Saliva Assays for HIV Antibody Diagnosis

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The worldwide human immunodeficiency virus (HIV) pandemic continues, with the cumulative number of HIV-infected adults now estimated by the World Health Organization (WHO) to be over 30 million people. The most common way to identify persons as being infected is to test serum for the presence of HIV antibodies using the enzyme-linked immunosorbent assay (ELISA). While others were still debating the name of the virus, in 1985 the first ELISA was licensed to screen donated blood and blood products for HIV antibodies. Since then, the ELISA has been used for screening concerned persons to identify those likely to be infected with the virus. During those early years, there was no time delay with endless speculation about the value of the test. The sensitivity and specificity were far from perfect. The test has not been a perfect indicator of HIV infection since it was measured against the occurrence of the disease in serum within months after initial infection. Yet epidemiologists and laboratory scientists who pushed for licensure of this important test recognized that they could not wait for a perfect test; given the toll that HIV was taking on the world population. This progressive spirit of scientifically developing and licensing an assay has not been evident with saliva testing.

Saliva assays

In 1987, Parry et al and colleagues reported their findings with saliva, rather than serum, as a testing medium for HIV antibodies. They used a simple free dip method to collect saliva. When compared to the conventional serum assay, Parry et al reported 100% concordance for two tests being used to detect antibody capture radioimmunossay (ACRIA) and G antigen capture enzyme-linked immunosorbent assay (GACELISA). A year later, Johnson and associates evaluated the effectiveness of four different assays on identifying HIV in saliva. They also used a free dip method of collection. In the years that followed, at least nineteen more articles have been published that evaluate saliva as an HIV antibody testing medium.

While most of the ELISA tests were intended for use with serum, one was specially designed for use with uncentrifuged samples of saliva, urine and dried blood spots. The Wellcozyme HIV 1+2 GACELISA (Murex Diagnostics Ltd, Dartford, UK). Since the concentration of IgG HIV antibodies in saliva is about 1/1000 of that in serum, the GACELISA test was optimized for low concentrations of antibodies. Eight publications have reported on the GACELISA. The sensitivity of the GACELISA with saliva was reported as 100% in seven of the eight published studies, and the specificity was 99.9-100% in seven of the eight studies. Other promising assays have not been studied so extensively.

Refrigeration is not necessary for several weeks. The collection pad is designed to hold 1 ml of fluid when fully saturated, to fit into the transport tube, and to allow contact with the transport medium. Extraction of fluid from the pad typically yields 1-3.5 ml of cell-free fluid. The device also features a color indicator to ensure that sufficient volume has been collected. The Orasure device uses a similar method, but according to the manufacturer, collects "oral mucosal transudate" rather than saliva. The Salivette consists of a cotton-wool roll that is chewed by the subject until sufficient saliva is absorbed.

Two measures are used to assess the validity of a test: sensitivity, or the proportion (or percentage) of persons with HIV antibodies who test positive, and specificity, or the proportion (or percentage) of persons without HIV antibodies who test negative. The findings for 36 ELISA tests that appeared in 21 studies published between 1987 and 1994 are shown in Figs. 1 and 2, for the sensitivity and specificity, respectively, of various saliva assays. In general, there is considerable variation in the sensitivity of the saliva assays (Fig. 1), although more than half of the report values lie between 98 and 100%. The specificity is even higher (see Fig. 2) with more than nine out of 10 of the reported assays exhibiting values of 99.5-100%.

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Uses of saliva

With a silent disease such as HIV infection, testing for HIV antibodies currently is the most cost-effective means for determining the incidence and prevalence of HIV infection in the community, and for identifying HIV infection in the individual. For such assessments, each with its own preferred characteristics—surveillance tests, screening tests, and diagnostic tests. For screening tests (notably in groups with true HIV prevalence of less than 10%), the HIV antibody assay should have high specificity, but only needs moderate sensitivity. Surveillance programs describe the prevalence of HIV infection in a group with few HIV-positive persons but many HIV-negative individuals. Accordingly, low specificity would result in many false-positives, thereby greatly inflating the HIV prevalence estimates. The result would be a waste of public funds as government intervention resources are channelled to the artificially-high-prevalence area.

Screening tests are used to identify HIV infection in the individual. The intention of a screening test is to identify those who are likely to be positive or negative for HIV. Persons who test negative are not measured again, while those who test positive are given a confirmatory test. Thus, screening tests need to have high sensitivity to avoid false-negatives, and if there is confirmatory testing, need only have moderate specificity since false positives will be identified at the next stage of testing. Of course, if there is no confirmatory testing, the screening test should have both high specificity and sensitivity.

While the findings of the cited studies are promising, WHO has been cautious in endorsing saliva as a medium for HIV antibody testing. Although some of their concerns are over technical issues, others are related to policy. With respect to the former, the WHO focuses primarily on the comparative value of the various collection methods, the effect of the collection devices on the validity of the ELISA, and the effect of oral disease or ingestion of food on the accuracy of the test. For the policy-related issues, WHO has expressed concern with determining appropriate testing objectives and strategies, and with maintaining the same ethical and legal standards with saliva testing as with blood testing.
With diagnostic testing, there are typically two components: a screening assay and a confirmatory assay. Once the screening assay has identified a person as HIV antibody-positive, a diagnosis is made with a confirmatory assay that should have equal sensitivity and higher specificity than the screening assay. To be diagnosed as HIV antibody-positive, the tested specimen must be reactive to both the screening assay and the confirmatory assay. HIV antibody-negative persons are those who either were nonreactive to the screening assay or were reactive to the screener and nonreactive to the confirmatory assay. If the sensitivity of the confirmatory assay is less than that of the screening assay, then the diagnostic testing procedure will result in too many false negatives.

Based on the results in the published literature, saliva would seem to be an ideal medium for surveillance testing. Most assay methods exhibit high specificity and moderate to high sensitivity, fulfilling the requirements of a surveillance test. Saliva is especially useful for surveillance of drug addicts who often resist giving blood, and of street prostitutes who may provide samples only to trusted colleagues, rather than official health department personnel. For screening purposes, however, more care would need to be taken in selecting an assay for saliva. At present, the GACELISA appears to be the most sensitive and specific assay than is available for saliva, although other tests are under development. Thus, saliva will likely not be used extensively for diagnostic testing, given the current acceptance of blood-based tests. Instead, what is likely is that saliva will be used as the initial screening test, followed by confirmatory testing using blood.

Saliva has several advantages over serum; it is safer for the health worker collecting the specimen; it is easy to gather from many people in a single setting; it is more acceptable to those who fear giving blood, and eliminates the vry problems typical of needle and syringe disposal programs. As a non-invasive method, saliva presents few of the biological hazards associated with handling potentially infected blood. Saliva contains antibodies to HIV, but the infectious virus is rarely present. Building on these advantages, attempts are underway to develop a low-cost saliva assay that can be marketed in developing countries for US$1.50 or less for both the saliva collection unit and the HIV antibody assay. Such an assay would need to have high sensitivity and specificity, and be easy to use in third world settings. Saliva is ideal as a collection medium since it does not require high-cost medical personnel, is safe to handle and, with a stabilizing buffer featured in some collection devices, does not need to be refrigerated for several weeks. Once a low-cost assay is tested and approved, saliva should become the HIV antibody testing medium of choice for much of the developing world.

Future trends
In the coming years, there are two uses for saliva testing that are now controversial and will be subject to considerable public debate. In the near term, saliva will very likely become important for blood donor screening. Contaminated blood appears to be the most effective way for the virus to spread, thus ELISA testing of blood by government agen-
IMMUNOLOGY: Saliva Assays, (cont'd)

cies is considered essential to most HIV control programs. In many instances, donors are screened with a set of questions to reduce the risk of contaminated blood being col-
lected. Instead of an interview that has low sensitivity and specificity, I foresee saliva testing of potential donors to identify per-
sons in the community who are deemed suit-
able for giving blood, followed by donations screening of the collected blood, as in current practice.

Even more controversial is the notion of widespread testing, either in the privacy of the home or in physicians' offices. In the develop-
ment world, driving the need for widespread testing will be the recognition that existing control strategies appear to have little sustain-
able impact, once highly funded, labor-intensive projects are no longer present. Rather than rely on underfunded government agen-
cies, public demand for inexpensive, noninvasive saliva tests to be offered in the private sector, either via local pharmacies or food markets, is foreseen. The primary de-
mand for such saliva kits will be for premar-
tial testing and for occasional testing of long-term sexual partners who demonstrate signs and symptoms of venereal diseases.

Most societies rely on two major efforts to prevent HIV infection: blood screening and avoidance of virus transmission via sexual

mean. While blood testing is widely advo-
cated, most countries do not place high prior-
ity on testing of potential long-term sexual
partners to determine if they are infected. 14 Yet the principle of protecting the unsuscepted from HIV remains the same, whether testing blood or people.

At the individual level, it is likely that saliva screening will have its greatest poten-
tial for reducing the spread of HIV infection. The infectivity of HIV via person-to-person contact appears to be very low, with prob-
abilities of transmission being estimated at
0.001 to 0.005 per sexual encounter. 15 al-
though a recent survey among military recruits in Thailand reported transmission probabili-
ties 30 to 50 times greater than previously estimated. 16 Many epidemiologic studies have shown that the virus will only spread through blood or sexual contact, with anal intercourse being a more infective route than vaginal intercourse. Sexually transmitted diseases that compromise the wall of the rectum or vagina also lead to increased infectivity. Finally, there may be a series of cofactors that contrib-
ute to the transmission of infection.

For individuals wanting protection from sexual transmission of HIV infection, there are currently few options. They could abstain from penetrative sexual intercourse altogether, as it is stressed in many societies where virgini-

ity prior to marriage and monogamy thereaf-
er is the rule rather than the exception, or as
is now done by some disinterested couples
where one spouse is HIV-positive and the
other is HIV-negative. 17 Or they could use
condoms, hoping that the condoms would not
break or slip, as has happened to many couples
who for decades have faced the unexpected
birth of a child. As a tool to avoid HIV infec-
tion, condoms appear to be between 90 and
95% effective, 18 and possibly even less. 19

If a vaccine becomes available, the risk to
the vaccinated individual would also be re-
duced, but likely only to be between 60 and
95%, depending on the effectiveness of the vac-
cines. In comparison, depending on the sensi-
tivity of the saliva assay and the length of the window period (i.e., first few months of infec-
tion when the person shows no HIV antibod-
ies in serum or saliva), testing of potential
long-term sexual partners would likely result in
a 95-98% reduction in the risk of HIV infec-
tion, far better than either condoms or poten-
tial vaccines. Of course, the person want-
ing maximum protection might both test po-
tential sexual partners and use a condom.

So what might stop such a valuable saliva test from becoming widely available to people as HIV-infected nations? Most likely, it would be concern about releasing a test for which there would be no assurance of confidential-
ity. The fear of ostracism or discrimination
against HIV-infected persons has convinced
many that widespread HIV antibody testing
would do more harm than good. Instead of
focusing on factors that created the fear of
HIV-infected persons, these critics deem HIV
testing to be the problem. 20 Yet, it is possible
that, in the future, social movements will take
place to normalize HIV/AIDS, especially in
developing countries, so that infected persons
are treated the same as others with life-threat-
ening ailments such as tuberculosis, cancer,
heart disease or diabetes. Of course, the main
difference will need to be recognized: namely that
HIV-infected persons can transmit the

virus to others via unprotected sexual inter-
course or sharing of contaminated blood. Once
public health, political, social and religious
leaders help remove the emotional element
from HIV/AIDS, it will be much easier for
societies to deal with HIV-infected people in
an open, caring manner, and to recognize
which behaviors can and cannot be done.

Conclusion
At this point, the emphasis of public health
officials would need to be shifted away from
safeguarding the identity of HIV-infected
persons and back to their primary function of
safeguarding susceptible persons from the
disease. Parents and their adult children would
be encouraged to test potential marriage or long-term sexual partners, but in the privacy of the home. If HIV antibody positive, the couple should be encouraged by community leaders and the mass media to go to a physician or testing center for confirmatory blood testing and to learn of treatment and prevension options. With the progress that has been made with ART as a testing medium, such a screening test will soon be available. What is needed now is to convince health workers, politicians, social scientists, and religious leaders, that HIV/AIDS is a disease rather than a sin, and that it needs to be dealt with in an open and caring manner. It is in this type of social environment that widespread antibody testing for HIV antibodies would have its greatest beneficial effect.

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REFERENCES