Poster #20

# New Raw Materials for the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test Design

**Graham D. Yearwood, Ph.D.**, Mark J. Fischl, Geraldine B. Guillon, Ph.D., Jay Lynn Unangst, Lisa A. Kurtz, Casey H. Snipes
OraSure Technologies, Inc., Bethlehem, PA 18015

OraSure Technologies, Inc.

## Introduction

The OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test is FDA approved to detect antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) in whole blood (from a finger puncture or a venipuncture), plasma, and oral fluid. The assay strip in the device consists of a membrane of nitrocellulose striped with control and test lines. The test line is striped with both HIV-1 and HIV-2 synthetic peptides bound to modified avidin replacement (a biotin-binding protein). Antibodies bound to the immobilized antigens are visualized by the binding of colloidal gold labeled with protein A. The presence of HIV-1 and/or HIV-2 antibodies in the tested specimen is indicated by the appearance of a reddish-purple colored band at the test line location of the device within 20 to 40 minutes.

In this study, the new test composition (modified avidin replacement with streptavidin BSA) was evaluated through design validation. Studies were conducted to characterize functional performance in selected samples, low titer panels, seroconversion panels (23), and unrelated medical conditions. In seroconversion panels, results were compared to 3rd generation Enzyme-Linked Immunosorbent Assay [EIA] and specifically to the Bio-Rad GS HIV-1/HIV-2 PLUS O published in the seroconversion panel package inserts EIA by assessment of the first consecutive reactive specimen to the respective FDA-approved EIA result. The OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test has the following Package Insert Claims.

| Claim | Sensitivity | Specificity |
|---|---|---|
| HIV-2 | 100% | - |
| Oral Fluid | 99.3% | 99.8% |
| Whole Blood | 99.6% | 100% |
| Plasma | 99.6% | 99.9% |

Centers for Disease Control Prevention (CDC) and the Association of Public Health Laboratories (APHL) have issued new recommendations based on HIV tests approved by the Food and Drug Administration (FDA) as of December 2012 and scientific evidence, laboratory experience, and expert opinion collected from 2007 through December 2013.

These guidelines will provide critical direction in ending the epidemic by directing the use of the most sensitive test available. OraSure Technologies Inc. has been a leader in the rapid diagnostic testing industry in both research and development of highly accurate and efficient methods of oral fluid and blood testing for HIV. The Company launched the nation's first FDA-approved oral fluid rapid CLIA waived HIV test in 2004, and has successfully conducted more than 35 million tests over the past 13 years with its laboratory partners.

These recommendations do not include the rapid HIV-1/HIV-2 antigen/antibody combination test approved by the FDA in August 2013 (for which evidence of performance in the algorithm was insufficient) or HIV-2 nucleic acid tests (NATs), which lack FDA approval (Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations[1]).

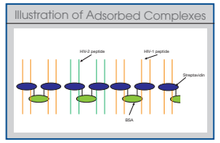
False Positive and False Negative results matter; in the absence of a suitably sensitive rapid HIV-1/HIV-2 antigen/antibody combination test, [2-9] rapid tests that perform similarly to laboratory antibody tests have a key role in proper diagnosis of HIV infection. The OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test design has proven patient acceptability; test acceptance is especially facilitated by specimen flexibility. The small sample size (5 µL) matters to patients and Oral fluid is still the patient preferred method of testing[10]. The test is simple, rapid HIV-1/2 antibody testing with results in 20 minutes; accurate, results with >99% sensitivity and specificity across all specimen types and versatile, testing platform suitable for both clinical and non-clinical settings.

In January 2009, OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test shelf life was improved initially to 12-months. The United States Food and Drug Administration (FDA) approval was based on process improvements made to the manufacturing and product packaging of OraQuick *ADVANCE®* resulting in this new version of the test. Subsequently, this allowed the test to gain 30 month expiry dating.

Following the approved process improvements, OraSure launched a Post-Market Surveillance program designed to monitor product performance with sentinel sites[10].The results include Whole Blood specificity of 99.98% (n = 1,040,342 samples) and Oral Fluid specificity of 99.94% (n = 1,097,869 samples).

This development effort identified optimal raw material properties of Avidin for the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test. High polymerization allows increased adherence to nitrocellulose for optimal sensitivity. Maximum deglycosylation; maintains specificity. OraSure Technologies Inc. developed a modified avidin replacement process based on the known critical to quality attributes, a conjugate of streptavidin (SA) and bovine serum albumin (BSA) which has the correct analytical and functional characteristics: recombinant streptavidin already lacks glycosylation and the controlled conjugation process ensures suitable physical and functional properties. It is combined with an enhanced blocker pad composition to maintain compatibility with Streptavidin-BSA Conjugate.

Schematically the passively adsorbed complexes at the test line are illustrated below:




The conjugate permits orientated adsorption of the complex such that the immunoreactive peptides are more available for detection.

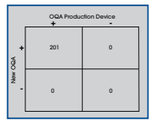
## Methods

Design validation has been completed via numerous Non-Clinical and Clinical studies, verifying the product claims have not been altered as a result of the process change to replace Modified Avidin with Streptavidin. There were a total of eight (8) non-clinical studies used to evaluate the overall performance of the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test (SA-BSA replacement for Modified Avidin): HIV-1 Sensitivity; HIV-2 Sensitivity; Specificity (fresh plasma); Low and Mixed Titer Panels; Worldwide Panels; Interfering Substances and Interfering Medical Conditions, Seroconversion Panels, and Reproducibility. There were a total of two (2) clinical studies executed to evaluate the overall performance of the device (Oral Fluid Specificity and Oral Fluid Sensitivity).

The performance of the Modified Avidin Replacement OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test was assessed with commercially available seroconversion panels. The samples were characterized by FDA licensed assays (Enzyme-Linked Immunosorbent Assay [EIA] and Western Blot). The results were compared to the current, approved OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test and to the Bio-Rad GS HIV-1/HIV-2 PLUS O enzyme immunoassay (3rd Generation EIA). This was determined by comparison of the first consecutive reactive specimen at the 20-minute read time, in the panel series to the respective FDA-approved EIA test results provided by the vendor Certificate of Analysis (CofA).

## Results

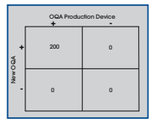
In 2016, OraSure Technologies received FDA approval to implement further raw material and process change with 30-month dating to OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test




**HIV-1 Infected Samples**

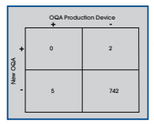
A total of 201 samples were tested and are summarized:

The Modified Avidin Replacement device demonstrated equivalent sensitivity to the current, approved OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test.




**HIV-2 Sensitivity**

Two hundred (200) HIV-2 banked blood samples were reactive with similar visual test line intensities for both the Modified Avidin Replacement Tests and OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Tests.



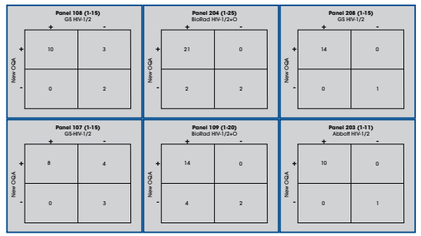

**Specificity**

Each of the three replacement validation lots were evaluated with unique shipments of ~250 fresh negative samples and tested contemporaneously with a current production control device.

Of the 749 fresh negative samples evaluated, the specificity results for the Modified Avidin Replacement validation lots were 100%, 100%, and 99.2%, with a cumulative result of 99.7%. The cumulative specificity for the production control lot was 99.3%.

**Low Titer and Mixed Titer Panels**

Six (6) commercially sourced Anti-HIV low / mixed titer panels were evaluated across two (2) validation lots of Modified Avidin Replacement and the results compared to the current production OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test.




The Modified Avidin Replacement lots displayed equivalent sensitivity to the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test lot (control) when these low and mixed titer panels were tested.[11] Sample 204-25 was characterized as an HIV-1 Western Blot indeterminate with varying reactivity in EIA assays and FDA approved HIV rapid tests. Sample 109-13 was characterized as an HIV-1 Western Blot indeterminate and negative on EIA and FDA approved rapid tests. The varying responses recorded for these two (2) samples that were nonreactive in the Modified Avidin Replacement lots suggests that these do not represent an actual differential reduction in sensitivity.

**Worldwide Panels**

This study evaluated the Modified Avidin Replacement Rapid HIV-1/2 Antibody Test device and the current, approved OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test with a commercially available worldwide panel.

All worldwide panel samples (305-01 to 305-20) were highly reactive with similar visual test line intensities for both the Modified Avidin Replacement Test and OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test.

**Specimens from Individuals with Potentially Interfering Medical Conditions and Specimens with Interfering Substances**

The following unrelated medical conditions were tested: Multiparous Women, Sjogrens, Lupus, Rheumatoid Arthritis, Cytomegalovirus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus, Hepatitis E Virus, Human T-Cell Lymphotropic Virus Type I and II and I/II, Rubella, Syphilis, Toxoplasmosis, Herpes Simplex Virus, Dialysis Patients, Chlamydia, Flu Vaccine, Anemia, Scleroderma, and Breast and Colon Cancer.

Of the 237 specimens from 27 unrelated medical conditions evaluated in this study, 223 specimens yielded concordant results with both tests at the 40-minute read time. There were 14 samples reactive at the 40-minute read time, with the current, approved OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test yet were nonreactive in the Modified Avidin Replacement Tests; however, at the time of the study, the specimens underwent multiple freeze–thaw cycles prior to testing. There are no studies available evaluating the effect of multiple specimen freeze-thaw cycles on the performance of the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test.

**Seroconversion Panels**

Data submitted to the FDA included testing on seroconversion panels using the average enzyme immunoassay (3rd Generation EIA) results. A total of 201 seroconversion panel member samples from 23 seroconversion panels were evaluated in this study. The delay days (i.e., the number of days the comparative method detected seroconversion after the EIA) and the 95% confidence intervals were calculated and summarized. The 95% Confidence Intervals (95% CIs) for the Modified Avidin Replacement OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test overlap and are within the 95% CIs range of the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test. Delay days were similar for the devices tested when compared to the FDA-approved EIA CofA data. Moreover, the 95% Confidence Intervals (95% CIs) for the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test are consistent with the performance being comparable to that of 3rd Generation EIA.

Results of testing with 23 seroconversion panels are as follows:

Seroconversion Panels Tested

Table 1:

| Seroconversion Panel | Current OQA Product | | New OQA Lot1 | | New OQA Lot2 | | New OQA Lot3 | | New OQA Average | EIA Average | Current OQA-EIA Average Δ | New OQA-EIA Average Δ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days | Bleed | Days | Bleed | Days | Bleed | Days | Bleed | Days | Days | Days | Days |
| 924 | 40 | 8 | 35 | 7 | 40 | 8 | 35 | 7 | 36.7 | 33 | 7 | 4 |
| 925 | 44 | 5 | 44 | 5 | 44 | 5 | 44 | 5 | 44 | 46.5 | -2.5 | -3 |
| 926 | 27 | 5 | 27 | 5 | 27 | 5 | 27 | 5 | 27 | 27 | 0 | 0 |
| 933 | 27 | 3 | 27 | 3 | 27 | 3 | 27 | 3 | 27 | 24 | 3 | 3 |
| 934 | 7 | 2 | 7 | 2 | 7 | 2 | 7 | 2 | 7 | 9 | -2 | -2 |
| 939 | 103 | 9 | 103 | 9 | 103 | 9 | 103 | 9 | 103 | 103 | 0 | 0 |
| 945 | 20 | 6 | 20 | 6 | 20 | 6 | 20 | 6 | 20 | 13 | 7 | 7 |
| 947 | 20 | 4 | 11 | 3 | 20 | 4 | 20 | 4 | 17 | 14.5 | 5.5 | 3 |
| 959 | 14 | 4 | 14 | 4 | 14 | 4 | 14 | 4 | 14 | 11.5 | 2.5 | 3 |
| 965 | 14 | 5 | 12 | 4 | 12 | 4 | 12 | 4 | 12 | 12 | 2 | 0 |
| 967 | 19 | 5 | 19 | 5 | 19 | 5 | 19 | 5 | 19 | 17 | 2 | 2 |
| 968 | 33 | 9 | 33 | 9 | 33 | 9 | 33 | 9 | 33 | 26 | 7 | 7 |
| 969 | 72 | 9 | 70 | 8 | 70 | 8 | 70 | 8 | 70 | 70 | 2 | 0 |
| 970 | 14 | 4 | 14 | 4 | 14 | 4 | 14 | 4 | 14 | 10 | 4 | 4 |
| 6243 | 32 | 9 | 32 | 9 | 32 | 9 | 32 | 9 | 32 | 32 | 0 | 0 |
| 9015 | DNS | | 35 | 9 | 35 | 9 | 35 | 9 | 35 | 34 | DNS | 1 |
| 9019 | 38 | 3 | 38 | 3 | 38 | 3 | 38 | 3 | 38 | 38 | 0 | 0 |
| 9032 | 49 | 12 | 36 | 10 | 36 | 10 | 36 | 10 | 36 | 34.7 | 14.3 | 1 |
| 9076 | 74 | 10 | 74 | 10 | 69 | 9 | 69 | 9 | 70.7 | 66 | 8 | 5 |
| 9077 | 52 | 14 | 45 | 12 | 45 | 12 | 45 | 12 | 45 | 54.5 | -2.5 | -10 |
| 9079 | 62 | 15 | 55 | 13 | 55 | 13 | 55 | 13 | 55 | 47 | 15 | 8 |
| 9084 | 49 | 4 | 49 | 4 | 49 | 4 | 49 | 4 | 49 | 49 | 0 | 0 |
| 12008 | 35 | 11 | 33 | 10 | 33 | 11 | 35 | 11 | 34.3 | 33 | 2 | 1 |
| | | | | | | | | | | | | |
| Average | 38.41 | | 36.22 | | 36.70 | | 36.48 | | 36.46 | 34.99 | 3.38 | 1.48 |

The delay days (i.e., the number of days the comparative method detected seroconversion after the EIA) and the 95% confidence intervals were calculated and summarized in Table 2 below:

Seroconversion Delay Days

Table 2:

| Number of Panels | Number of Concordant Results | Number Detected Earlier by EIA | Number Detected Earlier by OQA Current | Average Days EIA | Average Days OQA Current | Delay Days (OQA-EIA) (95% CIs) |
|---|---|---|---|---|---|---|
| 22 | 5 | 14 | 3 | 35.03 | 38.41 | 3.38 (1.2 - 5.5) |

| Number of Panels | Number of Concordant Results | Number Detected Earlier by EIA | Number Detected Earlier by New OQA | Average Days EIA | Average Days New OQA | Delay Days (NewOQA-EIA) (95% CIs) |
|---|---|---|---|---|---|---|
| 23 | 7 | 13 | 3 | 34.99 | 36.46 | 1.48 (-0.1 - 3.1) |

The OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test and the Modified Avidin Replacement OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test (new OQA), had the same number of seroconversion panels detected earlier by EIA (14 panels), the new OQA test had one (1) more seroconversion panel in which seroconversion was recorded (HIV 9015).

**Reproducibility**

Three (3) testing sites, with three (3) operators per site completed whole blood Panel Member (PM) testing according to the protocol and the Package Insert for the OraQuick *ADVANCE®* HIV-1/2 Antibody Test, over the course of three (3) days. Each operator performed five (5) blinded tests from a uniquely assigned kit on each validation lot on each of the three (3) dates.

Each Panel Member test kit consisted of five (5) blinded Panel Members; and each of the nine (9) new OQA device test kit types was assigned a unique number. There were five (5) Panel Member reactivity types. Operators at each site were also assigned an identity number. The same operator number at each site used the same test kit type on the same dates. Test kit types and testing assignments were designed so that each lot was tested once with each of the nine (9) Panel Member Test Kit Types, and nine (9) times with each of the five (5) Panel Member reactivity types per site.

There were 405 Panel Members tested (81 per each PM reactivity type) with 100% concordance results (95% CI: 99.1%, 100.0%) across all three (3) validation lots. Concordance for each of the five (5) reactivity types was 100.0% (95% CI: 95.6%, 100.0%) across all three (3) validation lots. The tests were highly reproducible across device lots, test sites, and test operators.

**Oral Fluid from HIV Negative Subjects**

A total of 768 unique subjects were enrolled in the study. Individuals were able to provide both an oral fluid and a fingerstick sample; however, some subjects only provided one sample (oral fluid or fingerstick).

There were 608 oral fluid tests and 601 fingerstick tests assessed across the three validation lots. Each test was evaluated and documented at 20 and 40 minutes, as per the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test Package Insert. Results from the 40-minute read times were evaluated for concordance and 596 oral fluid tests and 595 fingerstick tests were included in the Per Protocol Analysis.

Overall concordance for oral fluid was 99.8% (595/596, 95% confidence interval: 99.1% - 100.0%), and for fingerstick whole blood was 99.5% (592/595, 95% confidence interval: 98.5% - 99.9%).

**Oral Fluid from HIV Positive Subjects**

A total of twenty (20) unique subjects were enrolled in the study, and each provided three (3) oral fluid samples for a total of sixty (60) oral fluid samples. Twenty (20) samples were tested in each of three (3) validation lots. Each test was evaluated and documented at 20 and 40 minutes, as per the OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test Package Insert. Results from the 40-minute read time were evaluated and showed concordance.

Of the sixty (60) completed OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test validation lot tests, all were 100.0% in agreement (95% CI: 94.0% - 100.0%).

## Conclusions

The Modified Avidin Replacement OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test performance was comparable to that of current, approved OraQuick *ADVANCE®* Rapid HIV-1/2 Antibody Test. The successful design validation provides evidence that the product claims have not changed as a result of the revised process. Additionally, the new OraQuick *ADVANCE®* test performance in seroconversion panels is comparable to that of the Bio-Rad GS HIV-1/HIV-2 PLUS O enzyme immunoassay. OraSure Technologies has begun manufacturing this product and distribution has commenced. The new product has 30-month dating and as part of this switch, requires the use specific kit controls.

The additional practical significance of the raw material changes to the test performance are being explored further. OraSure Technologies Inc. is currently in the process of requesting FDA to approve changes to the test Package Insert to record the seroconversion performance. Until these changes are approved for implementation, the most obvious manifestation of the introduction of this new test will be the requirement to use specific kit controls (by iterated lot number). This change is necessitated to seamlessly integrate the new product with no obvious intensity difference in the Kit Control level.

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