

# Field Evaluation of Calypte's AWARE™ Blood Serum Plasma (BSP) and Oral Mucosal Transudate (OMT) Rapid Tests for Detecting Antibodies to HIV-1 and 2 in Plasma and Oral Fluid

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**Abstract:** As programs to prevent and care for HIV-infected persons are scaled-up in Africa, there is the need for continuous evaluation of the performance of test kits that could best support these programs. The present study evaluated the sensitivity, specificity, ease of use, and cost of AWARE™ Blood Serum Plasma (BSP) and Oral Mucosal Transudate (OMT) Rapid HIV-1/2 test kits using real-time and archived samples of HIV-infected persons from Cameroon. Matched whole blood and OMT specimens were collected prospectively from HIV-positive and HIV-negative persons from different regions of Cameroon and tested using the AWARE™ BSP and OMT test kits, respectively. These results were compared to the gold standard that included a combination of Determine HIV-1/2 and Enzygnost HIV-1/2. The BSP Rapid test kit was further evaluated using well characterized panels of HIV-2 and HIV-1 group O samples. Cost and end-user analysis of the OMT test kit was done by comparing its actual cost, consumables, safety, bench time and manipulation with other test kits. Of the 732 matched samples, 412 (56.3%) and 320 (43.7%) were from females and males, respectively. Of these samples, 23 (3.1%) gave discordant results between Determine HIV-1/2 and Enzygnost HIV1/2 and were excluded from the analysis. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the AWARE™ BSP were 100%. The AWARE™ OMT had 98.8% sensitivity, 98.9% specificity, 98.0% PPV and 99.4% NPV. The results of a well-characterized archived panel of HIV-2 (n=7) and HIV-1 group O (n=3) samples using the AWARE™ BSP Rapid test kit gave 100% concordance. Total per patient cost of the AWARE OMT rapid test kit was US\$4.72 compared to a mean cost of US \$7.33 ± 0.11 for the other test kits. Both the AWARE™ BSP and OMT Rapid test kits demonstrated high sensitivities and specificities on all samples tested and were well adapted for use in resource-constrained settings with high HIV heterogeneity such as Cameroon. The AWARE™ HIV-1/2 OMT Rapid test kit appears to be the cheapest, safest and easiest to use compared with other available test kits.

## BACKGROUND

There are concerted efforts by multilateral partners and international bodies such as the World Health Organization (WHO), the Global Fund for AIDS, Tuberculosis, and Malaria (GFATM), the World Bank (WB), and the US President's Emergency Plan for AIDS Relief (PEPFAR), to make antiretroviral drugs (ARVs) available in developing countries. To meet these goals, there is the need for improved strategies to counsel and test millions of people in different settings such as the voluntary counselling and testing (VCT) sites, antennal clinics, programs to prevent mother-to-child transmission (PMTCT), and Point-Of-Care (POC) sites who will eventually benefit from ARVs [1-3]. HIV rapid testing devices that are easy to use, do not require refrigeration, and are less evasive will be very suitable for this purpose. HIV rapid test kits have generally been shown to be less expensive to run, portable and with a longer shelf-life. They are more adaptable for field use [4, 5] and screening of high risk and far-to-reach populations [6] mostly located in resource-poor settings. In fact, they have reduced the time between

testing and release of results to minutes from days or weeks when compared with other assays; thus improving the rate at which people return for their results.

Rapid test kits have been shown in certain settings to be incapable of detecting infections by some genetic subtypes of the HIV-1 [7-9]. This calls for the need to regularly update diagnostic principles and methods, and the continuous evaluation of test kits to make sure that they can detect the newly emerging viral strains.

Cameroon is particular in terms of the HIV/AIDS pandemic because of the high heterogeneity of the virus. The two main types of the virus, HIV-1 and HIV-2, and the three main groups of HIV-1: Group M (M for Major), Group O (O for Outlier), and Group N (N for Non-M / Non-O or New), have been documented in Cameroon [10-15]. The HIV-1 Group N virus was first discovered in Cameroon and till date, it has not been reported elsewhere [15]. This complex HIV diversity may have an impact on the effectiveness of HIV diagnostic test kits on samples from Cameroon [16]. Efforts aimed at scaling up ARV treatment and early identification and careful orientation of newly HIV-infected persons are currently the main objectives of the Cameroonian National AIDS Committee. Because of this complex viral diversity within its population, there is the need for

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continuous rapid test kits selection, evaluation and proposal of simplified algorithms that could be used for HIV diagnosis within its far to reach population particularly those in the rural areas.

The use of blood as the sole medium for testing for HIV can be problematic in some groups of persons such as children, newborns, immune-compromised and obese individuals in whom getting blood samples can be particularly difficult. It has been shown that this problem could be eased by using other body fluids such as oral fluid [17-19] because its collection involves non-invasive techniques, unlike phlebotomy and fingertip pricking. This could be more adaptable in resource-poor settings with improved participant acceptability.

The aim of this study is to evaluate the sensitivity, specificity, ease of use, and cost of AWARE™ BSP and OMT HIV-1/2 Rapid Test kits [20] on whole blood and oral fluid using real-time and archived samples from Cameroon.

## MATERIALS AND METHODS

### Study Design and Population

Between August 2005 and January 2006, we carried out a cross-sectional evaluation of the AWARE BSP and OMT HIV-1/2 Rapid test kits during free screening campaigns for HIV infection from presumed HIV negative persons attending voluntary counselling and testing services (VCT) and from support groups of known persons living with HIV/AIDS (PLWHA) in Cameroon. These individuals were recruited from two different locations namely: the Cameroon Development Corporation (CDC) plantations, Tiko, a semi-urban area in South West Province, and Shemka Medical Foundation in the Nkolbisson neighborhood of Yaounde, the capital city of Cameroon. Initial contacts were made with leaders of PLWHA support groups during which the purpose of the study was clearly explained. This was followed by a larger meeting involving all members of the group and study team during which the objectives of the study were further explained. A subsequent appointment was taken for sample collection and testing. VCT campaigns were also organized for sample collection and testing from presumed HIV-negative persons during the last week of November 2005 as part of the activities marking the World AIDS day that occurred on December 1<sup>st</sup> 2005.

### Inclusion and Exclusion Criteria

Participants were eligible if any of the following criteria were met: >15 years of age; known HIV positive status; unknown HIV status; and willingness to sign an informed consent form. Participants were excluded if they had ever received ARV.

### Ethical Considerations

This study received approval from the Ethical Review Board of the Ministry of Public Health Cameroon. Concerning PLWHA, initial permission was obtained from various leaders of the groups, further explanation and clarification was obtained during the general assembly. Permission was also obtained from the Administration of the VCT centres from where participants were recruited. Furthermore, on the

day the samples were collected, all participants received a one-on-one pre-test counselling that was followed by reading and signing of consent forms for their acceptance to participate. Only those who accepted and signed the consent forms were enrolled into the study.

### Sample Collection and Testing

After collection of minimal socio-demographic information, participants were received by laboratory technicians for sample collection. Matched whole blood and oral mucosal transudate (OMT) specimens were collected from each participating individual. Approximately 5 mls of blood were collected by venu-puncture into EDTA-anticoagulant tubes bearing the corresponding code number of the participant. The OMT specimens were collected using the AWARE™ HIV-1/2 OMT collection device provided within the test kit. A portion of the whole blood and all of the OMT samples were tested on site using the AWARE™ HIV-1/2 BSP and OMT Rapid test kits. The remaining portion of the blood was transported in coolers containing ice packs to the Central Laboratory in Yaounde, centrifuged, plasma stored at -20 °C and later tested in parallel using Determine HIV1/2 rapid (Abbott Laboratories, Japan) and the Enzygnost HIV1/2 Integral ELISA (Dade Behring, Germany); the current diagnostic algorithm in Cameroon and considered the gold standard for this study. All testing was done following the recommendations of the test manufacturers' product insert.

### HIV-2 and HIV-1 Group O Panels

In order to determine the sensitivity of the AWARE™ HIV1/2 BSP on some rare HIV variants common in Cameroon, we further tested this kit using a well characterized panel of seven HIV-2 and three HIV-1 group O samples. Each of these panels had been well characterized using a variety of serologic and virologic assays (U.S. Food and Drug Administration).

### Quality Assurance and Quality Control

At the beginning of the study, all staff involved was trained on how to use the AWARE™ rapid test kits, the Determine HIV1/2 rapid test, and the Enzygnost HIV1/2 Integral ELISA according to their respective manufacturers' instructions. Sample collection procedures and laboratory safety measures were also reviewed and expiration dates of all kits checked. In-house controls and kit controls were run daily and the environmental temperature at which tests were run was noted daily. Results on test strips were read and cross-checked by another technician. Ten percent (10%) of the samples were selected at randomly during the course of the study and retested to ensure accuracy of the testing staff. All test results were entered in an excel spreadsheet and later on cross-checked for entry errors.

### Statistical Analysis

The sensitivity, specificity, positive and negative predictive values of each of the AWARE™ HIV1/2 Test kits were evaluated by using the results of the Determine HIV1/2 rapid test and the Enzygnost HIV-1/2 Integral ELISA as the gold standard. If a specimen was found to be discordant between

the gold standards, the sample set results were excluded from the calculation. Sensitivity was calculated as the number of HIV-positive samples detected by the assay under evaluation, divided by the total number of confirmed HIV-positive samples, multiplied by 100. Specificity was calculated as the number of HIV-negative samples detected by the assay under evaluation, divided by the total number of confirmed HIV-negative samples, multiplied by 100. The positive predictive value (PPV) was derived from the true positives (TP) divided by the sum of true positives (TP) and false positives (FP). The negative predictive value (NPV) was calculated as the true negatives (TN) divided by the sum of false negatives (FN) and true negatives (TN). All analyses were done in Microsoft Excel 2003 and 95% Confidence Intervals were calculated in OpenEpi Diagnostic or Screening Test Evaluation 1.0.

### Total Laboratory Cost

Cost and end-user analysis of the AWARE™ HIV-1/2 BSP and OMT Rapid test kits were compared with other commonly used blood and oral fluid test kits found in the Cameroon market. Parameters considered included cost of the test devices, needed for additional materials and supplies, and labor cost. The general safety of the devices in regard to the patient, counsellors, and laboratory staff was noted.

### RESULTS

The two kits evaluated were based on Immuno-chromatographic and either used recombinant or synthetic peptides from the gp36 and or 41 regions. The gold standard included the Enzygnost HIV1/2 Integral, a fourth generation ELISA test that detects both HIV antigens and antibodies. Of the 732 participants, 248 (34.98 %) and 461(65.02%) were confirmed positive and negative, respectively. Of these participants, 412 (56.3%) were female. A breakdown of the sero-status of the participants and their enrollment location are shown in Table 1. Results of 23 participants were discordant between the two gold standard test kits, and were considered invalid and excluded from the data analysis.

Of the 732 samples, 709 (96.9%) matched venous whole blood and oral fluid specimens were tested and included in the study results (Table 2). All 248 and 461 patients confirmed positive and negative blood samples respectively by gold standard were equally positive and negative by the AWARE™ HIV1/2 BSP test kits, thus the diagnostic accuracy of the AWARE™ BSP test kit was 100% sensitivity, specificity, positive and negative predictive values. The AWARE™ HIV1/2 OMT test detected a total of 246 positive samples, of which 243 were true positives; thus, a sensitivity of 98.8% and positive predictive value of 98%. Simi-

larly, of the 458 negative samples by the AWARE™ HIV1/2 OMT test, 453 were true negatives; thus, a specificity of 98.9%, and negative predictive value of 99.4 % (Table 2). The results of a well-characterized archived panel of HIV-2 (n=7) and HIV-1 group O (n=3) samples using the AWARE™ BSP Rapid test kit gave 100% concordance.

**Table 1. Overall Sero-Status of Participants Enrolled Per Collection Site**

Sero-Status	Yaounde	Tiko	Total
Confirmed HIV-Positive	128	120	248
Confirmed HIV-Negative	382	79	461
Discordant samples between gold standard	20	3	23
<b>Total</b>	<b>530</b>	<b>202</b>	<b>732</b>

A laboratory cost analysis between the AWARE™ test kits and other available kits in Cameroon showed that the AWARE™ HIV1/2 OMT had a total per patient cost of \$4.72 compared to \$7.33 ± 0.11, the mean cost of other tests and was the safest and easiest to use when compared with other blood and oral fluid test kits (Table 3).

### DISCUSSION

HIV serodiagnosis particularly in resource limiting-settings has been hindered by several factors, including the need for trained personnel, high cost of equipment and reagents, and lack of electricity and laboratory infrastructure [21]. This has undermined the importance of current efforts to scale-up ARV programs and other HIV preventive measures within these populations. Our field evaluation of the AWARE™ HIV-1/2 BSP and OMT Rapid test kits using samples from Cameroon have demonstrated that these kits have high sensitivity, specificity, are easy to use, and thus, could serve as POC rapid test, hence circumventing the current HIV diagnostic challenges.

The AWARE™ HIV1/2 BSP rapid test kit was 100% sensitive and specific with a 100% NPV and PPV. This is consistent with the results obtained in Thailand [22] where a sensitivity of 100% and a specificity of 100% were obtained in a similar field evaluation of the same test kit. Thailand is an area where only the HIV-1 B subtype predominates [23], while all HIV-1 subtypes have been shown to co-exist in Cameroon including recombinant forms and HIV-1 group N which has been reported only in this country [10-15]. The consistency in performance of this test kit in these populations of different viral diversity makes it a reliable tool for

**Table 2. Summary of Performance of the AWARE™ Rapid Test Kits**

Test	No of Samples		False Positive	False Negative	Sensitivity (95 CI,%)	Specificity (95 CI,%)	Predictive Value (%)	
	True Positive	True Negative					Positive (95 CI,%)	Negative (95 CI,%)
BSP	248	461	0	0	100 (98.5-100)	100 (99.2-100)	100 (98.5-100)	100 (99.2-100)
OMT	243	453	3	5	98.8 (96.5-99.6)	98.9 (97.1-99.5)	98.0 (95.4-99.1)	99.4 (98.1-99.8)

**Table 3. Total Laboratory Cost Between Calypte Products and other Commonly Used Rapid Test Kits in Cameroon**

Test Kits	AWARE OMT (\$)	AWARE BSP (\$)	Bioline (\$)	Determine (\$)	CAMSTIX (\$)	Oraquick (\$)
Cost of Test Device	3.000	1.700	1.700	1.34	1.49	5.77
Supplies not Included with Test	0.116	3.140	3.140	3.540	3.86	0.116
Personnel costs (based on 1 hour work/person)	1.60	2.50	2.50	2.50	2.50	1.60
<b>Total Cost (\$)</b>	<b>\$4.716</b>	<b>\$7.33</b>	<b>\$7.33</b>	<b>\$7.18</b>	<b>\$7.35</b>	<b>\$7.48</b>

HIV testing. In fact, the AWARE BSP test kit had a 100% concordance with a panel of well characterized HIV-2 and HIV-1 group O samples from Cameroon. These are rare non B subtype variants of the HIV that are common in the West and Central African countries [24]. This further shows that the AWARE™ HIV1/2 BSP test kit can also be reliably used for rapid HIV testing in countries within this sub region.

The AWARE™ HIV1/2 OMT rapid test kit had a sensitivity and specificity of 98.8% and 98.9% respectively in this study. Similar field evaluation of the same kit gave sensitivities and specificities of 100% each in Tanzania [25] and 99.6% and 100% respectively in South Africa [26]. Despite the high viral diversity that has been reported in Cameroon and which could have an impact on HIV test kits performance, current results obtained for the AWARE™ HIV-1/2 OMT evaluation still falls within the WHO recommended range for test kits to be used for HIV diagnosis [4]. A laboratory cost analysis showed that this test kit was the least expensive, safest and easiest to use compared with other available test kits. Historically, most countries have structured their HIV testing programs using blood-based tests that remain the “gold standard” of HIV testing. However, the effectiveness of these traditional blood tests is impaired by issues of patient appeal, risk to patients and risk to health care workers. The fact that the AWARE™ HIV1/2 OMT test uses oral fluid instead of blood, implies that its sample collection technique is non-invasive; thus making it very adaptable for use in settings where trained manpower, acceptability and collection devices may compromise HIV diagnostic initiatives. Despite this, it is important to note here that a recent literature search shows that the New York City Department of Health and Mental Hygiene (NYC DOHMH) noticed some unexplained sporadic false positive results using the OraQuick Advance Rapid HIV-1/2 Antibody oral fluid test [27]. These findings underscore the importance of confirming all reactive HIV tests, both from oral fluid and whole-blood specimens.

## CONCLUSION

In summary, this current evaluation shows that both the AWARE™ HIV-1/2 BSP and OMT Rapid test kits performed well on samples from Cameroon, a country with complex HIV genetic diversity against a gold standard kit which included a fourth generation ELISA that could detect both HIV antigens and antibodies. This performance coupled with the user friendliness of these kits, suggests that their inclusion in the national HIV testing algorithm of this country will have tremendous impact in scaling up its HIV diag-

nostic program with positive gains in the number of people to benefit from ARV drugs treatment programs and other care and prevention efforts. Because of these characteristics, we also suggest that these kits should further be evaluated in other regions of the world particularly in developing countries where their use could have significant public health impact on HIV prevention and clinical management.

## ACKNOWLEDGEMENT

This work was supported by Calypte grant #V010305R from Calypte Biomedical Lake Oswego, OR, USA.

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Received: December 6, 2008

Revised: January 23, 2009

Accepted: January 29, 2009

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