

In our study, urinary free-carnitine excretion declined with PEM, as did its rate of clearance. This result seems contrary to the findings of Maebashi *et al.*⁴ who described an increase in urinary carnitine excretion in fasting subjects. Wennberg *et al.*¹⁰ found that creatinine excretion was decreased in PEM. They found that malnourished patients showed increased excretion of free and total carnitine per urinary creatinine excretion compared with control patients. Petrykowski *et al.*¹¹ found that total carnitine excretion always exceeded glomerular filtration, indicating tubular secretion. This increased markedly at higher serum concentrations. In another study, carnitine levels (plasma and urinary) were found to be normal or above normal after 1 week of L-carnitine administration.¹²

Acceleration of incremental growth was seen in 22 of the 33 patients receiving carnitine who presented with failure to thrive.¹² Winter *et al.*¹² found that improved growth was in the group characterized by increasing muscle mass on L-carnitine treatment. They believed that the role of carnitine as a muscle growth factor in infancy should be explored.

Finally, carnitine can be used in the treatment of malnutrition, especially kwashiorkor, 5 day carnitine supplementation is sufficient.

References

1. Arslanina SA. Nutritional disorders: integration of energy metabolism and its disorders in childhood. In: Sperling MA (ed.), *Pediatric Endocrinology*. WB Saunders, Philadelphia, 1996; 523–49.
2. Nelson-Barness LA, Curran JS. Nutritional disorders. In: Behrman RE, Kliegman RM, Arvin AM (eds), *Nelson Textbook of Pediatrics*. WB Saunders, Philadelphia, 1996; 166–9.
3. Yalaz K, Epir S. Physical growth measurements of preschool urban Turkish children. *Turk J Pediatr* 1983; 25: 155–65.
4. Tietz NW. *Textbook of Clinical Chemistry*. WB Saunders, Philadelphia, 1987; 674–7.
5. Marquis KA, Fritz IB. Enzymological determination of free carnitine concentrations in rat tissues. *J Lipid Res* 1964; 5: 184–7.
6. Tanzer F, Uzunsel S, Atalay A. Plasma free carnitine levels in children with malnutrition. *Turk J Pediatr* 1994; 36: 133–7.
7. Lennon DLF, Shrago ER, Madden M, Nagle FJ, Hanson P. Dietary carnitine intake related to muscle and plasma carnitine concentrations in adult men and women. *Am J Clin Nutrition* 1989; 43: 234–9.
8. Khan L, Bamji M. Plasma carnitine levels in children with protein caloric malnutrition before and after rehabilitation. *Clin Chim Acta* 1977; 75: 163–7.
9. Maebashi M, Kawamura N, Yoshinaga K. Urinary excretion of carnitine in man. *J Lab Clin Med* 1976; 87: 760–6.
10. Wennberg A, Hyltander A, Siöberg A, *et al.* Prevalence of carnitine depletion in critically ill patients with under-nutrition. *Metabolism* 1992; 41: 165–71.
11. Petrykowski W, Ketelsen UP, Schmidt-Sommerfeld E, *et al.* Primary systemic carnitine deficiency under successful therapy: clinical, biochemical, ultrahistochemical and renal clearance studies. *Clin Neuropathol* 1985; 4: 63–71.
12. Winter SC, Szabo-Aczel S, Curry CJR, Hutchinson HT, Hogue R, Shug A. Plasma carnitine deficiency. Clinical observations in pediatric patients. *Am J Dis Child* 1987; 141: 660–5.

Stability of Saliva for Measuring HIV in the Tropics

by Min Thwe,^a Ralph R. Frerichs,^b Khin Yi Oo,^c Edward Zan,^a and Nora Eskes

^aAIDS Prevention and Control Program, Yangon, Myanmar

^bDepartment of Epidemiology, UCLA, Los Angeles, CA, USA

^cNational Health Laboratory, Yangon, Myanmar

Summary

If HIV is to be detected among pregnant women in remote regions of the tropics, HIV antibodies need to remain stable until specimens arrive at the laboratory. Our objective was to assess the stability of HIV antibodies in saliva held for up to 1 month at ambient temperature in Yangon, Myanmar. We gathered 10 saliva specimens from each of 102 HIV-infected persons with the Omni-Sal collection

Acknowledgements

This study was partially supported by the UCLA/Fogarty HIV/AIDS Training Program, the Ministry of Health, Myanmar and Saliva Diagnostic Systems Inc., Vancouver, WA, USA.

Drs Thwe, Frerichs, Oo, and Zan have no financial or other connections with Saliva Diagnostic Systems Inc., or other mentioned companies. Ms Eskes was formerly employed by Saliva Diagnostic Systems, and still owns stock in the company.

Correspondence: Professor R. R. Frerichs, Department of Epidemiology, UCLA, Los Angeles, CA 90095-1772, USA.

device (Saliva Diagnostic Systems, Inc.), and for each subject, divided the saliva into 15 portions. During 33 days, the 102 saliva specimens, kept at ambient temperature, were tested every 2–3 days for HIV antibodies (total 1530 assays) with the GACELISA (Murex Diagnostics Ltd), a highly sensitive test designed for use with saliva. We observed no reduction in test performance over 33 days, indicating that the antimicrobial and antiproteolytic transport medium in the Omni-Sal device can preserve HIV antibodies without refrigeration for up to a month before saliva specimens reach the laboratory.

Introduction

During the past year, nearly 600 000 children were estimated to be newly infected with the human immunodeficiency virus (HIV) by their mothers, most of whom live in the tropical societies.¹ When undetected, approximately one-third to one-quarter of HIV-infected women pass the virus on to their offspring either during pregnancy or birth,² or via breastmilk.³ Yet if HIV infection is detected in the potential mother, zidovudine (ZDV) therapy has been shown to reduce transmission by two-thirds when using a more extensive treatment,⁴ or more recently by half when using a less costly modified treatment.⁵ Advising HIV-infected women to not breastfeed has also been effective at reducing HIV infection and mortality in the offspring.⁶ Because of the beneficial effects of breastmilk, however, health professionals are reluctant to advise mothers not to breastfeed, although the advice usually changes when the mother is found by testing to be HIV infected.

Most HIV testing requires drawing of blood, an organized laboratory with a refrigerator, equipment, and kits for enzyme immunoassay (EIA) or particle agglutination (PA) assay for the presence of HIV antibodies. While such tests are typically done with

blood, saliva has also been shown to be an effective^{7,8} and acceptable⁹ medium for HIV antibody testing. Being non-invasive, saliva specimens are easier to gather by midwives or other health workers who typically do not draw blood. A major issue in tropical societies, however, is the need for refrigeration, since pregnant women may live far from laboratories. To address this issue, our study was designed to assess, in a warm climate, whether lack of refrigeration during prolonged transportation negatively affects the stability of HIV antibodies in saliva.

Materials and Methods

The study was done in Yangon, Myanmar, following a research protocol that was approved by the Human Subjects Protection committees of UCLA and the Myanmar Ministry of Health. After giving informed consent, 10 saliva specimens were gathered from 102 HIV-infected persons who had previously been tested at a government drug treatment centre. The saliva was collected with the Omni-Sal [Saliva Diagnostic Systems, Inc. (SDS), Vancouver, Washington, USA], a device that consists of a cotton pad and a tube containing

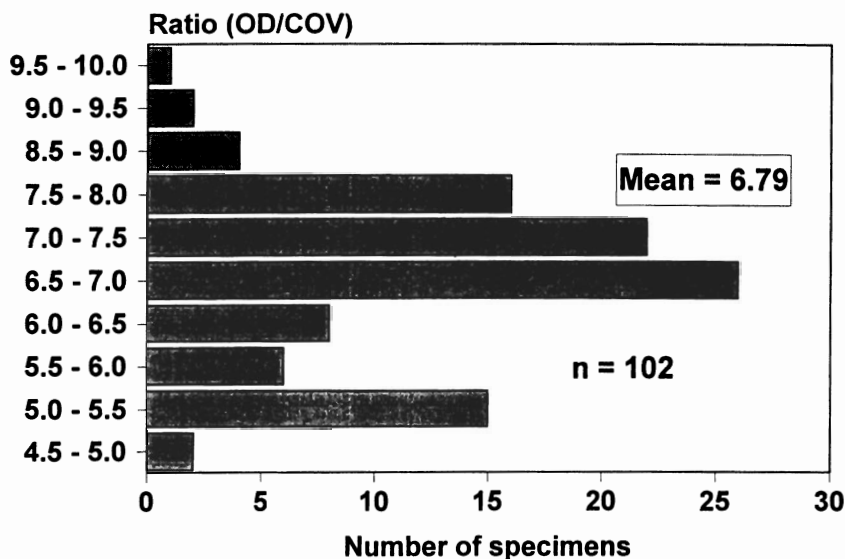


FIG. 1. Repeat measurements at time 1 of OD/COV for saliva-based HIV antibody test.

antimicrobial and antiproteolytic agents as transport medium. For each subject, the pooled saliva was divided into 15 portions, and kept at ambient temperature for the duration of the study. Testing for HIV antibodies was done by the staff of the National Health Laboratory in Yangon using the GACELISA (Murex Diagnostics, Ltd, Dartford, England, UK), a highly sensitive EIA specially designed for saliva.¹⁰ Using the GACELISA, laboratory personnel measure the optical density (OD) of the specimen and relate it to a cut-off value (COV) based on a control specimen. If the OD/COV ratio is at or greater than 1.0, the person is considered HIV reactive (or positive) and if less than 1.0, is considered HIV non-reactive (or negative). During 33 days, the 15 groups of 102 saliva specimens were tested for HIV antibodies every 2–3 days, for a total of 1530 assays. The daily ambient temperature in Yangon during the study ranged from an average high of 32.1°C (89.7°F) to an average low of 24.2°C (75.5°F), while the relative humidity ranged from an average daily high of 93.9 per cent to an average daily low of 74.8 per cent.

Results

On day 1 of the study, all of the 102 subjects were highly reactive to the GACELISA, with OD/COV ratios ranging from a low of 4.91 to a high of 9.30 and an average value of 6.79, well above the ratio of 1.00 used to separate the HIV infected from the non-infected (see Fig. 1). The same 102 specimens were again tested at days 3–33 (for a total of 15 test periods), remaining at ambient temperature throughout the study. As shown in Fig. 2, there was no observable reduction in the OD/COV ratio during the 33 day period, indicating

that the antimicrobial and antiproteolytic transport medium in the Omni-Sal device can preserve HIV antibodies without refrigeration for up to a month before saliva specimens reach the laboratory for testing.

Discussion

Saliva is a useful medium for HIV antibody testing in developing countries, and is especially important for viral detection in pregnant women. Test findings with saliva agree very well with those from serum, whether using more expensive laboratory assays such as the GACELISA,⁷ less expensive laboratory tests such as the Detect HIV 1/2 assay (BioChemical Immuno-systems, Inc., Montreal, Canada),⁸ or rapid assays more appropriate for use in rural clinics such as the SalivaCard HIV-1/HIV-2 and Orapette collection device (Trinity Biotech, Dublin, Ireland), or ImmunoComb II HIV-1 and HIV-2 (Organics Ltd, Israel) and OmniSal collection device (SDS, USA).¹¹

The stability of HIV antibodies has also been reported by others, with whole-blood spots stored in Zaire for up to 6 weeks,¹² with post-mortem whole blood stored at ambient temperature in Finland for 51–266 days,¹³ and with saliva stored in Germany at ambient temperature for up to 20 days and at 37°C (98.6°F) for up to 5 days.¹⁴ Finally, Wang and colleagues found that heating serum to 65°C (149°F) for 60 minutes had no effect on a particle agglutination test of three HIV-positive sera.¹⁵

In rural Myanmar, home visits for antenatal consultation are made by midwives or auxiliary midwives who do not collect blood, but could easily be trained to obtain saliva specimens. The saliva would be taken to the local

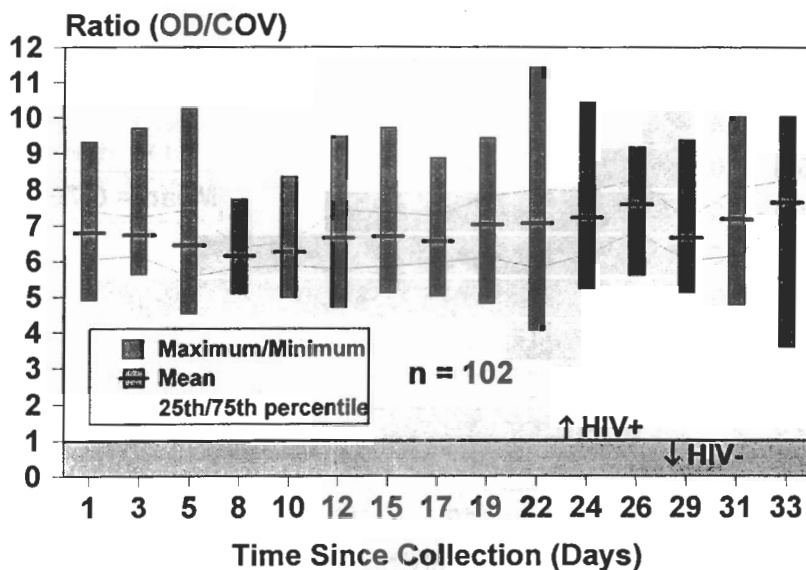


FIG. 2. Repeat measurements for 33 days of OD/COV for saliva-based HIV antibody test.

township hospital, where HIV testing would be done. Not having to rely on refrigeration during the transportation is important in such settings. Our study of saliva from 102 HIV positive subjects shows that with the Omni-Sal transport medium, HIV antibodies are stable for up to 33 days, providing sufficient transportation time to enable HIV testing of pregnant women in remote areas.

References

1. Anonymous. Global AIDS surveillance—Part I. *Wkly Epidemiol Rec* 1997; 72: 357–60.
2. Kuhn L, Abrams EJ, Matheson PB, et al. Timing of maternal–infant HIV transmission: associations between intrapartum factors and early polymerase chain reaction results. *New York City Perinatal HIV Transmission Collaborative Study Group. Aids* 1997; 11: 429–35.
3. Kreiss J. Breastfeeding and vertical transmission of HIV-1. *Acta Paediatrica (Suppl.)* 1997; 421: 113–17.
4. Connor EM, Sperling RS, Gelber R, et al. Reduction of maternal–infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. *Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. New Engl J Med* 1994; 331: 1173–80.
5. Anonymous. Administration of zidovudine during late pregnancy and delivery to prevent perinatal HIV transmission, Thailand, 1996–1998. *MMWR* 1998; 47: 151–4.
6. Bobat R, Moodley D, Coutsooudis A, Coovadia H. Breastfeeding by HIV-1-infected women and outcome in their infants: a cohort study from Durban, South Africa. *AIDS* 1997; 11: 1627–33.
7. Frerichs RR, Silarug N, Eskes H, et al. Saliva-based HIV antibody testing in Thailand. *AIDS* 1994; 8: 885–94.
8. Wongba C, Pagcharoenpol P, Eskes N, Frerichs RR, Silarug N. HIV saliva test for surveillance and surveys [letter]. *AIDS* 1995; 9: 1104–5.
9. Matee MI, Lyamuya EF, Simon E, et al. Detection of anti-HIV-1 IgG antibodies in whole saliva by GACELISA and Western blot assays. *East Afr Med J* 1996; 73: 292–4.
10. Connell JA, Parry JV, Mortimer PP, Duncan J. Novel assay for the detection of immunoglobulin G anti-human immunodeficiency virus in untreated saliva and urine. *J Med Virol* 1993; 41: 159–64.
11. Saville RD, Constantine NT, Holm-Hansen C, Wisnom C, DePaola L, Falker WAJ. Evaluation of two novel immunoassays designed to detect HIV antibodies in oral fluids. *J Clin Lab Anal* 1997; 11: 63–8.
12. Behets F, Kashamuka M, Pappaioanou M, et al. Stability of human immunodeficiency virus type 1 antibodies in whole blood dried on filter paper and stored under various tropical conditions in Kinshasa, Zaire. *J Clin Microbiol* 1992; 30: 1179–82.
13. Karhunen PJ, Brummer-Korvenkontio H, Leinikki P, Nyberg M. Stability of human immunodeficiency virus (HIV) antibodies in postmortem samples. *J Forens Sci* 1994; 39: 129–35.
14. Stark K, Warnecke C, Brinkmann V, Gelderblom HR, Bienzle U, Pauli G. Sensitivity of HIV antibody detection in saliva. *Med Microbiol Immunol* 1993; 182: 147–51.
15. Wang GR, Yang JY, Lin TL, Chen HY, Horng CB. Temperature effect on the sensitivity of ELISA, PA and WB to detect anti-HIV-1 antibody and infectivity of HIV-1. *Chinese Med J (Taipei)* 1997; 59: 325–33.

Surrogate Markers of Disease Progression in HIV-infected Children in Rio de Janeiro, Brazil

by M. B. Ortigão-de-Sampaio,^a T. F. Abreu,^b M. I. Linhares-de-Carvalho,^c A. Ponce de Leon,^d and L. R. R. Castello-Branco^a

^aLaboratório de Imunologia Clínica, Departamento de Imunologia, IOC-FIOCRUZ, Rio de Janeiro, Brazil

^bInstituto de Pediatria e Puericultura Martagão Gesteira, Universidade Federal do Rio de Janeiro, Brazil

^cAmbulatório do Banco da Providência, Rio de Janeiro, Brazil

^dDepartamento de Estatística, IME, Universidade do Estado do Rio de Janeiro, Brazil

Summary

In order to test the predictive value of immune complex-dissociated p24 antigenaemia (ICD-p24Ag), β 2 microglobulin (β 2-M), and neopterin as markers of disease progression, 53 HIV-1 infected

Acknowledgements

This study was funded by Fundação Banco do Brasil and CNPq.

We would like to express our gratitude to the nursing and laboratory staff of IPPMG/UFRJ and Ambulatório do Banco da Providência, to Dr Mauro Schechter of the Laboratório Petrobrás-HUCCF/UFRJ for performing the CD4 counts, and to the Fundação Banco do Brasil and CNPq for financial support.

Correspondence: Dr Maria Beatriz Ortigão-de-Sampaio, Laboratório de Imunologia Clínica, Departamento de Imunologia, IOC/FIOCRUZ, Av. Brasil, 4365, Mangunhos, CEP 21045-900, Rio de Janeiro, Brasil. Tel. +55 21 280 1486; Fax +55 21 280 1589. E-mail <ortigao@gene.dbm.fiocruz.br>.