REVIEW ARTICLE

SALIVARY SAMPLES FOR HIV 1 & 2 ANTIBODY TESTING-CONSIDERATION OF ADVISABILITY OF THE OPTION
S. V. Lavanya¹, C. Subhashini²

HOW TO CITE THIS ARTICLE:
S. V. Lavanya, C. Subhashini. “Salivary Samples for HIV 1 & 2 Antibody Testing-Consideration of Advisability of the Option”. Journal of Evolution of Medical and Dental Sciences 2015; Vol 4, Issue 64, August 10; Page: 11232-11239, DOI: 10.14260/jemds/2015/1617

ABSTRACT: Being one of the personnel working in medical diagnostics since the past twenty one years, I feel responsible to use the most viable test procedure to save the patient’s interest. Simultaneously, our team of medical teachers, bear the responsibility to screen the immense literature available, and present only those details that need to be known to an average under graduate student. Thus, when a statement in a popular textbook was noticed, it became necessary for our team to consider all possibilities and probabilities before popularizing saliva as sample for HIV antibody testing. It was concluded that, in India, it was advisable to continue using serum as sample for HIV antibody testing. Using saliva as sample involves more cost and more elaborate procedure, and there would be a disturbance in the understanding of the Indian public about the disease.
KEYWORDS: HIV antibody testing, Saliva sample, Cost reduction, Patient compliance, Test reliability.

INTRODUCTION: This article was prepared considering the necessity to address the issue of whether to use saliva as a sample for HIV antibody testing. The experienced personnel, sharing their views, included people who have worked in the field of interest, both in the laboratory and the clinical sides of patient care, and included ICTC workers. It was necessary to study the logistics involved, because the idea of using saliva as sample for HIV antibody testing appeared to be a tenable (Less labour intensive) alternative to using blood as a sample.
In a popular microbiology textbook for undergraduate students, (Textbook of Microbiology by Prof C. P. Baveja),¹ the following information was given—that, besides blood and serum, saliva can also be used as a sample, while testing for anti-HIV Antibodies. The practitioner can easily perform the test in his clinic, while doing away with the services of a phlebotomist. Mandel had claimed concordance between anti HIV antibodies in saliva and serum way back in 1990.²
This set us thinking about the various possibilities if we followed this option. A search for references was initiated. Both textbook references and references of publications were compiled. Reference for results of such a procedure followed (Study conducted in 1993 by A. J. Hunt et al.),³ yielded 96.2% sensitivity and 100% specificity (Preliminary test by competitive ELISA, confirmed by indirect ELISA).
Suggestions and opinions were taken from another senior microbiologist in the department, and a technician, who looks over the sample collection. Opinion of a few educated patients attending the STD out-patient department in the government hospital, and also those attending a private clinic, was taken, about the use of saliva as sample for HIV antibody testing. The patients’ response was that of disbelief, surprised that their physician was suggesting such an impossible or unreliable test. Later, we had an inside out discussion about the pros and cons of the procedure.
The development of increasingly sophisticated virology and immunologic techniques has further enhanced our ability to diagnose HIV-1 infection early and accurately. Despite the potential
value of newer techniques, various modifications of the original solid phase serologic methods have remained the standard means by which most HIV-1 infections continue to be diagnosed in the United States and in many developed nations.\(^4\) (Devita). These solid phase methods use serum as sample for the test.

The aspects, that decide the advisability of a procedure, could be classified into physiological, technical, social, economic and inter-related of the above four.

Serological tests can be done using blood or oral fluids for the presence of HIV antibodies\(^5\) (Jawetz). There is no evidence that the virus is transmitted through casual contact.

Dry kissing does not transmit HIV.\(^6\) (Arora D. R.) Saliva in adults contains some non-specific inhibitory substances like fibronectins which can prevent cell to cell transfer of the virus. Thus, saliva is not a likely vehicle for transmission of HIV.

Saliva is an acceptable and often a favorable alternative to serum for HIV antibody testing. Blood of HIV infected individuals is a hazardous substance that occasionally leads to HIV infection among health workers. Saliva is a safer medium than blood for 4 reasons, viz:

- HIV is rarely found in the saliva of HIV infected individuals.
- When found in the saliva, the concentration of HIV tends to be very low.
- Factors present in saliva appear to inhibit viral infectivity.
- There is no possibility of needle stick injury during specimen collection.

Saliva is especially useful for HIV testing among groups such as injectable drug users who may have collapsed blood vessels and those who refuse to give a blood sample. ELISA test, using saliva as a sample, has a sensitivity and specificity of 98% and 99.4% respectively.

HIV is however not transmitted by casual contact, touching, hugging, kissing, coughing, sneezing, insect bites, water, food, utensils, toilets, swimming pools, or public baths.\(^7\) (Murray, Rosenthal). Rapid screening tests are available that detect specific antibody in blood or oral fluids from a swab of the gums.

The immunoglobulin content of oral fluid is similar to that of blood, but their levels are lower. However, the use of an HIV IgG antibody capture assay, designed specifically for testing oral fluids, has produced encouraging results.\(^8\) (KMK Masthan).

Virus may be present in the saliva, but at lower titres than in the blood.\(^9\) (Greenwood)

Considering the procedure of sample collection, it seems easier to collect a saliva sample, because it is a non-invasive procedure. But, after a detailed consideration of the reference studies, it was realized that collecting saliva was not as simple as collecting sputum for a smear study. Fluids collected for the salivary tests (i. e., fluids obtained from the oral cavity), consisted of saliva and gingival crevicular fluid.\(^8\) (KMK Masthan). The volume of saliva required for the test was significant, and it required the patient to be more involved in the sample collection procedure. According to A. J. Hunt et al,\(^3\) the sample was collected using a small cotton swab, which the patient was asked to chew for half a minute.

Also it was necessary that the patient should not have consumed anything orally during at least the last half hour before collecting the sample. The study group included homosexual men and IV drug abusers. These regulations before collecting the sample may be a minor issue for this study group. However in a routine Out Patient Department, a patient getting himself checked for HIV status will be apprehensive and therefore not enough co-operative, and it would be easier to secure a blood sample.
The swab was sealed in a plastic collection tube, which was packed into a larger plastic tube. The sample had to be collected by trained interviewers. In another study, conducted by W Schramm et al., a special sample collection device was recommended. It consisted of a cellulose pad held at the end of a thin plastic stem. This pad had to be held sublingually until the indicator panel turned blue (when approximately 1ml of saliva gets collected in the pad). Further processing of the sample involved introducing the pad into a phosphate buffer, containing sodium azide and avian serum. Further proceeding with the test includes the use of a piston filter to extract completely the saliva-buffer mixture from the cellulose pad. (These reagents and equipment meant a significant expenditure which would perhaps double the test cost.)

The test proper carried out by A. J. Hunt for HIV 1 Abs in saliva, included ELISA and RIA. W. Schramm made use of ‘test strips’. In both the studies, the results were repeatedly compared with those using serum to decide the false positivity or false negativity (Specificity and sensitivity respectively). However, knowledge of HIV status for an individual is of utmost importance, and cannot be misinterpreted, for the sake of opting for a non-invasive sample collection procedure.

The procedure of giving a blood sample was described to be traumatic. However 3.6% (19 of the 534 samples) of the study group did not submit a sufficient volume of the saliva sample to conduct the test. Also, the saliva results of two serum positive subjects were equivocal i. e. not conclusively positive. The participants of the study concluded that it was acceptable to have non-medical personnel to collect the samples; that insufficient sample collection was within acceptable levels of frequency; that more regular training could be given to the sample collectors, that simpler sample collection devices could be designed etc.

But all these endeavours are energy & finance consuming and will not be viable in a developing country like India. No single test in the history of modern medicine has had such a momentous impact on the lives of the individuals who rely on it. Since the beginning of the AIDS crisis, people have had very dramatic responses to the test, lapsing into severe chronic depression and anxiety. (Shibani Shetty et al). If such is the impact of the test result on the psyche of the patient, one cannot opt for a test that frequently gives results that are ‘equivocal’. However, the authors of the study suggest saliva, gingival crevicular fluid (GCF), oral mucosal transudate and urine as alternative samples to blood, to carry out non-invasive methods of testing. Oral fluids need to be diluted 1 to 2 (Unlike serum which is diluted 1 to 75). Further research for the improvement of these alternative techniques is required.

A more recent study carried out in Zimbabwe by Pascoe, S. J. et al in 2006 (Feb to July), on patients presenting for VCT (Voluntary Counseling & Testing) at rural clinics showed a prevalence of 29.8%. Limitations with the assay were false negativity in early stages of infection, or with reduced viral load; and altered accuracy in pregnancy. However, these limitations also apply to other rapid assays.

Another study conducted in Namibia, by Raph L Hamers et al, (June & July 2006) to assess 2 oral mucosal fluid based tests, showed high diagnostic accuracy, thus supporting utility in surveillance. The study group was adult antenatal women, among whom 25.6% positivity was noted (7/273). However, steps were needed to be taken to prevent collection of inadequate sample. Moreover, the procedure involved the use of oral collection devices. Also, it was stated that only the reference tests conducted on blood-plasma, tested for the presence of both HIV-1 and HIV-2 antibodies.
Discussion at our centre, about this alternate test procedure, with the technical staff, engaged in sample collection and processing, revealed a new possibility. There is a substantially big chance of these technical staff, and similarly the public, as well as the undergraduate medical student, to have a tendency to understand that HIV is transmissible through saliva. A brainstorming session with all the members involved in the discussion revealed this possibility. It became necessary to explain to them and to ensure them that only antibodies are passed into the salivary secretions (and not the virus) in a HIV+ve person. A search to confirm the point revealed otherwise! The saliva may variably carry the virus. According to Per Brandzaeg, Atkinson et al reported elevated IgA levels in submandibular but not in parotid secretions in such patients. Also, serum IgA levels rose in HIV+ve patients; and salivary IgA levels were not consistent, especially when the patients have candidiasis or other oral health problems.

Salivary IgA antibodies to viral p24 and to gp160 are reduced in symptomatic patients. The level of IgG Abs to p24 in whole saliva correlates with that in serum. Also, salivary IgG has been reported to carry specific anti-gp 160 activity 25 fold higher than that of serum IgG, suggesting some local production – perhaps in tonsils, inflamed gingivae, or submandibular and minor salivary glands, which contain a higher density of IgG and plasma cells than the parotid glands. Thus commercial kits available measure salivary IgG antibodies to HIV. These workers preferred saliva to blood for antibody screening in clinical settings and particularly for 'home testing'! Such an option i.e. home testing is something that is best avoided in the Indian scenario.

A study on self-medication practices by Shyam Sunder Kesari et al, reveals that in India, more male patients used self-medication compared to females, contrary to data from Western reports. If the same mindset prevailed in this situation too, that is, while testing for HIV status using a 'home testing' method, more often than not, the male has the freedom to self-test himself whenever he has a doubt about his HIV status. This will embolden him to put himself at risk again and again and increase his chance of acquiring the virus. Also, if he is in the window period, he would unknowingly transfer the virus to his partner or partners. Source of drug information, in the study on self-medication, were doctors, chemists and advertisements. However, in the case of HIV self-testing, it would be advertisements (If they come to be as the case is now with self-testing for pregnancy), doctors (if they ever start prescribing self-testing) and lab technicians performing the test (where the patient himself could be one.) Which ever the case may be, counseling, which is one of the important part and entity of HIV testing, is given a miss if the patient self-tests himself and keeps the information to himself and does not approach a counselor.

An acquaintance, who is a technician, acting as a source of information is more likely, if the given individual is a male than if she is a female. This puts the female partners to the given male patient, at a disadvantage of exposure for a longer period of time. Also, just as symptoms of a minor illness may cause a major illness if not diagnosed properly, an early diagnosis without treatment may shorten the life-expectancy that is possible with early onset of treatment Anti-retroviral therapy also reduces viral load and infectivity (Arora DR, Meghna Maheshwari et al). Saliva samples are suitable for carrying out rapid point-of-care testing, but the ethical framework surrounding handling the results requires careful consideration.

Noninvasive methods, where oral fluids are used as specimen, need devices for collecting these fluids. The very fact that the study was carried out in a dental college, points to the fact that in the process of avoiding the services of a phlebotomist, we are unwittingly making the services of a dentist mandatory in carrying of the test. The microbiologist opinion is still always necessary and
cannot be done away with17 (NACO Guidelines, March 2007). According to these guidelines, saliva is not as good as blood/plasma/serum for antibody detection. Antibody level in oral fluids is less than 1% of the serum levels. Secretory IgA is the isotype present, and appropriate ELISA kits need to be used. Issues regarding confidentiality, counseling and follow up need to be resolved. The kits need to be validated in India. Also, testing without consent of the patient has been proven to be counterproductive, because the target people get driven underground, and launching interventions becomes impossible. As the procedure involves more number of equivocal results, it becomes difficult to decide the category of posttest counseling to be done for each of the patients. So is the case with the receiving of consent to carry out the test-it is of no use if the test itself is not enough reliable.

Infrastructure for HIV testing laboratories includes a phlebotomy/blood collection point, a needle destroyer, tourniquet, medicated spirit, etc, all of which will become obsolete if the sample to be collected is altered.

On further search for behavioral patterns among patients, we came across studies detailing the approach of patients (Females in this case) to the use of E.C. pill (Emergency contraceptive pill). These studies made it clear that the E. C. pill serves its purpose in a reasonably healthy manner only if both the partners involved are not having multiple partners (Or are a married couple), and are free from STDs. Similarly, self-testing for HIV Abs is advisable only if it is made sure that a patient, if tested positive, will approach a counselor, and also that the mechanisms for referral and follow-up are in place. These systems are already in practice using the blood sample and it is best to continue with the same, at least for the present and the immediate future.

It may be argued that empowering individuals to manage their HIV risks will dramatically increase rates of disease detection in communities that have proven difficult to reach and to link to appropriate care. But the authors offer a more cautious perspective21 (Walensky R P et al). According to what is already known about the market demand for over-the-counter HIV testing kits, their costs, and the performance of rapid HIV tests in the market, the authors do not anticipate that the rapid home test will have a profound impact either on the HIV public health crisis or on the populations in greatest need. Home HIV testing will attract a predominantly affluent clientele, composed disproportionately of HIV-uninfected new couples and "worried well" persons, as well as very recently infected persons with undetectable disease. The authors illustrate how testing in these populations may have the perverse effect of increasing both false-positive and false-negative results. A poorly functioning home HIV test may thereby undermine confidence in the reliability of HIV testing more generally and weaken critical efforts to expand HIV detection and linkage to lifesaving care for the persons with unidentified HIV infection. Response to the FDA approval on July 3rd, 2012, of an over-the-counter home HIV testing kit, by A David Paltiel et al is tempered.22 Besides the above mentioned disadvantages, known HIV positive patients may seek to monitor therapy or pursue a misperception that treatment has reversed their seropositivity!.

CONCLUSION: The study team, involved in the discussion, had a difficult time to finally conclude that in the present Indian scenario, it is not advisable to suggest saliva as an alternative sample to blood sample for HIV antibody testing.

The main advantages in using saliva are non-invasive sample collection procedure; higher antibody concentration in saliva than in blood, at least during some phases of the HIV infection, as claimed by some of the studies, (While others point out that the antibody concentration is higher in the blood throughout the course of the disease).
However, the disadvantages far outweigh the advantages. Considering the Indian rural population and their psychology, it is best to continue with the systems that are now in place. The AIDS pandemic has played havoc not only with the health but also with the psyche of the Indian public during the two decades, from 1990 to 2010. At present, though the prevalence of the viral infection is just as high as before, there is a sense of acceptance of the disease in the society. The associated social stigma, is now not as obvious. The common man now understands that HIV is not transmitted through ‘casual contact’-thanks to the active campaigning by the public health department. But if the method of sample collection is altered now, there will be a total confusion and commotion in his understanding of the disease. There is no doubt a high level of illiteracy in the country, but the common man in India is fairly intelligent and has a reasonable level of inquisitiveness to think over these possibilities. It is difficult to explain to him that there is a difference in the saliva being positive for antibodies and the saliva being positive for the virus. Also, it is even more untenable to tutor him about the different stages of AIDS and that the saliva is positive for the virus only in the fourth stage of the disease.

Increase in the cost of sample collection and sample processing procedures was noted. Cost of training of the personnel who take up the new method is also considerably high. These are of significance in a funds deprived country like India. Also problems such as an insufficient volume of collected sample and of results being equivocal may be minor as a percentage of a study result, but is of 100% importance to the individual patient, and so are best to be avoided. So, in the interest of the individual patient, it would be better to go through a ‘traumatic’ sample collection procedure than to miss a correct diagnosis.

ACKNOWLEDGEMENTS: This article was written to provide a discussion on the various factors that decide the advisability of the option of using saliva samples for testing for anti-HIV 1 and 2 antibodies. Dr T. V. Ramani (Senior Microbiologist) and Mr S. Suresh (Technical personnel) are immensely thanked for their participation in discussions and for the suggestions given by them. It is an understood fact that the student community is the main motivation behind the initiation of this writing, as it is the duty of the teacher to draw an informed conclusion in such situations, before giving voice to his opinion.

REFERENCES:
1. Dir. Prof. C. P. Baveja; Textbook of Microbiology, 4th Edition, 2012, pgs 517, 521; Arya Publications.
2. Mandel ID, The Diagnostic uses of saliva, Journal of Oral Pathol Med. 1990 Mar; 19(3); 119-25.
3. A. J. Hunt, J. Connell, G. Christofinis, J. V. Parry, P. Weatherburn, F. C. l. Hickson, A. P. M. Coxon, P. M. Davies, T. J. McManus, S. Sutherland; The testing of saliva samples for HIV-1 antibodies: reliability in a non-clinic setting Genitourinary Medicine 1993; 69: 29-30.
4. Vincet T. Devita Jr, Samuel Hellman and Steven A Rosenberg (Editors), James Curran, Max Essex and Anthony S Fauci (Asso Editors), AIDS: Aetiology, Diagnosis, Treatment and Prevention., 4th Edition, Editors: Lippincott-Raven Publishers, N. Y. 1997.
5. Jawetz, Melnick & Adelberg’s Medical Microbiology by Geo. F. Brooks, Karen C. Carroll, Janet S. Butel, Stephen A. Morse, Timothy A. Mietzner; pg 620, 25th Edition, 2010; LANGE.
6. D. R. Arora & Dr Brij Bala Arora; Textbook of Microbiology; 4th Edition, 2012, pgs 589, 596, CBS Publishers and Distributors Pvt Ltd.
7. Patrick R. Murray, Ken S. Rosenthal & Michael A Pfaller; Medical Microbiology; pgs 576, 578; 7th Edition, 2013, Elsevier Saunders.
8. KMK Masthan, Textbook of Oral Microbiology, N. Aravindha Babu; 1st Edition, 2011; pg 122, CBS Publishers.
9. David Greenwood, Mike Barer, Richard Slack & Will Irving; Medical Microbiology; pg 573; 18th Edition, 2012; Churchill Livingstone (ELSEVIER).
10. Willfried Schramm, Gustavo Barriga Angulo, Patricia Castillo Torres, Anthony Burgess-Cassier; A Simple Saliva-Based Test for Detecting Antibodies to Human Immunodeficiency Virus, Clinical Diagn Lab Immunol. 1999 Jul; 6(4): 577-580.
11. Shibani Shetty, Sudeendra Prabhu, Kaveri Hallikeri and Rekha Krishnapillai; Laboratory Tests for HIV: Diagnosing, Monitoring and Managing AIDS – An Overview (Review article), International Journal of Oral and Maxillofacial Pathology; 2011:2(1):20-28.
12. PASCOE, S. J.; LANGHAUG, L. F.; Mudzori, J.; Burke, E; HAYES, R.; Cowan, F. M.; Field Evaluation of Diagnostic Accuracy of an Oral Fluid Rapid Test for HIV, Tested at Point-of Service Sites in Rural Zimbabwe, AIDS Patient Care STDS, 2009; 23(7): 571-6.
13. Raph L. Hamers, Ingrid H. de Beer, Harold Kaura, Tang. Rinke de Wit,. Diagnostic accuracy of two oral mucosal fluid based tests for HIV surveillance in Namibia, Journal of Acquired Immunodeficiency Syndrome, 2008; 48(1):116-118.
14. Per Brandtzaeg; Secretory Immunity with special reference to the Oral Cavity, study supported by the Research Council of Norway (GLOBVAC Project No 185872/Ent Vac) and Oslo University Hospital, Rikshospitalet, assisted by Ms Hege Eliassen.
15. Kesari S S, Kesarwani P, Mishra M; Prevalence and Pattern of Self-medication Practices in Rural Area of Barabanki, Community Medicine, Indian Journal of Clinical Practice; 25(7), December 2014.
16. D.R. Arora, Maheshwari M and B Arora., Rapid Point of Care Testing for Detection of HIV and Clinical Monitoring ISRN AIDS 2013.
17. NACO, Guidelines for HIV Testing, March, 2007, Ministry of Health and Family Welfare, Govt of India.; and Manual for HIV Testing, March 2007, NACO.
18. Behavioral and Biomedical Research, Family Health International, PO Box 13950, Research Triangle Park, North Carolina, USA, NC 27709.
19. Repeat use of Emergency Contraceptive Pills in Urban Kenya and Nigeria;
20. Emergency Contraception: Knowledge and Attitudes of Nursing Students; Public University in Golas (a state in Brazil).
21. Walensky RP, Paltiel AD., Rapid HIV testing at home: does it solve a problem or create one? Annal InternMed 2006 Sep 19; 145(6):459-62.
22. A David Paltiel, PhD and Rochelle P Walensky, MD, MPH, Home HIV Testing: Good News but Not a Game Changer.
AUTHORS:
1. S. V. Lavanya
2. C. Subhashini

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Microbiology, NRI Institute of Medical Sciences, Sangivalasa, Visakhapatnam.
2. Assistant Professor, Department of DVL, Andhra Medical College.

FINANCIAL OR OTHER COMPETING INTERESTS: None

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:
Dr. S. V. Lavanya,
Associate Professor,
NRI IMS, Visakhapatnam Dist,
Andhra Pradesh.
E-mail: venkatalavanyasayam@gmail.com

Date of Submission: 13/06/2015.
Date of Peer Review: 15/06/2015.
Date of Acceptance: 13/07/2015.
Date of Publishing: 08/08/2015.