

## Diagnosis of HIV infection using rapid oral fluid detection kit and evaluation of its diagnostic accuracy – a case control study

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### Abstract

**Method:** The informed consent was obtained and samples were collected from two groups. Each group consists of 60 samples. The first group involved known HIV positive patients. The second group involved the control group of patients who were above 18 years of age and patients who were found negative by rapid assay test.

Participants were excluded if they were pregnant, had other systemic diseases which compromise immunity and with chronic debilitating condition that would preclude an informed consent.

This rapid test is a manually performed, visually read, 20 minute lateral flow immunoassay test for the qualitative detection of antibodies to HIV-1 in oral fluid. The device plastic housing holds an assay test strip comprised of several materials that provide the matrix for the immunochromatography of the specimen and the platform for indication of the test results.

**Results:** The results were 100 % for both seropositive and seronegative samples. The results obtained by the rapid test using oral fluid were the same as that obtained by serum tests.

**Conclusion:** Our study finds 100% result with regards to sensitivity and specificity hence oral fluid can be used for screening of HIV infection as it is comparable to serum test.

**Key Words:** HIV, Oral fluid, Rapid test

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### Introduction

Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are two words that conjure up an image of fear, social stigma, suffering and finally death. The first case of AIDS was reported in U. S in the morbidity and mortality weekly report on June 5, 1981<sup>1</sup>. The Centre for disease control and prevention introduced the term AIDS in September 1982<sup>2</sup> which is caused by HIV. Two types of HIV are recognized: HIV- 1 and HIV-2. The virus was originally referred as Lymphadenopathy – associated virus (LAV) and Human T- cell Lymphotropic virus type - 3 (HTLV). The unifying name HIV was adopted by the International committee on taxonomy of viruses in the year 1986<sup>2</sup>. AIDS is characterized by immune suppression associated with a spectrum of clinical manifestations that include opportunistic infection, secondary neoplasm and neurological manifestations<sup>3</sup>.

Laboratory diagnosis can either be by direct or indirect method. In indirect method detection of anti – HIV antibodies is the main parameter in testing for HIV. In the direct method, diagnosis is made either by demonstrating the presence of the virus or detection of nuclear material or antigen<sup>4</sup>. The most commonly used specimen for detection of HIV is blood. Other fluids like plasma, serum, oral fluid and urine could also be used<sup>5</sup>.

Oral fluid offers an alternative to serum as a biological fluid that can be analyzed for diagnostic purposes. Oral fluid contains locally produced and serum derived markers that are useful in diagnosis of variety of systemic disorders like autoimmune disorders, cardiovascular diseases, steroid levels, drug

monitoring, and various infectious diseases<sup>6</sup>. Oral fluid fits the role for rapid testing admirably and it is feasible to use oral fluid for the detection of HIV infection using sophisticated techniques such as IgG antibody capture enzyme linked immunosorbent assay (GACELISA)<sup>7</sup>.

Oral fluid has various advantages it is a safe sample to collect, the procedure is noninvasive and painless, Infectious virus in oral fluid is rare making oral fluid sample more readily disposable, adequate oral fluid sample can be collected. Considering the numerous advantages, oral fluid based rapid HIV testing can be used as an alternative proposal for Indian scenario. This study is undertaken to get a more comprehensive picture to evaluate the diagnostic accuracy of OraQuick rapid detection kit using oral fluid.

### Aims

The aims of the study include determination and evaluation of the diagnostic accuracy of oral fluid, patient acceptability of oral fluid when compared to finger stick and to evaluate the feasibility and usage of the rapid oral fluid test for diagnosis of HIV infection with the objectives to determine the Sensitivity, specificity, positive and negative predictive value.

### Materials used

Developer Solution Vial, reusable Test Stand, test Device, absorbent Packet, timer, antiseptic wipe, disposable gloves and biohazard disposal container.

## Method

This study was undertaken to evaluate the performance of the rapid test utilizing oral fluid as a diagnostic medium for HIV infection. The study was initiated after obtaining the ethical committee clearance of Vinayaka Mission's Sankarachariyar Dental College, Salem. The informed consent was obtained and samples were collected from two groups, the first group involved 60 HIV positive patients attending a nongovernmental organization. They were tested by rapid assay test and were under treatment. CD4 count available by the patient at the time of the procedure was also recorded. The second group involved 60 patients attending Vinayaka Missions Kirupanandavariyar Medical College, Salem who were above 18 years of age and patients who underwent HIV screening and found negative by rapid assay test were subjected to oral fluid test.

Participants were excluded if i) they were pregnant or breast feeding, ii) had other systemic diseases which compromise immunity like tuberculosis, bone marrow suppression iii) associated with chronic debilitating condition iv) they had mental health disorders that would preclude informed consent.

**Biological principles of the test<sup>8</sup>:** The OraQuick® Rapid HIV-1 antibody test is a manually performed, visually read, 20 minute immunoassay for the qualitative detection of antibodies to HIV-1 in human whole blood/ saliva. The test device containing the flat pad is taken and the sample is collected by swabbing the flat pad between the gingiva and upper and lower lips.(Fig. 1)

Oral fluid sample/ whole blood specimen collected is transferred into the vial of developer solution, followed by the insertion of the test device. The developer solution facilitates the flow of the specimen into the device and onto the test strip. As the diluted specimen flows through the device, it rehydrates the protein-A gold colorimetric reagent contained in the device. As the specimen continues to migrate up the strip, it encounters the T zone. If the specimen contains antibodies that react with the antigens immobilized on the nitrocellulose membrane, a reddish-purple line will appear, qualitatively indicating the presence of antibodies to HIV-1 in the specimen. Further up the assay strip, the sample will encounter the C zone. This built-in procedural control serves to demonstrate that a specimen was added to the vial and that the fluid has migrated adequately through the test device. A reddish-purple line will appear in the C zone during the performance of all valid tests, whether or not the sample is positive or negative for antibodies to HIV-1. The test results are interpreted after 20 minutes but not more than 60 minutes after the introduction of the test device into the developer solution containing the test specimen.

## Interpretation of results

If a line appears in the result window in the area adjacent to the triangle labeled "C" and a line appears in the area adjacent to the triangle labeled "T", the result is considered Reactive.(Fig. 2) If a line appears in the result window in the area adjacent to the triangle labeled "C", and no line appears in the area adjacent to the triangle labeled "T", the result is Non-Reactive.(Fig. 3)

A test is Invalid if any of the following occurs: no line is present in the area adjacent to the "C" triangle a red background in the result window makes it difficult to read the results after 20 minutes any of the lines appear outside of the areas adjacent to the "C" or "T" triangle.

## Results

This study was undertaken to evaluate the performance of the rapid test utilizing oral fluid as a diagnostic medium for HIV infection. With this aim, 120 oral fluid samples were collected, out of these 60 were seropositive and 60 seronegative on standard testing algorithms. In our study, the test acceptability was 100%. Out of the 60 seropositive samples all the 60 samples were correctly identified positive by the test kit using oral fluid. Of the 60 seronegative samples, all the 60 samples were identified negative by the test kit using oral fluid. The results were 100% for both seropositive and seronegative samples. The results obtained by the OraQuick test kit using oral fluid were the same as that obtained by serum tests. Thus there were no false positives or false negatives.

The utility of a given test kit can be judged by evaluating how often it's results are correct in two groups: a) a group in whom HIV infection is known to be present and therefore test result should be positive and b) a group in whom HIV infection is absent and therefore the test results should be negative.

To assess the quality of a test it needs to be compared to a method which is widely accepted as being the best available, also known as the 'gold standard' which in our case was the standard algorithm for HIV testing using serum. For the purpose of the study the gold standard is considered to have 100% sensitivity and specificity.



Fig. 1: Procedure



**Fig. 2: Result Interpretation Reactive**



**Fig. 3: Result Interpretation Non-reactive**

## Discussion

“There is clearly room for further studies using salivary testing”<sup>9</sup>, taking cue from this statement of Dr. Scully, this study was undertaken to evaluate the performance of a rapid immunoassay specifically designed for HIV testing using saliva, as applicable to Indian scenario.

Since late 1980’s interest in salivary antibody testing has been elicited with the pioneering work of Archibald et al<sup>10</sup> and Parry et al<sup>11</sup> followed by numerous other methods devised towards salivary antibody testing which include ELISAs, antibody capture assays, rapid assays and western blots. The early studies documented the presence of HIV -1 antibodies in saliva and reported that, although a salivary test was feasible, it yielded unreliable results.

Various studies have reported the sensitivity of early tests in detecting HIV antibodies in oral fluid from seropositive individuals was between 50%-100%, an unacceptably low range for a diagnostic modality. Tests designed for use with serum samples were used with saliva without any modifications which gave less than optimal indices whereas at the same time the use of sophisticated tests such a RIPA and GACELISA<sup>12</sup> was providing excellent results. The turning point in the utilization of saliva as a sample was the recognition of the differing levels of IgG (HIV specific antibody) in serum and saliva. It was found that saliva demonstrates about 1:1000 level of IgG as compared to serum from then on, with the introduction of various specialized

collection devices, efforts were made to obtain a sample rich in IgG. The tests, were also modified to take the lower concentration of immunoglobulin into consideration which included increased sample volume, decreased diluent and changed cutoff value, in case of serum ELISAs. In a benchmark study by Gallo et al<sup>13</sup> involving specimens from 3570 subject, the sensitivity was reported to be 99.9% by this method.

Specific tests for saliva were developed. Grant et al<sup>14</sup> used Omni Sal and a filter paper assay with a reported sensitivity of 95% and specificity of 99%. Leow et al<sup>15</sup> used OraScreen saliva dipsticks the sensitivity and specificity of this test was 94.7% and 99.5% respectively. In a study in India the Oraquick HIV 1/ 2 rapid test was used with low sensitivity (75%), which was thought to be attributed to dehydration<sup>16</sup>. Later studies after 2004 found Oraquick test to be highly sensitive and specific.

This study was carried out using oral fluid samples, the kit performed well with all the HIV positive cases reporting positive and all HIV negative cases reporting negative no false positives or false negatives were reported. Thus our study compares well with the results of Reynolds<sup>17</sup>, Bulterys<sup>18</sup>, Landrum<sup>19</sup>, Delaney<sup>20</sup>, Jamieson<sup>21</sup> and pant pai<sup>22</sup> who, in different studies, reported sensitivities as 100%, 100%, 100%, 99.1%, 100% and 100% respectively and sensitivities of 100%, 99.9%, 100%, 99.6%, 99.92%, and 100% respectively.

## Conclusion

This study was undertaken to evaluate the performance of the OraQuick rapid detection kit in the diagnosis of HIV, utilizing oral fluid as a diagnostic medium for HIV infection. The test results were compared with standard serum testing procedures. The various advantages of using oral fluid over serum were educated to both the control and the experimental group. The acceptability of the test was also analyzed and the test was found to be highly acceptable by the subjects. Our findings support the results of other published studies, utilizing rapid oral fluid testing for detection of HIV infection. Sensitivity, specificity, positive and negative predictive values have been calculated to be 100% thus oral fluid can be used for screening of HIV infection as it is comparable to serum test. Thus we conclude that rapid oral fluid testing is feasible and can be used for surveillance as well as part of a diagnostic algorithm, within the limit applicable to standard tests used for antibody detection.

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