIV DNA PCR	Amplicor HIV-1 DNA
HIV-exposed infants				
4 months	+	3	3/3	3/3
	-	29	0/29	1**/29
6 months	+	1	1/1	1/1
	-	14	0/14	0/14
9 months	+	2	2/2	2/2
	-	21	0/21	0/21
Symptomatic HIV-infected children	+	15	14***/15	15/15
Total				
	+	21	20/21	21/21
	-	64	0/64	1/64

* HIV infection status is defined by at least 2 HIV antibody tests performed during 12-18 months of age

** 1 false positive test by Amplicor HIV-1 DNA method

*** 1 false negative test by in-house HIV DNA PCR method

Table 4. Diagnostic value of in-house HIV DNA PCR and Amplicor HIV-1 DNA in 62 infants under 9 months of age who were born to HIV-infected mothers who received ZDV prophylaxis.

PCR Test	In-house PCR			Amplicor HIV-1 DNA		
	No.	%	95% CI	No.	%	95% CI
Sensitivity	6/6	100	54-100	6/6	100	54-100
Specificity	56/56	100	94-100	55/56	98	90-100
Positive predictive value*	6/6	100	54-100	6/7	86	42-100
Negative predictive value*	56/56	100	94-100	55/55	100	94-100

* The positive predictive value and negative predictive value were calculated by using the transmission rate of 8.2 in calculating the prevalence rate.

in-house HIV DNA PCR should be considered as an alternative for use in developing countries for the early diagnosis of HIV-1 infection in children.

A meta-analysis of the HIV DNA PCR method found an overall sensitivity of 94.9 per cent and specificity of 93.2 per cent in the diagnosis of HIV infection in infants⁽¹³⁾. The present findings were consistent with the study of Vongsheree, et al⁽¹⁰⁾ who reported the sensitivity and specificity of the in-house HIV DNA PCR in diagnosing HIV infection within 6 months of age, using the same primer JA17/JA20 and JA18/JA19, of 100 per cent and 94.4 per cent, respectively. In a large multicenter prospective evaluation of the Amplicor HIV-1 DNA by Bremer, et al⁽¹⁴⁾ using 1209 specimens from 483 infants from 1 to 36 months of age, the sensitivity and specificity of the test were 95 per cent and 97 per cent respectively.

There are several possibilities to explain the false-positive and false-negative test results obtained

in the present study. The single false-negative result by in-house HIV PCR may be explained by the primer sets. The primer set for *pol* gene, a more variable gene, has been reported to have a lower sensitivity compared to *gag* gene for the detection of HIV-1 by PCR in Thai patients⁽¹⁵⁾. The false-positive result using the Amplicor HIV-1 DNA test might be caused by contamination⁽¹⁴⁾.

As part of this study, the authors performed a subgroup analysis that focused on a specific population. These were children, under 9 months of age, born to HIV-infected mother who received ZDV prophylaxis and feeding with infant formula. These children represent the population targeted for implementation of the in-house HIV PCR as a diagnostic tool in Thailand. The negative predictive value of a single negative PCR test result was 100 per cent (95% CI 94-100%), and the positive predictive value of a single positive PCR test result was 100 per cent (95% CI 54-100%). Therefore, a single negative PCR result

in HIV-exposed infants who are older than 4 months might be useful to define the infection status in this clinical setting in an area of limited resources. However, using two separate tests to verify infection status for clinical purposes is still the best approach in an unlimited resource setting⁽¹⁶⁾.

The authors have recognize several limitations to the present study. Firstly, this was a pilot study to investigate the possibility of implementing an in-house HIV DNA PCR as a diagnostic tool in the medical service at the Department of Pediatrics, Chiang Mai University; therefore, the number of subjects is limited. Secondly, the PCR technique requires high quality control; therefore, the ability to generalize this diagnostic value of the test from the present study should take into account the variability in the quality control of different laboratories. As part of the efforts to improve the early diagnosis of perinatal HIV infection, further studies should be conducted 1) to evaluate the diagnostic value of in-house HIV DNA

PCR in HIV-exposed infants at 1 month of age 2) to evaluate the efficacy of in-house HIV DNA PCR by using a dried blood specimen collected on filter paper, which could serve as a powerful tool for use in hospitals in a rural setting.

In summary, the present study provides evidence that the in-house HIV DNA PCR test, which is inexpensive, has a sensitivity and specificity comparable to those of the commercial test (Amplicor HIV-1 DNA), in the early diagnosis of HIV infection in infants born to HIV-infected mothers in Thailand.

ACKNOWLEDGEMENTS

The authors wish to thank the pediatricians and nursing staff of the Pediatrics Department, Chiang Mai University Hospital for their research cooperation and we also wish to thank the mothers and their children who participated in this study. This study was supported by the Faculty Endowment Fund of the Faculty of Medicine, Chiang Mai University.

(Received for publication on February 9, 2002)

REFERENCES

1. Simonds RJ, Teeraratkul A, Kanshana S, et al. Estimating the burden on perinatal HIV infection and the effect of interventions in Thailand (Abstract 375). 2nd Conference on global strategies for the prevention of HIV transmission from mothers to infants, Montreal; 1999.
2. Shaffer N, Chuachoowong R, Mock PA, et al. Short-course zidovudine of perinatal HIV-1 transmission in Bangkok, Thailand: A randomized controlled trial. Bangkok collaborative perinatal HIV transmission study group. *Lancet* 1999; 353: 773-80.
3. Lallemand M, Jourdain G, Le Coeur S, et al. A trial of shortened zidovudine regimens to prevent mother-to-child transmission of human immunodeficiency virus type 1. Perinatal HIV prevention trial (Thailand) investigators. *N Engl J Med* 2000; 343: 982-91.
4. Centers for Disease Control and Prevention. Guidelines for the use of antiretroviral agents in pediatric HIV infection. *MMWR Morb Mortal Wkly Rep* 1998; 47: 1-43.
5. Thaineua V, Sirinirund P, Tanbanjong A, et al. From research to practice: Use of short course zidovudine to prevent mother-to-child HIV transmission in the context of routine health care in Northern Thailand. *Southeast Asian J Trop Med Public Health* 1998; 29: 429-42.
6. Kanshana S, Thewanda D, Teeraratkul A, et al. Implementing short-course zidovudine to reduce mother-infant HIV transmission in a large pilot program in Thailand. *AIDS* 2000; 14: 1617-23.
7. Evaluation and medical treatment of the HIV-exposed infant. American Academy of Pediatrics. Committee on Pediatric AIDS. *Pediatrics* 1997; 99: 909-17.
8. Benjamin DK Jr, Miller WC, Fiscus SA, et al. Rational testing of the HIV-exposed infant. *Pediatrics* 2001; 108: E3.
9. The Joint United Nations Programme on HIV/AIDS (UNAIDS). Mother-to-child transmission of HIV-UNAIDS Technical Update; 1998: 1-11.
10. Vongsheree S, Ruchusatsawat N, Saguanwongse S and Warachit P. Diagnosis of perinatal HIV-1 infection by in-house PCR. *Asian Pac J Allergy Immunol* 1997; 15: 199-204.
11. Albert J, Fenyo EM. Simple, sensitive, and specific detection of human immunodeficiency virus type 1 in clinical specimens by polymerase chain reaction with nested primers. *J Clin Microbiol* 1990; 28: 1560-4.

12. Centers for Disease Control and Prevention. 1994 revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep* 1994; 43: 1-19.
13. Owens DK, Holodniy M, McDonald TW, Scott J, Sonnad S. A meta-analytic evaluation of the polymerase chain reaction for the diagnosis of HIV infection in infants. *JAMA* 1996; 275: 1342-8.
14. Bremer JW, Lew JF, Cooper E, et al. Diagnosis of infection with human immunodeficiency virus type 1 by a DNA polymerase chain reaction assay among infants enrolled in the women and infants' transmission study. *J Pediatr* 1996; 129: 198-207.
15. Sutthent R, Foongladda S, Likanonkul S, et al. Detection of HIV-1 proviral DNA by polymerase chain reaction: A preliminary study in Bangkok. *J Med Assoc Thai* 1996; 79: 142-8.
16. Centers for Disease Control and Prevention. CDC guidelines for national human immunodeficiency virus case surveillance, including monitoring for human immunodeficiency virus infection and acquired immunodeficiency syndrome. *MMWR Morb Mortal Wkly Rep* 1999; 48: 29-31.

ประสิทธิภาพของวิธี in-house HIV DNA PCR ในการวินิจฉัยการติดเชื้อเอชไอวี ในเด็กเล็ก†

ธัญวีร์ ภูธนกิจ, พบ*,
ชัชวาลย์ อภิชาติปิยะกุล, ประด**, วิรัตน์ ศิริสันธนะ, พบ*

ผู้วิจัยนำเลือด 85 ตัวอย่างจากเด็กที่เกิดจากมารดาติดเชื้อเอชไอวีโดยเก็บตัวอย่างเลือดเมื่อเด็กอายุ 4 เดือน หรือ 6 เดือน หรือ 9 เดือน และจากเด็กที่มีอาการเจ็บป่วยที่เป็นผลจากการติดเชื้อเอชไอวีแล้ว (HIV-related illness) โดยเก็บตัวอย่างเลือดเมื่อเด็กเข้ารับการรักษาในโรงพยาบาล นำไปตรวจหา DNA ของเชื้อเอชไอวีโดยวิธี in-house nested PCR และวิธี Amplicor HIV-1 DNA ซึ่งการหาค่าความแม่นยำของการตรวจทั้งสองวิธีนั้นทำโดยเปรียบเทียบกับภาวะการติดเชื้อเอชไอวีของเด็กซึ่งวินิจฉัยจากการตรวจ HIV antibody เมื่ออายุ 12 -18 เดือน ผลการวิจัยพบว่าวิธี in-house HIV DNA PCR และ Amplicor HIV-1 DNA มีความไวของการทดสอบ ร้อยละ 95.2 และ ร้อยละ 100 ตามลำดับ และมีความจำเพาะของการทดสอบร้อยละ 100 และ ร้อยละ 98.4 ตามลำดับ ในการวิเคราะห์ข้อมูลจากตัวอย่างเลือด 62 ตัวอย่างโดยคิดเฉพาะเด็กที่เกิดจากมารดาติดเชื้อเอชไอวีและได้รับการป้องกันการถ่ายทอดเชื้อจากแม่สู่ลูกโดยการรับยา และการงดนมมารดา พบว่าวิธี in-house HIV DNA PCR มีค่าความไวของการทดสอบ ค่าความจำเพาะของการทดสอบ ค่าการทำนายโอกาสที่จะเป็นโรคเมื่อผลการทดสอบเป็นบวก และค่าการทำนายโอกาสที่จะเป็นโรคเมื่อผลการทดสอบเป็นลบ ร้อยละ 100 ผู้วิจัยเห็นว่าการตรวจหา HIV DNA โดยวิธี in-house nested PCR มีความแม่นยำเทียบได้กับวิธี Amplicor HIV-1 DNA และมีค่าใช้จ่ายต่อการทดสอบที่ถูกลง ดังนั้นจึงเห็นควรที่จะนำไปประยุกต์ใช้เพื่อการวินิจฉัยการติดเชื้อเอชไอวีในเด็กที่เกิดจากมารดาซึ่งติดเชื้อเอชไอวีต่อไป

คำสำคัญ : เอชไอวี ดีเอ็นเอ พีซีอาร์, การวินิจฉัยการติดเชื้อเอชไอวี, เด็ก, ภาวะติดเชื้อเอชไอวี

ธัญวีร์ ภูธนกิจ, ชัชวาลย์ อภิชาติปิยะกุล, วิรัตน์ ศิริสันธนะ
จดหมายเหตุทางแพทย์ ๙ 2546; 86: 758-765

* ภาควิชากุมารเวชศาสตร์,

** ภาควิชาจุลชีววิทยา, คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่, เชียงใหม่ 50200

† นำเสนอในการประชุมใหญ่งานกุมารเวชศาสตร์ครั้งที่ 50 พัทยา, ชลบุรี เมื่อ 26-28 เมษายน 2543