



**FIG. 1.** Platelet counts in an HIV-infected patient showing the effect of IVIg (arrows) and didanosine therapy (shaded area).

ous treatment. There was no history of bleeding or purpura. Clinical examination was normal without spleen enlargement. CD4 cell count was  $0.45 \times 10^9/L$ . There was no detectable circulating p24 antigenemia and no decrease in the serum level of anti-p24 antibodies. Serum concentration of  $\beta_2$  microglobulin was 2.8 mg/L.

The patient was initially treated with two consecutive courses, 4 weeks apart, of intravenous immunoglobulin (IVIg) (Biotransfusion, France), 400 mg/kg/day given for 4 days. Platelet count transiently increased in response to therapy and then decreased to  $<20 \times 10^9/L$  within 2 weeks after the infusions. At that point, zidovudine was started at 750 mg/day, but it was stopped after 1 month because it had no effect on the platelet count. The patient received two additional courses of IVIg and was then treated with ddI at 400 mg/day. The platelet count increased progressively, reaching  $80 \times 10^9/L$  after 3 months with no associated therapy; it remained stable after 9 months.

Zidovudine is considered the first-line treatment for HIV-related thrombocytopenia (1,2). Didanosine, another antiretroviral nucleosidic analog that exhibits less bone marrow toxicity than zidovudine, was recently shown to be beneficial for patients with progressive disease who are either refractory or intolerant to zidovudine (3). Patients have shown significant improvement in their baseline hemoglobin levels, granulocyte, and platelet counts in retrospective data collected from 170 patients in phase I trials of ddI (4). Two adults with HIV-related thrombocytopenia that had been successfully treated with zidovudine, however, recently suffered a relapse after switching from zidovudine to ddI (5). On the other hand, Butler et al. have reported a long-lasting increase in platelet counts in three children with thrombocytopenia who had been enrolled in a study of ddI in symptomatic HIV-infected children (6).

This is the first report of a successful treatment of HIV-related thrombocytopenia with didanosine in an adult patient. Didanosine represents an alternative to zidovudine for the treatment of thrombocytopenia in HIV patients

intolerant or resistant to zidovudine. Its use among this patient population warrants further investigation.

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## Validity of Three Assays for HIV-1 Antibodies in Saliva

*To the Editor:* Human immunodeficiency virus (HIV) antibody testing is routinely done using serum collected with invasive venipuncture or fingerstick methods. A noninvasive alternative to serum is testing of saliva, using a simple collection device that requires very little training, is acceptable to participants, and avoids the possibility of needlestick injuries of health care workers (1-4). Earlier we reported that saliva was an effective testing medium for identifying the prevalence of HIV-1 antibodies in a sentinel surveillance program in Myanmar (formerly Burma) (5). We based our assessment on comparison with a "gold standard," serum tested in a blind manner in the United States using the World Health Organization (WHO) confirmatory Strategy III, screening with one enzyme-linked immunosorbent assay (ELISA) test followed by confirmation with two different ELISA tests (6). The saliva assay we used had high specificity but only moderate sensitivity, suggesting that the assay was good for HIV surveillance testing of a group but inadequate for HIV screening of individuals. Here we report a blind reanalysis of the Myanmar surveillance samples, comparing the sensitivity and specificity of our original

saliva assay and two new promising tests, one of which is designed specifically for urine and saliva.

## SUBJECTS AND METHODS

Included in the present study was serum and saliva collected from 468 members of high-risk groups (intravenous drug users, patients at sexually transmitted disease clinics, and prostitutes), and 1,012 members of low-risk groups (new military recruits and women attending antenatal clinics), both drawn from townships in the southern and central regions of Myanmar. Excluded from our earlier analysis due to insufficient sera or saliva were 7 high-risk persons and 14 low-risk subjects. Myanmar health officials, following an established sentinel surveillance protocol, selected persons for anonymous testing during April–May 1992. Vials of blood and saliva were collected from each individual using a vacutainer system and the Omni-sal saliva collection device (Saliva Diagnostic Systems, Vancouver, WA, U.S.A.), respectively. The device consists of a cotton pad and a tube containing a transport medium. Antimicrobial and antiproteolytic agents in the transport medium stabilize the specimen during the time between collection and testing. The collection pad is designed to hold 1 ml of fluid when fully saturated, to fit into the transport tube and to allow maximum contact with the transport medium. Extraction of fluid from the pad typically yields 1–1.5 ml of cell-free fluid.

The vials of serum and saliva were identified with a numbered label that provided no information as to the linkage of the specimens or the identity of the persons. In Myanmar, the National Health Laboratory in Yangon (formerly Rangoon) received half the volume of serum and half of saliva, the other half being immediately frozen ( $-20^{\circ}\text{C}$ ) and then shipped to the Saliva Diagnostic Systems Laboratory in Vancouver, Washington, United States. Additional details are presented elsewhere (5). Only specimens shipped to the United States, thawed for the original analysis, and then refrozen ( $-20^{\circ}\text{C}$ ) were used for the present analysis.

### Gold Standard One

Serum was analyzed independently with two confirmatory methods, the first using once-thawed sera (original analysis) (5) and the second using twice-thawed sera (present analysis). The first, termed "gold standard 1," followed the WHO recommendation for HIV antibody testing of one ELISA as a screening test, and two different ELISA methods for confirmation (Strategy III) (6). The first assay for serum was Cambridge BioTech Recombigen HIV-1 (*env* and *gag*) enzyme immunosorbent assay (EIA) test (Cambridge BioTech Corporation, Worcester, MA, U.S.A.), conducted singly and then in duplicate, whereas the second and third assays, each retested in triplicate, were the Abbott HIV AB HIV-1 EIA (Abbott Diagnostic Division, Abbott Park, IL, U.S.A.) and the Wellcozyme HIV 1 + 2 (Murex Diagnostics Limited, Dartford, England), respectively. If nonreactive on the first test, the specimens were deemed negative. Those clearly reactive or in the "gray zone" ( $\geq 70\%$  of the cutoff value) were retested in duplicate. If

again reactive or in the gray zone, the specimens were confirmed using the two different ELISA methods. If repeatedly reactive with both of the confirmatory ELISAs, the sera were reported as positive. Those tests not clearly positive or negative were termed inconclusive.

### Gold Standard Two

Because the validity of the "gold standard" is highly dependent on the initial screening test, we derived a second gold standard for the twice-thawed sera using another ELISA test as the screening test and the Western Blot as the confirmatory test. The screening test was the Abbott HIV AB HIV-1 EIA (see above) and the confirmatory test was either the Cambridge HIV-1 Western Blot (Cambridge BioTech Corporation, Worcester, MA, U.S.A.) or the Biotech/Du Pont HIV-1 Western Blot (Biotech Research Laboratories, Inc., Rockville, MD, U.S.A.). Coding was similar to "gold standard 1" except for the Western Blot, in which three outcomes were possible: positive (reactive pattern specified by manufacturer), negative (no bands present), or indeterminate (one or more bands present, but not the reactive pattern specified by the manufacturer).

### ELISA Methods Using Saliva

The saliva specimens were analyzed with three different ELISA assays. The first analysis, done with the Cambridge BioTech Recombigen HIV-1 (*env* and *gag*) EIA test on once-thawed saliva (see above), was the same test as presented elsewhere (5), but recoded based on single assay findings for the present study. The second analysis of twice-thawed saliva was with the Abbott HIV AB HIV-1 EIA (see above). Both the Cambridge and Abbott assays are the same as those used with serum but with modifications of package instructions to optimize the kits for use with saliva. These included increasing sample volume, decreasing diluent volume, and for the Cambridge but not the Abbott, lowering the optical density (OD) cutoff value (COV) to 0.7 of the serum value. The third analysis was with the Wellcozyme HIV 1 + 2 GACELISA (Murex Diagnostics Limited, Dartford, England), a test intended for epidemiologic and other research use with unconcentrated samples of saliva (wherein the concentration of IgG HIV antibodies is  $\sim 1/1,000$  of that in serum) (7), urine and dried blood spots. No change was made in the COV specified by the manufacturer in the package instructions. If the initial assay is nonreactive, the specimen is considered negative. If the optical density reading for the sample is  $> 0.7$  times the cutpoint (gray zone), the specimen is retested in duplicate. If the sample is reactive in at least two of the three repeated tests it is considered positive, and otherwise negative.

Analysis was done by one of us (R.R.F.) using Quattro Pro (a spreadsheet program) and EpiInfo (a data management and analysis program). The identification codes linking serum and saliva were maintained by two of us (R.R.F. and M.T.H.) to ensure that the laboratory personnel did the analysis in a blind manner.

## RESULTS

The two "gold standard" assay methods using sera from the 1,480 subjects were in complete concordance, thereby validating the HIV status of the specimens and showing that freezing and thawing had no discernible effect on the measurement of HIV antibodies. Both methods independently reported the same 75 persons as HIV positive and 1,405 as HIV negative; hence the two "gold standard" methods are treated as one.

The three assay methods using saliva are compared with the combined gold standard in Table 1. The Cambridge assay using once-thawed saliva had an exceptionally high specificity (99.7–100%), but only a moderate sensitivity (93.2%). The Abbott test using twice-thawed saliva shows a much higher sensitivity (98.6%) than the Cambridge but has a slightly lower specificity in both the high- (98.7%) and low-risk (99.3%) groups. Finally, the twice-thawed Wellcozyme assay, specially designed for saliva, had 100% sensitivity and nearly as high specificity (99.7–99.9%) in the two risk groups.

## DISCUSSION

The collection and analysis of saliva specimens for HIV antibodies in developing countries has many advantages over serum, as we and others have noted elsewhere (1,4,5). A major advantage of saliva is safety for occasionally careless health care workers due to the absence of needle-stick injuries and near-total absence of HIV in the collected specimen (8,9). A second advantage is being able to use unskilled personnel for collection of specimens. For example, in sentinel surveillance programs, we could

use members of high-risk groups such as prostitutes or intravenous drug users to gather specimens from reluctant colleagues, using the Omni-sal device that features a simple indicator of sufficient saliva volume and a buffer to preserve HIV antibodies. Others report successfully using a similar saliva collection device with drug addicts (10). A third advantage is the positive effect of a noninvasive medium on participation in surveys, surveillance, or screening programs, assuming people are more willing to provide saliva specimens than blood samples (1,2,4). Finally, saliva specimens gathered with the Omni-sal collection device do not require refrigeration between the time of collection and analysis. Preliminary testing by Saliva Diagnostic Systems indicates that saliva with the correct buffer can be maintained at tropical temperatures for several weeks with no deterioration in the quality of the HIV assay.

In our earlier publication we demonstrated that a saliva HIV assay is very effective for surveillance testing (5). Here we have shown that the sensitivity and specificity of the Wellcozyme GACELISA used with saliva is comparable to that of high-quality serum HIV antibody assays and thus would be useful for screening purposes as well. Our findings with the GACELISA are similar to those reported for saliva testing by others (3,10–14). The present investigation, however, features more HIV-positive subjects and far more HIV-negative persons than the other published studies, and includes mainly "first-time" testers typically found in sentinel surveillance programs.

An issue of interest is whether HIV antibodies appear first in saliva or serum. In small studies of seroconverters, Major et al. reported complete concordance of ELISA findings between saliva and serum in five subjects (1), whereas Behets et al. found that saliva was ELISA positive before serum in one person, serum was ELISA positive before saliva among five subjects (but three showed a p24 band on a saliva Western Blot), and eight persons were ELISA positive for serum and saliva at the same time (2). Although our sentinel surveillance data are cross-sectional, the absence of false positives with the GACELISA test suggests that serum is not positive for HIV antibodies before saliva. Conversely, the presence of one false positive with the Cambridge ELISA (also a false positive with the Wellcozyme GACELISA) suggests that HIV antibodies may appear in saliva before serum, but only on rare occasion. Hence we believe that in a field setting typical of screening or surveillance programs, the timing of HIV antibody conversion in saliva versus serum seems of minor importance. When taking into account the shortcomings of ELISA tests, there is near total concordance between serum and saliva in the presence or absence of HIV antibodies.

Although the present results are very encouraging, we are continuing to evaluate the accuracy of saliva assays in a field setting. Starting in December 1992, we have conducted a blind study to compare the value of ELISA tests with saliva versus serum in 4 of the 73 sentinel sites in Thailand. This study of high-risk sentinel groups involves ~1,950 subjects with a combined HIV prevalence of 15%, and is the largest study of its kind to date. For saliva we use the Wellcozyme GACELISA, the preferred HIV an-

TABLE 1. Sensitivity and specificity of saliva human immunodeficiency virus (HIV) antibody tests among 468 high- and 1,012 low-risk persons in Myanmar

Saliva assay	Serum gold standard <sup>a</sup>					
	High-risk persons		Low-risk persons		Total persons	
	(+)	(-)	(+)	(-)	(+)	(-)
Cambridge						
(+)	69	1	1	0	70	1
(-)	5	393	0	1,011	5	1,404
Total	74	394	1	1,011	75	1,405
Sensitivity	93.2		—		93.3	
Specificity	99.7		100.0		99.9	
Abbott						
(+)	73	5	1	7	74	12
(-)	1	389	0	1,004	1	1,393
Total	74	394	1	1,011	75	1,405
Sensitivity	98.6		—		98.7	
Specificity	98.7		99.3		99.1	
Wellcozyme						
(+)	74	1	1	1	75	2
(-)	0	393	0	1,010	0	1,403
Total	74	394	1	1,011	75	1,405
Sensitivity	100.0		—		100.0	
Specificity	99.7		99.9		99.9	

<sup>a</sup> Two independent serum testing procedures: Cambridge (screening test) and Abbott plus Wellcozyme (confirmatory test); Abbott (screening test) and Western blot (confirmatory test)—see text.

tibody assay based on the present study. Our standard of comparison is the existing serum ELISA test done in Thailand, with Western Blot confirmation of all HIV-positive samples. If the blinded field experiment in the Thai sentinel surveillance program provides similar results to our current findings with the Myanmar specimens and to those reported by others, then saliva might well become the testing medium of choice for HIV antibodies, especially in developing countries.

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## Effects of Intravenous Drug Use and Gender on the Cost of Hospitalization for Patients with AIDS

*To the Editor:* Severity adjustment in AIDS research is an important and somewhat complex matter. Three basic questions must be kept in mind: What is severity of illness, what are the outcomes of interest, and what are the variables under study?

Severity-of-illness adjustments attempt to estimate, at a predetermined baseline, the probability that there will be mortality and/or morbidity in certain subgroups of patients. A perfect system of severity adjustment would match an experienced clinician's accuracy in predicting short- and long-term morbidity and mortality for a sample of patients (1). Due to the practical difficulties of measuring morbidity, most severity systems focus on predicting mortality. Baseline adjustment for severity in clinical research helps ensure that the results of a study are not biased because of baseline differences in risk of death. If the only outcome of interest is mortality, no additional adjustment is needed.

When a study focuses on outcomes other than mortality, severity adjustment alone may not be sufficient to avoid bias. Severity of illness is only one of many baseline factors likely to influence outcomes such as length of stay and hospital costs. Other important influences may include the therapeutic requirements of a particular admission diagnosis, the availability of social support networks to facilitate continuity of care after discharge, the patient's desire to go home, and the efficacy of in-hospital evaluation and treatment. It is important to distinguish severity adjustment from other kinds of adjustment because severity of illness is always a potential confounder in medical research. In contrast, other baseline variables (or confounders) only require inclusion if they are known to be associated with both the outcome of interest and the variables under study.

Dr. Seage and colleagues have concluded that "instruments to assess severity of illness in AIDS [i.e., The Justice AIDS Severity of Illness System (2) and SCAH (3)] should incorporate information on intravenous drug use" (4). It appears that they have confused severity adjustment with the more general process of adjusting for baseline differences to avoid confounding (5). Their study does not report that intravenous drug use was independently associated with increased morbidity or in-hospital mortality. Instead it reports an independent association with increased length of stay and hospital costs. Furthermore, the authors recognize that baseline differences, other than severity, may fully explain the differences in these economic outcomes and state that "we have minimized the possibility [that longer length of stay for intravenous drug users was due to greater severity] by controlling for severity using two systems." To then recom-