

IMMUNOLOGY

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) as 14 million or more persons.¹ During most of the time course of HIV infection, the condition remains silent with no definitive signs or symptoms, until the final appearance of acquired immune deficiency syndrome (AIDS).² 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,³ in 1985 the first ELISA was licensed to screen donated blood and blood products for HIV antibodies.⁴ Thereafter, the ELISA was also 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 ELISA, even though the sensitivity and specificity were far from perfect. The ELISA was partially flawed as an indicator of HIV infection since it was measuring HIV antibodies that often do not appear in serum until 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 to commercially develop and license an assay has not been as evident with saliva testing.

Saliva assays

In 1987, Parry and colleagues reported their findings with saliva, rather than serum, as a testing medium for HIV antibodies.⁵ They used a simple free drip method to collect saliva. When compared to the conventional serum assay, Parry et al reported complete concordance for two tests being analyzed: G antibody capture radioimmunoassays (GACRIA) and G antibody capture enzyme-linked immunosorbent assays (GACELISA). A year later, Johnson and associates evaluated the effectiveness of four different assays at identifying HIV in saliva.⁶ They also used a free drip 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,⁷⁻²⁵ some of whom used free drip while others used commercial collection devices.

At least three collection devices are currently being commercially marketed: Omnisal (Saliva Diagnostic Systems, Inc., Vancouver, WA, USA), Orasure (Epitope, Inc., Beaverton, OR, USA), and Salivette (Sarstedt, Leicester, UK). The Omnisal 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 so that

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 pad typically yields 1-1.5 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 (see 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%.

While most of the ELISA tests were intended for use with serum, one was specially designed for use with unconcentrated 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,²⁶ the GACELISA test was optimized for low concentrations of antibodies. Eight publications have reported on the GACELISA.^{5,12,13,16,18,20,21,25} 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.

While the findings of the cited studies are promising, WHO has been cautious in endorsing saliva as a medium for HIV antibody testing.^{27,28} Although some of their concerns are over technical issues, others are related to policy. With respect to the former, the WHO focus is 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.

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, there are three types of tests, each with its own preferred characteristics: surveillance tests, screening tests, and diagnostic tests. For surveillance testing (notably in groups with true HIV prevalence of less than 10%), the HIV antibody assay should have high specificity, but only needs moderate sensitivity.¹⁹ Surveillance programs describe the prevalence of HIV infection for 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 estimate. 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 negative or positive 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 have only 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.

Figure 1

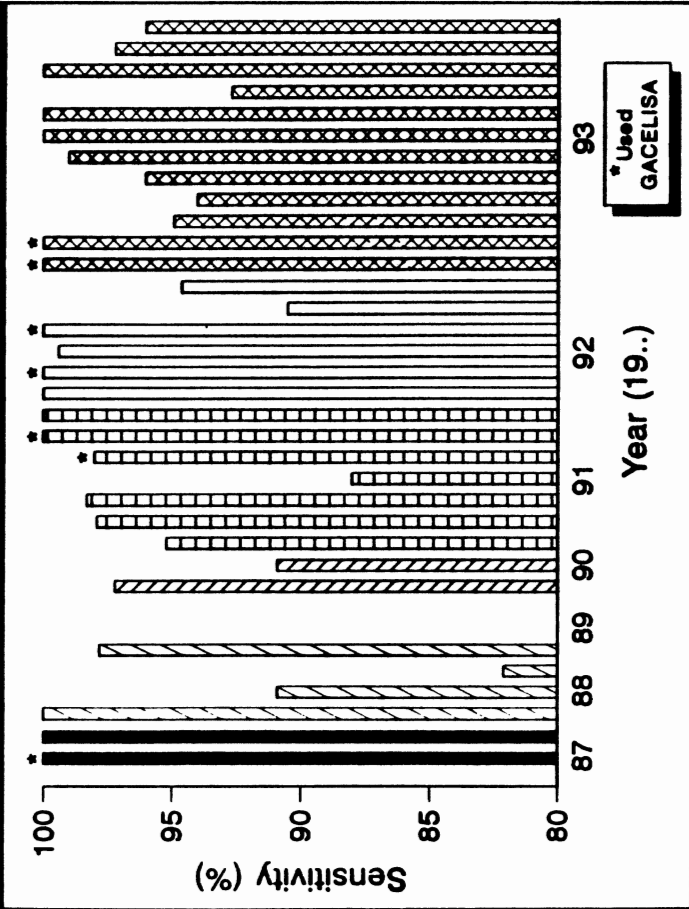
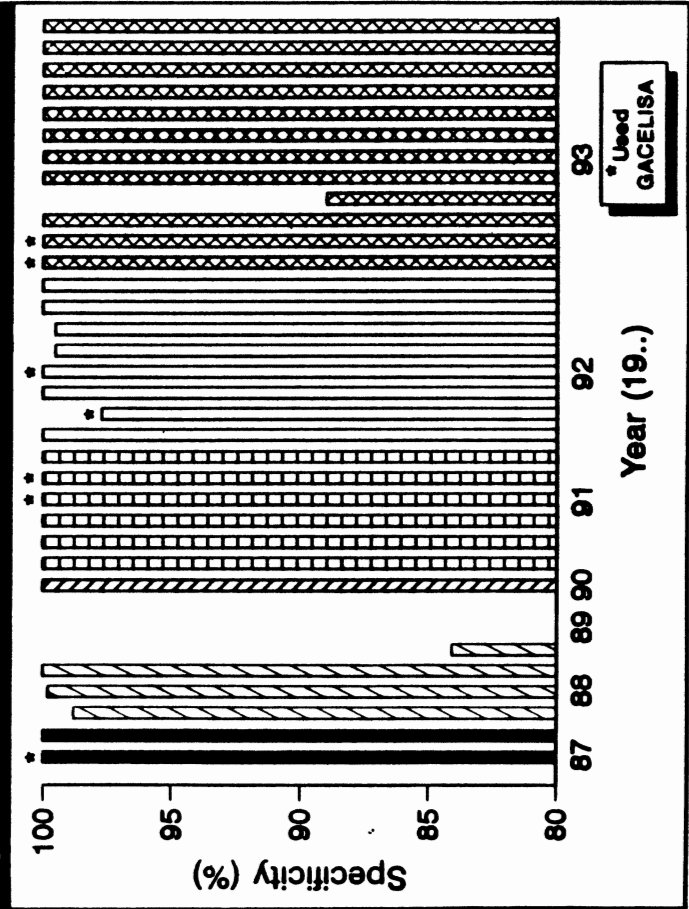


Figure 2



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 that 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 tests, 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 very 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.²⁹ 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-

Fig. 1: Sensitivity of published HIV assays using saliva, 1987-1994.

Fig. 2: Specificity of published HIV assays using saliva, 1987-1994.

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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 collected. Instead of an interview that has low sensitivity and specificity, I foresee saliva testing of potential donors to identify persons in the community who are deemed suitable for giving blood, followed by donations screening of the collected blood, as is current practice.

Even more controversial is the notion of widespread testing, either in the privacy of the home or in physicians' offices. In the developing world, driving the need for widespread testing will be the recognition that existing control strategies appear to have little sustainable impact, once highly funded, labor-intensive projects are no longer present. Rather than rely on underfunded government agencies, public demand for inexpensive, noninvasive saliva test kits to be offered in the private sector, either via local pharmacies or food markets, is foreseen. The primary demand for such saliva kits will be for premarital 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

means. While blood testing is widely advocated, most countries do not place high priority on testing of potential long-term sexual partners to determine if they are infected.³⁰ Yet the principle of protecting the uninfected from HIV remains the same, whether testing blood or people.

At the individual level, it is likely that saliva screening will have its greatest potential for reducing the spread of HIV infection. The infectivity of HIV via person-to-person contact appears to be very low, with probabilities of transmission being estimated as 0.001 to 0.005 per sexual encounter,³¹ although a recent study among military recruits in Thailand reported transmission probabilities 30 to 50 times greater than previously estimated.³² 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 contribute 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 is stressed in many societies where virgin-

ity prior to marriage and monogamy thereafter is the rule rather than the exception, or as is now done by some discordant couples where one spouse is HIV-positive and the other is HIV-negative.³³ 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 infection, condoms appear to be no more than 90-95% effective,³⁴ and possibly even less.³⁴

If a vaccine becomes available, the risk to the vaccinated individual would also be reduced, but likely only to between 60 and 95%, depending on the effectiveness of the vaccines. In comparison, depending on the sensitivity of the saliva assay and the length of the window period (i.e., first few months of infection when the person shows no HIV antibodies in serum or saliva), testing of potential long-term sexual partners would likely result in a 95-98% reduction in the risk of HIV infection, far better than either condoms or potential vaccines. Of course, the person wanting maximum protection might both test potential sexual partners and use a condom.

So what might stop such a valuable saliva test from becoming widely available to people in 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.³⁵ 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-shortening 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 intercourse 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

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be encouraged to test potential marriage or long-term sexual partners, but in the privacy of the home. If HIV antibody positive, the couple would 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 prevention options. With the progress that has been made with saliva as a testing medium, such a screening test will soon be available. What is needed next 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 saliva testing for HIV antibodies would have its greatest beneficial effect.

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