EDITORIAL BOARD

EDITOR IN CHIEF
Professor G I Muguti

ASSOCIATE EDITORS
Professor IT Gangaidzo
Dr S P Munjanja

EDITORIAL BOARD MEMBERS

Professor MM Chidzonga	*(Zimbabwe)*
Professor P Jacobs	*(South Africa)*
Dr R A Kambarami	*(Zimbabwe)*
Professor A S Latif	*(Zimbabwe)*
Professor P R Mason	*(Zimbabwe)*
Professor CT Musabayane	*(Zimbabwe)*
Professor KJ Nathoo	*(Zimbabwe)*
Mr L Nystrom	*(Sweden)*
Dr S Siziya	*(Zambia)*

PAST EDITORS
Professor M Gelfand (1953-1985)
Professor H M Chinyanga (1985-1990)
Professor J A Matenga (1991-1999)

ADMINISTRATIVE AND OFFICE STAFF
Director of Publications: Mr Munani S Mtetwa
Administrative Manager: Mr Christopher Mashavira
Technical Editor: Mrs Ling M Cooper
Statistical Advisor: Mr S Rusakaniko
Secretary: Mrs Patricia Bhunhu

All manuscripts will be prepared with the International Committee of Medical Journal Editors - Uniform requirements for manuscripts submitted to Biomedical Journals, 1993.

Manuscripts submitted for publication are accepted on the understanding that they are contributed exclusively to *The Central African Journal of Medicine*. A statement to that effect should be included in the letter accompanying the manuscript.

Communications concerning editorial matter, advertising, subscriptions, change of address, etc. Should be addressed to the Administrative Manager, P. O. Box A195 Avondale, Harare, Zimbabwe.

The subscription rate for **surface transmission** including postage for year 2002 is Z$3 100.00 locally; Africa US$210.00 for individuals and US$265.00 for institutions; and US$260.00 for individuals and US$280.00 for institutions for the rest of the world per annum. The subscription rate for **airmail transmission** for year 2002 in Africa is US$300.00 for individuals US$320.00 for institutions and US$70.00 for postage; and US$330.00 for individuals US$350.00 for institutions and US$70.00 for postage for the rest of the world per annum.

Owned and published by the *Central African Journal of Medicine* in conjuction with the Faculty of Medicine

University of Zimbabwe

Printed by **Mazongororo Paper Converters (Pvt.) Ltd.**

Harare

A cost-effective particle agglutination assay to detect viral antibodies in dried blood spots — a simple solution to HIV and HCV screening

*J J JOURBERT, **J B DEWAR, ***J WEINBERG, **M DE BEER, ****J S PARKER, **A D STEELE

Abstract

Objectives: To conduct a serological survey of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in Gabon and Ga-Rankuwa, South Africa. A secondary objective was to test a novel, simple, inexpensive agglutination assay for anti-HIV IgG and anti-HCV IgG from blood samples stored as spots dried onto filter paper.

Design: Blood from heel pricks was dried onto filter paper and stored. Blood was eluted from the spots and serum antibody was then assayed using a modified agglutination assay — blood was added to gelatin agglutination beads that had been sensitised with viral antigen. A positive result showed as an agglutination pattern while a negative result appeared as a tight bead.

Subject: This was a hospital-based study involving 271 neonates at Ga-Rankuwa Hospital, South Africa, and 856 patients ranging in age from three months to over 50 years who attended clinics in Gabon.

Results: Seroprevalence to HIV was determined in Ga-Rankuwa to be just under 14% (13.8%). Antibodies to HCV were not detected. In Gabon, the prevalence to HIV was just under 1% (0.82%) with a relatively high incidence of HCV, nearing 4% (3.79%).

Conclusion: The sensitivity of the agglutination assay compared favourably to enzyme immune assay (EIA) with respect to sensitivity, simplicity and cost. This assay may be useful in sero-epidemilogical assays in developing countries.

Cent Afr J Med 2003;49(11/12):127-30

Introduction

During the 1980s epidemiological data available on HIV indicated a low HIV seroprevalence in South Africa compared to the rest of Africa.[1] In support of this, a study indicated a negative HIV incidence among adult Venda men and women diagnosed with sexually transmitted diseases.[2] This correlated, at the time, with a negative incidence of HIV in the Kwa-Zulu region of South Africa.[3] Other studies compared the incidence of HIV in Kwa-Zulu in 1985 and again in 1991. These authors[4] reported an extremely low HIV statistical incidence (12/100 000) in 1985, but by 1991 the incidence of HIV in Kwa-Zulu had increased almost 200-fold, to 2 200/100 000. Nine years later, the situation was alarmingly different, with the South African Department of Health assessing that about 10% of the South African population was HIV positive.[5]

A rapid increase in HIV seroprevalence prompts the need for a simple, rapid and effective method of screening blood samples for epidemiological survey. A valid assessment of HIV seroprevalence is aided by unlinked anonymous screening — informed consent and councelling is not necessary — making use of simple but accurate assays. Trends in HIV seroprevalence can be determined by repeat surveys.[6]

One such method for HIV seroprevalence testing makes use of a simple method for obtaining blood samples from heel pricks. The blood is spotted onto filter paper and

*Department of Medical Virology
University of Stellenbosch Medical School
Tygerberg
Cape Town
South Africa
**Department of Virology and MRC Diarrhoeal Phathogens Research Unit
Medical University of South Africa
P O Box 173
Medunsa 0204
South Africa
Tel: 27 12 512 4117
Fax: 27 12 512 3535
***AMS Ahwll Gabon
Expat Mail
Shell Centre
London SE1 7NA
United Kingdom
****Virology Department
Camelia Botnar Laboratories
Great Ormond Street Hospital for Sick Children
London WC1N 3JH
United Kingdom

Correspondencie to:
Dr JB Dewar

stored as dried blood spots (DBS). An advantage of this is that the DBS can be simply stored or placed in an envelope prior to posting before being eluted to assay for HIV nucleic acids or antibodies.[7] Blood that has been eluted from the DBS can be assayed by the polymerase chain reaction (PCR) or EIA to determine HIV seroprevalence. Anonymous testing of DBS eluates with methods such as PCR and ELISA has been used to determine HIV prevalence in Asia,[8] in Canada,[9] in Spain,[10] in Sierra Leone[11] and in Zambia.[12] This method has also been used to diagnose malaria in Singapore[13] and Malaysia,[14] trypanosomosis in Zambia,[15] cysticercosis in Bolivia[16] and toxoplasmosis in Denmark and Massachusetts.[17,18]

A simple agglutination assay, involving the addition of blood eluted from DBS to agglutination beads sensitised with HIV antigen, has been shown to be a sensitive and specific alternative to PCR and ELISA in assays to determine HIV seroprevalence. In this way, unlinked anonymous testing of DBS using this modified agglutination assay has been achieved in determining HIV seroprevalence in three Thames regions[19,20] and in Edinburgh, Scotland.[21] Agglutination beads sensitised with HCV antigens have also been used to test for HCV seroprevalence in Parkistan.[22]

This article describes the use of a simple, inexpensive agglutination assay to detect anti-HIV IgG and anti-HCV IgG in blood samples that are efficiently obtained and stored as spots dried onto filter paper.

Materials and Methods

Dried blood spots were obtained from 271 neonates attending Ga-Rankuwa Hospital, Gauteng, South Africa and from 856 Gabonese patients from the age of three months to over 50 years.

The elution and testing of sera from DBS was performed as described.[19] Blood samples were routinely taken by heel prick from newborn babies, or from finger pricks from adults, and spotted onto Whatman™ 3MM filter paper. A 5,5mm punch of DBS was eluted in flat-bottomed 96 well master plates into 100 ml elution buffer (PBS) pH 7.2, 0.05% Tween 20, 0.05% sodium azide — PBS-Tw). The plates were shaken slowly for 30 minutes at room temperature and then stored overnight at 4°C. The next morning the plates were shaken fast for three minutes. Note that only 11 of the 12 rows of the plate were used to test samples and the 12th row was used to titrate the positive control. The eluates from the DBS were diluted one in five in PBS-Tw and then 30 ml of each was transferred to each V-shaped well of a test microtitre plate. This means that each plate comprised 88 test sera and eight dilutions of the positive control serum. The positive control consisted of HIV posititve serum in freshly drawn HIV negative whole blood. This was spotted onto filter paper and allowed to dry at room temperature for 48 hours. A control DBS was eluted into the first well in a master plate and then a 100 ml serial doubling dilution of this was prepared using PBS-Tw. The dilutions were then transferred to the test plate.

To the sera were added 25 ml of a 10 dilution of gelatine particles (Fujirebio™) sensitised with HIV-1 (or HCV) antigens. The plate was then covered, shaken at 260g for two minutes, left at room temperature for 15 minutes and then placed on a light box at an angle of 70°. The results were read after 10 minutes. A negative result showed as a tight bead while a positive result showed as a discrete agglutination pattern. Reactive eluates were re-tested. If again reactive they titrated across the endpoint and then aspirated from the plate for storage at –20°C.

Results

The DBS samples were tested for anti-HIV IgG and anti-HCV IgG as described in the methods section. Within the Ga-Rankuwa neonates, seroprevalence to HIV was determined to be 36/271 = 14%. The seroprevalence to anti-HCV IgG was similarly performed and was not detectable.

The DBS from Gabon were tested as to HIV and HCV seroprevalence using this modified agglutination assay. Overall, anti-HIV IgG was demonstrated in 7/856 (under 1%) of the cohort while anti-HCV IgG was determined in 25/856 (over 3% of the patients). The seven HIV positives were confirmed with enzyme immunoassay (EIA) as were the 25 samples that tested positive for HCV.

Discussion

The incidence of HIV in South Africa contrasts to HIV seroprevalence in the West African country of Gabon. Between 1986 and 1994, the seroprevalence to HIV in the general population in Gabon was reported as low and remained stable at just above 2%.[23] A more recent report, published in 1998, confirmed this in the general population of Gabon.[24] In contrast to those figures, use of DBS and the agglutination assay in the current study indicated a reduction in the seropositivity to HIV in Gabon — with an overall incidence of less than 1%. These contradictory findings may be ascribed to the fact that the figures published in 1996 and 1998 reflected the seroprevalence to HIV-1 amongst the sexually active in the general Gabon population. The present study involved testing blood samples from the general population, including neonates, as well as individuals over the age of 50 years. Although relatively low, published figures cautioned as to a doubling in the seroprevalence to HIV-1 within eight years in Gabon.[24]

The current study indicates a low seroprevalence to HCV in South African neonates. This correlates with published data[25,26] that reported an HCV prevalence of 1.8% and 0.64% in black South Africans, respectively. While the seroprevalence to HCV as reported by Schoub *et al.*[25] was low in males (at nearly 3%) this figure was significantly higher than the seroprevalence in females (at under 1%).

In comparison to the HCV seroprevalence in South Africa, the current study shows a relatively high HCV seroprevalence in Gabon (at over 3% of the general population). This indicated that in Gabon, more than three times as many females are infected with HCV (over 4% of total) compared to males (over 1% total) tested. This contrasts with results obtained to 1991 where an ELISA was used to determine the prevalence rate of HCV in Gabon. In this study the HCV seroprevalence was determined in blood donors (mainly men) and pregnant women to be over 2% and under 1% respectively.[27] While these figures indicate almost a doubling in the incidence of HCV in Gabon in the eight years to 1998, it shows almost a sixfold increase in the prevalence of HCV in Gabonese women in this time period.

In Lahore, Pakistan, another developing country, the above described agglutination assay of DBS-eluted blood was compared to EIA as well as a third generation RIBA test as to their detection of antibodies to HCV in DBS samples. These results indicate that 6,7% of 417 mothers and 1.3% of their 538 children were HCV seropositive.[22] These authors indicate that the use of the modified agglutination assay is comparable in sensitivity to the EIA and RIBA test but that the antigens used in the agglutination assay should be appropriate to the viral strain endemic in any particular country. Pakistan has a high incidence of HCV compared to other countries and this may be ascribed to increased risk of transmission due to therapeutic use of contaminated needles and the practice of transfusion with unscreened blood.

Conclusion

The DBS/agglutination method is very simple and cost-effective, at approximately 10% of the cost of a standard indirect ELISA test. Results using this technique have been confirmed by repeat testing of blood samples using EIA and RIBA. The RIBA test is an example of an expensive technique requiring well-trained staff and an appropriately appointed modern laboratory. Results using the DBS/agglutination assay can be obtained under much simpler conditions, reinforcing the suitability of such technology in developing countries and rural areas. As more vaccines are developed for use in developing countries, there is an ongoing need to develop sensitive assays that allow for rapid, appropriate testing of both antibodies and antigens. The assay described here is not a replacement for EIA or PCR diagnosis, but, as it combines low cost, simplicity, accuracy and sensitivity, it is appropriate in determining sero-epidemiological trends.

References

1. Schoub B, Smith A, Lynos S. Epidemiological considerations of the present status and future growth of the acquired immune deficiency sydrome in South Africa. *S A Med J* 1988;74:153-7.
2. Taylor M, Swanevelder C, Carlier N ,Coetzer P. HIV in Venda. *S A Med J* 1989;75(7):348-9.
3. Hainsworth M, van Heyningen C. HIV surveys in Kwa-Zulu. *S A Med J* 1988;73:639.
4. Karim S, Karim Q. Changes in HIV seroprevalence in a rural black community in Kwa-Zulu. *S A Med J* 1992;82:484.
5. Department of Health Summary Report. National HIV seropositivity surveys of women attending antenatal clinics in South Africa, 199. Pretoria: DOH, 2000.
6. Miotti P, Chiphangwi J, Dallabetta G. The situation in Africa: *Baillier's Clin Obstets Gynaecol* 1992;6(1):165-186.
7. Smit E, de Beer M, Steele A. The use of dried blood spots and a modified particle agglutination test for cost-effective seroepidemiological HIV surveys. *SA J HIV Med* 2001;4:31-3.
8. Cassol S, Weniger B, Babu P, Salminen M, Zheng X, Htoon M, Delaney A, *et al.* Detection of HIV type 1 env subtypes A, B,C, and E in Asia using dried blood spots: a new surveillance tool for molecular epidemiology. *AIDS Res Hum Retrov* 1996;12(15):1435-41.
9. Cassol S, Butcher A, Kinard S, Spadoro J, Sy T, Lapointe N, *et al.* Rapid screening for early detection of mother-to child transmission of human immunodeficiency virus type 1. *J Clin Micro* 1994;32(11): 2641-45.
10. Canosa C, Grady G, Cabelolo M, Comeau A, Schwerzler M, Contreras M, *et al.* Anonymous testing of newborn infants for HIV antibodies as a basis for estimating prevalence of HIV in childbearing women: the 1991-1994 study in Spain. *Acta Paed Suppl* 1997;421:67-71.
11. Boillot F, Peeters M, Kosia A, Delaporte E. Prevalence of the human immunodeficiency virus among patients with tuberculosis in Sierra Leone, established from dried blood spots on filter paper. *Int J Tuber Lung Dis* 1997;1(6):493-7.
12. Smyth A, tong C, Carty H, Hart C. Impact of HIV on mortality from acute lower respiratory tract infection in rural Zambia. *Arch Dis Child* 1997;77(3):227-30.
13. Tham J, Lee S, Tan T, Ting R, Kara U. Detection and species determination of malaria parasites by PCR: comparison with microscopy and with ParaSight-F and ICT malaria Pf tests in a clinical environment (199). *J Clin Micro* 1999;37(5):1269-73.
14. Cox-Singh J, Mahayet S, Abdullah M,Singh B. Increased sensitivity of malaria detection by nested polymerase chain reaction using simple sampling and DNA extraction. *Int J Parasit* 1997;27(12):1575-77.
15. Hopkins J, Chitambo H, Machila N, Luckins A, Rae P, van den Bossche P, *et al.* Adaption and validation of antibody-ELISA using dried blood spots on filter paper for epidemiological surveys of tsetse-

transmitted trypanosomosis in cattle. *Prev Vet Med* 1998;37(1-4):91-9.

16. Jafri H, Torrico F, Noh J, Bryan R, Balderrama F, Pilcher J, Application of the enzyme-linked immunoelectrotransfer blot to filter paper blood spots to estimate seroprecalence of cysticercosis in Bolivia. *Am J Trop Med Hyg* 1998;58(30):313-15.
17. Eaton R, Petersen E, Seppanen H, Tuuminen T. Multicenter evaluation of a fluorometric enzyme immunocapture assay to detect toxoplasma-specific immunoglobulin M in dried blood filter paper specimens from newborns. *J Clin Micro* 1996;34(12):3147-50.
18. Lebech M, Peterson E. Detection by enzyme immunosorbent assay of *Toxoplasma gondii* IgG antibodies in dried blood spots on PKU-filter paper from newborns. *Scand J Infect Dis* 1995;27(3):259-63.
19. Peckham C, Tedder R, Briggs M, Ades A, Hjelm M, Wilcox A, *et al.* Prevalence of maternal HIV infection base on unlinked testing of newborn babies. *Lancet* 1990;335:516-19.
20. Ades A, Parker S, Berry T, Holland F, Davison C, Cubitt D, *et al.* Prevalence of maternal HIV-1 infection in three Thames regions: results from anonymous unlinked neonatal testing. *Lancet* 1990; 337:1562-65.
21. Johnstone F, Goldberg D, Tappin d, Mathie L, Cameron S, Brown A, *et al.* The incidence and prevalence of HIV infection among childbearing women living in Edinburgh city, 1982-1995. *AIDS* 1998;512(8):911-18.
22. Parker S, Khan H, Cubitt D. Detection of antibodies to hepatitis C virus in dried blood spot samples from mothers and their offspring in Lahore, Pakistan. *J Clin Micro* 1999;37(6)2061-63.
23. Delaporte E, Janssens W, Peeters M, Buve A, Dibanga G, Perret J. Epidemiological and mulecular characteristics if HIV infection in Gabon, 1986-1994. *AIDS* 1996;10(8)903-10.
24. Betherat E, Georges-Courbpt M, Nabias R, Georges A and Renaut A. Seroprevalence of four sexually transmitted diseases in a semi-urban population of Gabon. *Int J Sex Trans Dis and AIDS* 1998;9(1):31-6.
25. Schoub B, Johnson S, McAnerney J,Blackburn N. The role of sexual transmitted of hepatitis virus in black South Africans. *Trans R Soc Med Hyg* 1992;86:431-3.
26. Basetse H, Peenze I, Aspinall S. Hepatitis C virus antibodies in the black population of Ga-Rankuwa. *Med Tech* 1993;6(5):88-9.
27. Develoux M, BoniG, Aguessay Ahyi B, Gnahoui L, Delaporte E. The prevalence of anti-hepatitis C antibodies in pregnant women and blood donors in Benin. *Bull Soc Path Exot* 1995;88(3):115-6

This work is licensed under a
Creative Commons
Attribution – NonCommercial - NoDerivs 3.0 License.

To view a copy of the license please see:
http://creativecommons.org/licenses/by-nc-nd/3.0/

This is a download from the BLDS Digital Library on OpenDocs
http://opendocs.ids.ac.uk/opendocs/

Institute of
Development Studies