

## Original Article

## HIV Antigen Testing

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**Abstract :** After exposure to HIV-1, the core protein, p24, of HIV can be found in serum of an infected individual shortly after the infection on an average of 5 or 6 days before the appearance of antibodies. As virus specific antibodies are produced, they are complex with free antigens so that the level of free antigens in the circulation tends to decline or disappear during the asymptomatic period of the disease, while the levels of envelopped antibody continue to rise to a plateau over a period of several weeks, remaining stable through later phases of AIDS.

As the most serious phase of disease develops the level of anti-p24 declines. With this antibody decline, there is a reappearance of the p24 antigen, therefore the objective of the detection of p24 antigen in the early antigenemia is an aid in the diagnosis of early infection, while in the later stage is of prognostic order and therapeutic follow up.

The biological principles of HIV Ag testing is antibody sandwich ELISA, all positive samples are retested in duplicate and all repeatedly positive samples have been tested in a neutralization test. A specimen is considered positive if the reduction in absorbance of the neutralized specimen is 50% or greater when compared to the non-neutralized control.

HIV antigen screening test appeared to be somewhat effective in narrowing the HIV windows. The consideration for HIV antigen screening in each country should be upon the prevalence of HIV infection and the presence of HIV infectious window.

**Key Words :** ● HIV Ag ● p24 antigen ● Prevalence of HIV-1 antigen in blood donors

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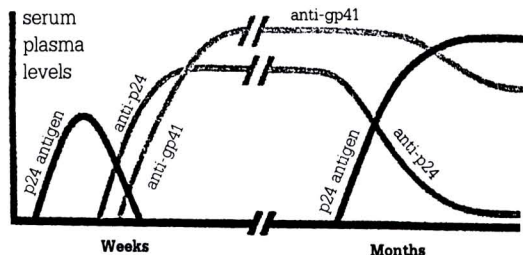
Human immunodeficiency virus type 1 (HIV-1) is recognized as the etiological agent of acquired immunodeficiency syndrome (AIDS). The virus is transmitted by sexual contact, ex-

posure to infected blood, certain body fluids or tissues, and from mother to fetus or child during the perinatal period<sup>1-6</sup>. After exposure to the virus, HIV-1 infection is characterized by an early period of antigenemia in which HIV-1 antigen is detectable in blood<sup>7-11</sup>. One of the viral antigens presenting in blood during anti-

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genemia is the core protein, p24, the major internal structural protein of HIV-1. For some infected people, this period of early antigenemia proceeds the appearance of detectable antibodies in blood (seroconversion) by days or weeks<sup>12-15</sup>. The core protein, p24, of HIV can be found in serum of an infected individual shortly after the infection on an average 5 or 6 days before the appearance of antibodies<sup>16</sup>. As virus specific antibodies are produced, they are complex with free antigens so that the level of free antigens in the circulation tends to decline or disappear during the asymptomatic period of the disease.<sup>17</sup> As the disease progresses, significant antibody levels are observed to the enveloped protein of HIV (gp 160, gp 120, gp 41). The levels of enveloped antibody continue to rise to a plateau over a period of several weeks, remaining stable through later phases of AIDS to death.

As the most serious phase of the disease develops, the level of anti-p24 declines. With this antibody decline, there is a reappearance of the p24 antigen. The accurate measurement of p24 antigen provides an important insight into the HIV infection process (Fig.1).



**Fig.1** Events associated with post-HIV exposure

**Objective :** The objective of the detection of p24 antigen in this study the early antigenemia is an aid in the diagnosis of early infection, while in the later stage is of prognostic order and therapeutic follow up.

## Material & Methods

### Biological Principles of HIV-Ag testing<sup>18</sup>

They are all immunoenzyme methods based on sandwich principle. They use monoclonal antibody on the solid phase and poly-clonal antibodies as the conjugate, the substrate reagents containing OPD or tetramethylbenzidine (TMB). The enzyme reaction is stopped by addition of acid. Color absorbance is measured using spectrophotometer.

All positive samples are retested in duplicate and all repeatedly positive samples have been tested in a neutralization test which utilizes the principle of specific antibody neutralization to confirm the presence of HIV-1 antigen. A specimen is considered positive if the reduction in absorbance of the neutralized specimen is 50% or greater when compared to the non-neutralized control.

### Interpretation of the HIV-I p24 antigen ELISA test system<sup>19</sup>

1. Specimens with absorbance values less than the cut-off value are considered non-reactive and may be considered negative for HIV-1 p24 antigen. Further testing is not required.

2. Specimens with absorbance values greater than or equal to the cut-off value are considered initially reactive and should be

tested in duplicate before final interpretation.

3. Upon retesting an initially reactive specimen, the specimen is considered, repeatedly reactive if either or both duplicate determination(s) is(are) reactive, i.e. greater than or equal to the cut-off value. Before final interpretation and for the management of donors, specimens that are repeatedly reactive should be tested using the additional, more specific HIV-1 p24 antigen neutralization kit.

4. Upon retesting an initially reactive specimen, the specimen is considered negative for HIV-1 p24 antigen if both duplicate determinations are non-reactive, i.e. less than the cut-off value further testing is not required.

5. Specimen that are repeatedly reactive in HIV-1 p24 antigen ELISA test-system which are neutralized by HIV-1 p24 antigen neutralization kit are considered positive for HIV-1 p24 antigen.

6. Specimens that are repeatedly reactive in HIV-1 p24 antigen ELISA test system but do not have a valid neutralization test, i.e. specimens that are incubated with the negative neutralized control reagent that produce an absorbance less than the assay cut-off value, are considered indeterminate. Indeterminate

results should be followed up by repeat testing of the original specimen and testing a fresh specimen obtained at least 8 weeks later.

7. Specimens that are repeatedly reactive in HIV-1 p24 antigen ELISA test system, but are non-neutralizing are considered negative for the neutralization test and indetermination for HIV-1 p24 antigen. The interpretation of results of specimens found to be repeatedly reactive in the HIV-1 p24 antigen ELISA test system and negative in the additional, more specific neutralization assay is unclear. The majority of such specimens do not contain HIV-1 p24 antigen, however, false negative neutralization tests can occur with some, specimens that do contain HIV-1 p24 antigen. Further clarification may be obtained by testing a fresh specimen for HIV-1 p24 antigen. Reactivity associated with seroconversion and the HIV-1 "window period" may be resolved by obtaining a fresh specimen after 8 weeks and testing for HIV-1 antigen and antibody

### Results (Table 1)

200 fresh specimens obtaining from blood donors who had the indeterminate of HIV-1 p24 antigen were tested for anti-HIV, 12 speci-

**Table 1** Prevalence of HIV-1 antigen in thai blood donors at NBC, TRCS

| Year         | No.of test       | IR           | RR         | No.<br>Neutralized | Follow up indeterminate |            | can not<br>contact |
|--------------|------------------|--------------|------------|--------------------|-------------------------|------------|--------------------|
|              |                  |              |            |                    | Anti-HIV+               | Anti-HIV-  |                    |
| 1993         | 274,099          | 732          | 74         | 36                 | 2                       | 30         | 6                  |
| 1994         | 273,431          | 533          | 45         | 21                 | 1                       | 16         | 7                  |
| 1995         | 299,454          | 521          | 68         | 20                 | 4                       | 33         | 11                 |
| 1996         | 335,196          | 471          | 96         | 16                 | 1                       | 60         | 19                 |
| 1997         | 368,553          | 817          | 90         | 13                 | 4                       | 49         | 24                 |
| <b>Total</b> | <b>1,550,733</b> | <b>3,074</b> | <b>373</b> | <b>106</b>         | <b>12</b>               | <b>188</b> | <b>67</b>          |

mens were positive<sup>20</sup>, therefore blood that are repeatedly reactive for HIV-1 p24 antigen must not transfused in order to reduce the risk of HIV-infection through blood transfusion.

### Discussion and Recommendations

1. Currently, blood donors are screened with tests that detect HIV antibodies, which typically appear within three months after infection and indicate the body is responding to the infection. In contrast, antigens, which are the virus' own proteins, can be detected about one week earlier than antibodies.

2. Principles of HIV Ag testing is ELISA based on sandwich principle. All positive sample should have been tested in a neutralization test to confirm the presence of HIV Ag.

3. Specimen that are repeatedly reactive in HIV-1 p24 antigen ELISA test system must be tested using the additional neutralization testing.

4. False negative results may occur if free antigen levels are below the lower limit of detection of the assay. This may occur during the earliest stage of infection before antigen reaches detectable levels.

5. False positive results may occur due to nonspecific binding to assay materials and not from infection with HIV-1.

6. The antigen test to screen blood donations is not recommended to replace the HIV antibody test for routine testing. Antibody testing is still the most efficient way to routine diagnose of HIV infection.

7. The HIV-antigen test should increase public confidence in the safety of blood supply.

8. By reducing the so-called "window period" when donors may be HIV-infected but still have negative antibody test, it has been estimated that HIV-1 antigen screening could prevent approximately 25% of current cases of AIDS transmitted by transfusion. HIV-screening test appeared to be somewhat effective in narrowing the HIV windows.

9 In order to provide safe blood to patients, testing for antibodies to HIV-1 and HIV-2 will be performed in addition to other recommended safeguards to reduce the chance of HIV-contaminated blood entering the blood supply. Other safeguards include the questioning of potential donors about risk behaviors.

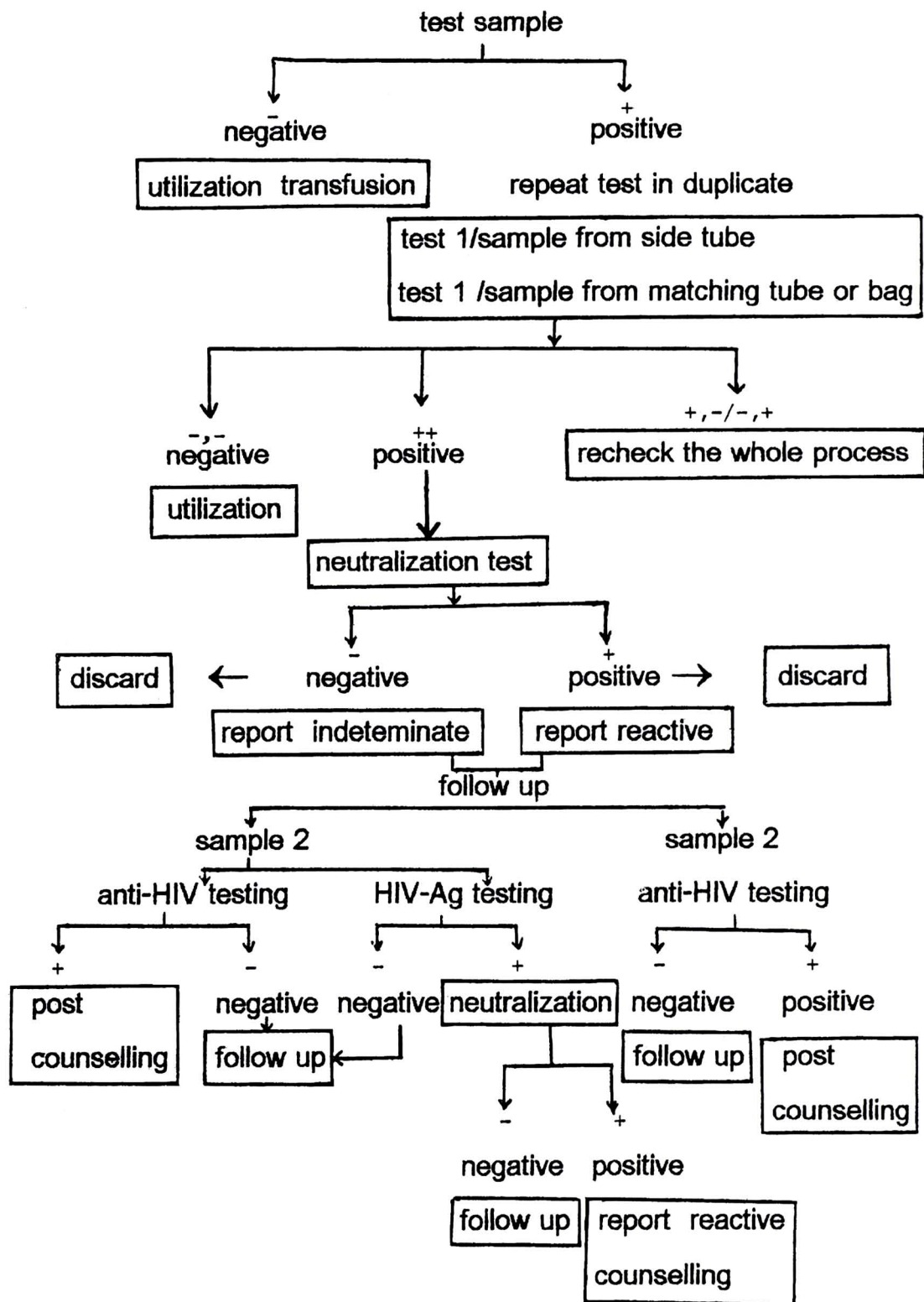
10. It has been a debating issue whether HIV antigen screening is cost-effective. The consideration should be upon the prevalence of HIV infection and the presence of HIV infectious window in each country. HIV Ag testing is recommended in countries or regions where the risk rate (HIV Ag positive with anti-HIV negative) is high.

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## Algorithm of HIV antigen testing



## การตรวจ HIV antigen

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ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย

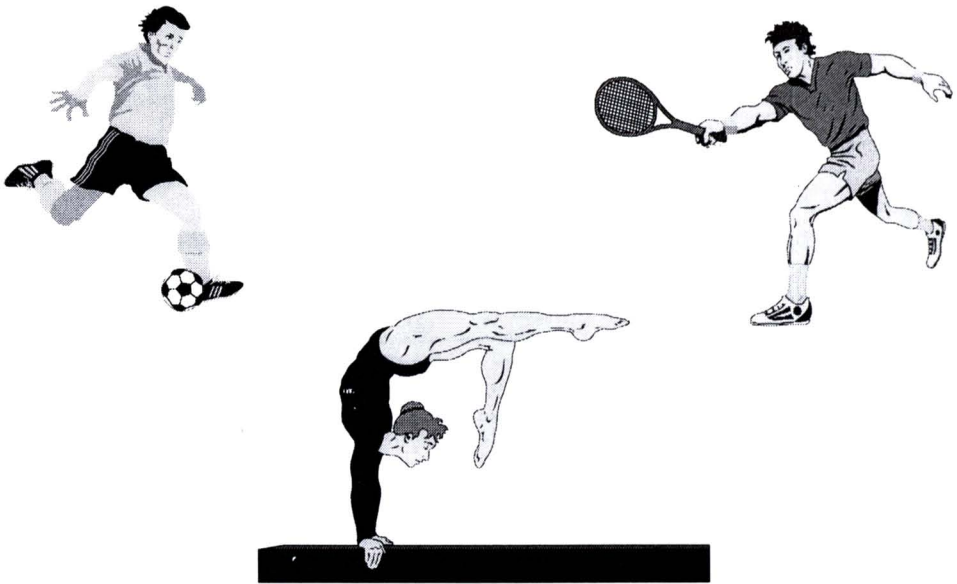
**บทคัดย่อ :** หลังจากได้รับเชื้อ HIV-1 ในระยะแรก จะตรวจพบ HIV antigen ในกระแสโลหิตได้ ทั้งนี้ส่วน core protein p24 antigen จะตรวจพบโดยวิธี ELISA ประมาณ 5-6 วัน ก่อนตรวจพบแอนติบอดี ผู้ติดเชื้อจะสร้างแอนติบอดีต่อส่วน envelop ซึ่งจะตรวจพบได้ตลอดไป แอนติบอดีที่เกิดขึ้นจะไปจับกับ free antigen ทำให้ free antigen p24 ลดลงหรือตรวจไม่พบ จนถึงระยะสุดท้ายเมื่อเชื้อไวรัสเพิ่มมากขึ้น แอนติบอดีจะลดลง และจะตรวจพบ p24 Ag ได้อีก ฉะนั้นจุดประสงค์ของการตรวจ p24 Ag ในระยะแรก เพื่อช่วยในการวินิจฉัยการติดเชื้อ HIV-1 ส่วนการตรวจ p24 Ag ในระยะหลัง เพื่อใช้พยากรณ์โรคและการรักษา

ในการตรวจ HIV Ag ใช้หลักการ antibody sandwich ELISA ซึ่งต้องยืนยันผลการตรวจด้วยวิธี neutralization การแปลผลเป็นบวก เมื่อ neutralized specimen มีค่า OD ลดลง 50% หรือมากกว่าเมื่อเปรียบเทียบกับ non-neutralized specimen

การตรวจ HIV Ag ได้รับการยืนยันว่าเป็นการลด window period ทำให้เลือดที่ใช้มีความปลอดภัยสูงขึ้น อย่างไรก็ตามก็ตีการพิจารณาความคุ้มค่าของการตรวจ HIV Ag ขึ้นอยู่กับอุบัติการณ์ของการพบเชื้อ HIV-1 และ HIV infectious window ของแต่ละท้องถิ่น

**Key Words :** ● HIV Ag ● p24 antigen ● Prevalence of HIV-1 antigen in blood donors

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2541;8:223-9.



แข่งเอ้ย แข่งขัน  
แข่งกันบริจาคโลหิต  
หมั่นทำบุญเป็นนิตย์  
สร้างกิจทุก ๓ เดือน

สร้อยสอางค์ พิกุลสด