HIV-1 p24 antigen testing in blood banks: results from Saudi Arabia

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ABSTRACT

HIV-1 p24 antigen testing was introduced to increase sensitivity in the early detection of HIV infection in blood donors. Since the introduction of HIV-1 p24 antigen testing in Saudi Arabia, we have failed to detect a single positive case. Over a three-year period, only four indeterminates were detected out of 24,654 blood donors. All four proved negative by confirmatory testing. Based on this experience, we believe that resources would be better directed to pooled nucleic acid testing and that p24 serological testing should be abandoned.

KEY WORDS: Blood banks. HIV core protein p24. Nucleic acids.

Introduction

Both the US Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) have issued guidelines on screening and confirmatory testing of human immunodeficiency virus (HIV)-1 p24 antigen in blood donations.[1,2] The sequence of testing involves enzyme immunoassay followed by a neutralisation assay, and results are interpreted as HIV-1 p24 antigen-positive, -negative or -indeterminate.

It is estimated that the cost of p24 antigen testing in the USA is $75-100 million per annum, and only prevents about eight HIV infections per year.[3] Addition of the polymerase chain reaction (PCR) could prevent a further 16 cases at an additional cost of $96 million per annum.[4]

In Saudi Arabia, 414 HIV cases have been reported up to 1999,[5] with the majority seen in patients over 15 years of age. However, the World Health Organization (WHO) estimate is 1100 symptomatic and asymptomatic cases. Annual incidence, based on the reported cases in the last five years, varies between 0.1-0.4 cases per 100,000 population,[5] which suggests that HIV infection is rare in Saudi Arabia.

Since the introduction of HIV p24 antigen testing in US blood banks in 1996,[6] many hospital blood banks in Saudi Arabia, including that at King Fahad National Guard Hospital, have introduced this test. Here, we present the data on p24 antigen testing of blood donors in Riyadh, Saudi Arabia.

Materials and methods

Donor selection

Blood bank donors at King Fahad National Guard Hospital undergo strict selection criteria in accordance with the American Association of Blood Banks (AABB) and College of American Pathologists (CAP) standards. Donors complete a questionnaire and are interviewed prior to donation.

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Routinely, haemoglobin level is tested to ensure it meets the established US FDA standard. The purpose of the questionnaire and the brief physical examination is to identify potential donor health problems or the presence of infection that could be transmitted to the recipient of the transfused unit.

In this study, 24,654 blood bank donors were analysed for the presence of HIV p24 antigen.

p24 antigen assay

A sandwich solid-phase enzyme immunoassay (Abbott Laboratories, Chicago, IL, USA) was used to detect HIV-1 p24 antigen according to the manufacturer's instructions.

Briefly, HIV-1 virions, when present in the test sample, were disrupted by the addition of specimen diluent containing Triton X-100. Beads coated with monoclonal antibody to HIV-1 p24 were incubated with serum samples and rabbit antibody to HIV-1. Horseradish peroxidase (HRPO)-conjugated goat antibody to rabbit IgG was then incubated with the beads. O-phenylenediamine (OPD) substrate solution containing hydrogen peroxide was used as the chromogen.

Intensity of the colour formed was proportional to the amount of uncomplexed HIV-1 p24 antigen in the sample. Specimens with absorbance equal to or greater than the cut-off value were considered to be reactive (positive). Specimens that gave a reading within 20% below the cut-off value were considered to be indeterminate.

Repeat reactive specimens (including indeterminate samples) were further tested using HIVAG-1 monoclonal blocking antibody (Abbott Laboratories). This detects uncomplexed HIV-1 p24 antigen in human serum or plasma by means of specific antibody neutralisation, and is based on an enzyme immunoassay (EIA), using the HIV-1 blocking antibody. Briefly, the assay involved room temperature neutralisation followed by the HIV-1 p24 antigen procedure.

Table 1. Results of HIV-1 p24 antigen testing in blood bank donors, 1999-2001

Year	Total tested	HIV-1 p24 antigen results		
		Negative	Positive	Indeterminate*
1999	7,319	7,318	0	1
2000	8,020	8,017	0	3
2001	9,315	9,315	0	0

*All indeterminate results by EIA were negative by the confirmatory test.

Results

Results from 1999-2001 failed to show a single confirmed HIV-1 p24 antigen-positive blood sample among donors at King Fahad National Guard Hospital (Table 1). Nearly 25,000 blood donors were tested over the three-year period, and only four samples gave borderline (indeterminate) results. These were re-tested with the specific neutralising assay and all four samples were confirmed as negative.

Discussion

The FDA implemented HIV-1 p24 antigen testing for blood donations in 1996. In the first year of testing, only one case was identified as p24 antigen-positive and HIV antibody-negative out of some 12 million volunteer blood donors.[7,8] This was attributed to the declining incidence of HIV and self-deferral of donors at recent risk of HIV infection.[9] Since HIV-1 p24 antigen testing of blood donations was adopted at King Fahad National Guard Hospital, not a single confirmed p24 antigen-positive blood unit has been identified.

False-positive p24 results from blood donors have also been documented. Of 6.75 million donations tested by the American Red Cross, 44 false-positives detected by neutralisation assay were documented in the first year of testing. These results were confirmed by RNA PCR and the absence of seroconversion and risk factors on follow-up.[7] Additionally, 1520 p24 antigen-positive, neutralisation assay-negative cases were identified, of which, 1216 were tested by PCR and found to be negative.[8] Busch and Stramer[3] suggested that the term indeterminate be changed to antigen confirmatory test-negative.

Recently, nucleic acid testing (NAT) was proposed as an alternative to p24 testing. This is a very sensitive assay that can detect HIV RNA levels down to 50-100 copies/mL. In addition, HIV RNA can be detected five days earlier than p24, and some 11-13 days prior to antibody seroconversion.[10]

Regulatory agencies in Europe and the US have promoted and mandated the screening of mini-pools of plasma. Giachetti *et al*.[11] described an NAT assay for the simultaneous detection of hepatitis C virus (HCV) and HIV, which has been approved by the FDA for testing blood donors. By August 2000, nearly 11 million donors had been tested using this assay at different donor centres in the USA, and three HIV-1 NAT-positive, p24 antigen-negative, seronegative donations were identified.[11]

Interestingly, the first transfusion-related transmission of HIV by RNA-screened blood was described recently, and the report concluded that even NAT testing in the early stages of HIV infection might not detect the virus and that even at this low level of viremia (pre-seroconversion) the blood might be infectious.[12] Nonetheless, it is estimated that NAT testing would reduce the risk of HIV transmission through blood donation.

In 2001, a report from Saudi Arabia suggested that serological testing for p24 is the way forward screening blood donations, as it has not produced a single positive out of 400 donors tested by NAT.[13] However, as NAT is more sensitive than serological testing, we suggest that it should replace the traditional EIA technique for HIV-1 p24 testing in blood banks in Saudi Arabia and in other countries where HIV is a low-risk infection. □

References

1 Food and Drug Administration, Center for Biologics Evaluation and Research: Memo to All Registered Blood and Plasma Establishments. Recommendations for Donor Screening with a Licensed test for HIV-1 Antigen. August 8, 1995.

2 Food and Drug Administration, Center for Biologics Evaluation and Research: Memo to All Registered Blood and Plasma Establishments. Additional Recommendations for Donor Screening with a Licensed test for HIV-1 Antigen. March 14, 1996.

3 Busch MP, Stramer SL. The efficiency of HIV-1 p24 antigen screening of US blood donors: projections versus reality. *Transfus Med Hemotherapy* 1998, **25**: 194-7.

4 AuBuchon JP, Birkmeyer JD, Busch MP. Cost-effectiveness of expanded HIV test protocols for donated blood. *Transfusion* 1997; **37**: 45-51.

5 UNAIDS. Epidemiological Fact Sheets on HIV/AIDS and sexually transmitted infections. 2000.

6 US Public Health Service guidelines for testing and counseling blood and plasma donors for human immunodeficiency virus type 1 antigen. MMWR Recomm Rep 45(RR-20), 3-1-1996: 1-9.

7 Stramer SL, Aberle-Grasse J, Brodsky JP, Busch MP, Lackritz EM. US blood donor screening with p24 antigen (Ag): one year experience. (Abstract) American Association of Blood Banks, 50th Annual Meeting, Denver, Oct 1997.

8 Kleinman SH, Busch MP, Stramer SL. First year experience with HIV-1 p24 antigen (Ag) testing: Ag indeterminate donors are not infected with HIV. (Abstract) American Society for Hematology, 39th Annual Meeting, San Diego, Dec 1997.

9 Schrieber G, Glynn S, Busch M, Kleinman S. for the Retrovirus Epidemiology Donor Study. Delayed donations in repeat blood donors who HIV seroconvert may explain low p24 antigen yield. (Abstract) American Association of Blood Banks, 50th Annual Meeting, Denver, Oct 1997.

10 Le Pont F, Costagliola D, Rouzioux C, Valleron AJ. How much would the safety of blood transfusion be improved by including p24 in the battery of tests? *Transfusion* 1995; **35**: 542-7.

11 Giachetti C, Linnen JM, Kolk DP *et al*. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. *J Clin Microbiol* 2002; **40**: 2408-19.

12 Delwart E, Kalmin N, Jones S, Ladd D, Tobler L, Busch M. First case of HIV transmission by an RNA-screened blood donation. (Abstract) 9th Conference on Retroviruses and Opportunistic infections, 2002.

13 Akhter J, Roberts GT, Perry A, Gaucher JS, Howman PA. Use of nucleic acid testing for blood donor screening of human immunodeficiency virus and hepatitis C virus in the Saudi population. *Saudi Med J* 2001; **22**: 1073-5.