treatment. There was no history of bleeding or pu-
pura. Clinical examination was normal without spleen en-
largement. CD4 cell count was 0.45 × 10^9/L. There was no
detectable circulating p24 antigenemia and no de-
crease in the serum level of anti-p24 antibodies. Serum
concentration of β₂-microglobulin was 2.8 mg/L.

The patient was initially treated with two consecutive
courses, 4 weeks apart, of intravenous immunoglobulin
(IgV) (Biotransfusion, France), 400 mg/kg/day given for
4 days. Platelet count transiently increased in response to
therapy and then decreased to <20 × 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 IgV and was then
treated with ddl at 400 mg/day. The platelet count in-
creased progressively, reaching 80 × 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, an-
other antiretroviral nucleoside analog that exhibits less
bone marrow toxicity than zidovudine, was recently
shown to be beneficial for patients with progressive dis-
ease who are either refractory or intolerant to zidovudine
(3). Patients have shown significant improvement in their
baseline hemoglobin levels, granulocytosis, and platelet cell
counts in retrospective data collected from 170 patients in
phase I trials of ddl (4). Two adults with HIV-related
thrombocytopenia who had been successfully treated with
zidovudine, however, recently suffered a relapse after switching
from zidovudine to ddl (5). On the other hand, Butler et al.
have reported a long-lasting increase in plate-
let counts in three children with thrombocytopenia who
had been enrolled in a study of ddl in symptomatic-
HIV-infected children (6).

This is the first report of a successful treatment of HIV-
related thrombocytopenia with didanosine in an adult pa-
tient. 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 possibil-
ity 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 antibod-
ies in a sentinel surveillance program in Myanmar (for-
merly Burma) (5). We based our assessment on compar-
ison with a “gold standard,” serum tested in a blind man-
ner 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 inade-
quately 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 ante-
natal clinics), both drawn from townships in the southern and central regions of Myanmar. Excluded from our ear-
lier analysis due to insufficient sera or saliva were 7 high-
risk persons and 14 low-risk subjects. Myanmar health ofﬁcials, following an established sentinel surveill-
ce 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 Omi-
sal saliva collection device (Saliva Diagnostic Sys-
tems, 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 de-
signed 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 typi-
cally 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°C) and then shipped to the Saliva Diagnostic Sys-
tems Laboratory in Vancouver, Washington, United States. Additional details are presented elsewhere (5).

Only specimens shipped to the United States, thawed for the original analyses, and then refrozen (−20°C) were used for the present analysis.

Gold Standard One

Serum was analyzed independently with two conﬁrma-
tory methods, the ﬁrst using once-thawed sera (original analysis) (5) and the second using twice-thawed sera (run-in analysis). The ﬁrst, termed “gold standard 1,” followed the WHO recommendation for HIV antibody testing of one ELISA at a screening test, and two differ-
ent ELISA methods for conﬁrmation (4). The ﬁrst assay for serum was Cambridge BioTech Rec-
ombigen HIV-1 (env and gag) enzyme immunoassay assay (EIA) test (Cambridge BioTech Corporation, Worcester, MA, U.S.A.), conducted singly and then in duplicate, whereas the second and third assays, each repeated in triplicate, were the Abbott HIV AB HIV-1 EIA (Abbott Diagnostics Division, Abbott Park, IL, U.S.A.) and the Wellcome HIV 1+2 EIA (Wellcome Diagnos-
tics Limited, Dartford, England), respectively. If non-
reactive on the ﬁrst test, the specimens were deemed neg-
ative. Those clearly reactive or in the “gray zone” (40–70% of the cutoff value) were repeated in duplicate. If

Gold Standard Two

Because the validity of the “gold standard” is highly de-
pendent on the initial screening test, we derived a sec-
ond gold standard for the twice-thawed sera using another
ELISA test as the screening test and two different ELISA tests 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 (Cam-
bridge BioTech Corporation, Worcester, MA, U.S.A.) or the BiotechDx Plas IV HIV-1 Western Blot (Biotech Re-
search Laboratories, Inc., Rockville, MD, U.S.A.). Cod-
ing was similar to “gold standard 1” except for the West-
ern 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 manu-
ufacturer).

ELISA Methods Using Saliva

The saliva specimens were analyzed with three differ-
ent ELISA assays. The ﬁrst analysis, done with the Cam-
bridge BioTech Recombigen HIV-1 (env and gag) EIA test on once-thawed saliva (see above), was the same test as presented elsewhere (5), yet recorded 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 these used with serum but with modiﬁcations of package instructions to optimize the kits for use with saliva. These included increasing sample vol-
ume, 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 Welcorzyme HIV 1+2 GACEILISA (Murex Diagnostics Limited, Dartford, England), a test intended for epidemiologic and other research use with uncentrusted samples of saliva (wherein the concentra-
tion of IgG HIV antibodies is 1/3,000 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 non-reactive, the spec-
imen is considered negative. If the optical density reading for the sample is >0.7 times the cutoff (gray zone), the specimen is retested in duplicate. If the specimen 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 Quatro Pro (a spreadsheet program) and EpiInfo (a data manage-
ment 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 per-
sonnel 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 77 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 (98.67%) than the Cambridge but has a slightly lower specificity in both the high (98.7%) and low-risk (99.39%) groups. Finally, the twice-thawed Wellcome 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 sera, and we and others have noted elsewhere (1,4,5). A major advantage of saliva in 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 specificity and sensitivity of the Wellcome 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 serocverters, 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 positive band on a serum Western Blot), and eight persons were ELISA positive for serum but negative for 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 Wellcome 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 accuracy of ELISA tests with saliva versus serum in 4 of the 7 sentinel sites in trials. Preliminary findings indicate that high-risk sentinel groups involve 1,450 subjects with a combined HIV prevalence of 15%, and is the largest study of its kind to date. For saliva we use the WeIcome GACELISA, the preferred HIV anti-
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 findings with the Myanmar specimens and to that 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 Intraocular 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 one of many base-line 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 and 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 “instrumental to assessing severity of illness in AIDS [i.e., the Joint 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 recon-