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I  Product Description

The Aware™ line of oral fluid rapid tests includes the Aware™ HIV-1/2 OMT (professional kit) and Aware™ HIV-1/2 OMT single pack rapid tests. Both tests are single-use, qualitative, visually read, in vitro immunoassays for the detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in human oral mucosal transudate specimens. The Aware™ HIV-1/2 OMT test is a professional-use product intended as a point-of-care aid in the clinical diagnosis of HIV infection. Aware™ HIV-1/2 OMT single pack test is the same test as Aware™ HIV-1/2 OMT but has been packaged singly and includes special instructional materials to facilitate at-home private testing. Both versions of the test are available in multiple languages.

1.1  Aware™ HIV-1/2 OMT Kit Composition

The Aware™ HIV-1/2 OMT test is available in kit sizes of 100 tests. Each kit contains:

1. Package Inserts (one (1) English plus local language translations)
2. 100 individual test sets each in a plastic pouch that contains:
   □ One (1) foil pouch containing one Oral Fluid Test Strip and one desiccant
   □ One (1) capped test tube containing Oral Fluid Sample Buffer (1 mL)
   □ One (1) Oral Fluid Sample Collection Swab
3. Three (3) disposable test tube stands
4. Three (3) test procedure cards

1.2  Aware™ HIV-1/2 OMT single pack Kit Composition

The Aware™ HIV-1/2 OMT single pack test comes individually packaged. Each test kit contains all materials and supplies required to collect and test one (1) oral fluid sample:

1. One (1) package insert
2. One (1) foil pouch containing one Oral Fluid Test Strip and one desiccant
3. One (1) capped test tube containing Oral Fluid Sample Buffer (1 mL)
4. One (1) Oral Fluid Sample Collection Swab

1.3  Principles of Procedure

The assay test strip contains recombinant proteins representing the immunodominant regions of the HIV-1 gp41 and HIV-2 gp36 transmembrane proteins and a goat anti-human IgG F(ab’)2 fragment antibody-capture procedural control immobilized onto the nitrocellulose membrane in the Test Zone and the Control Zone, respectively.

To perform the assay, an oral fluid specimen collection swab is wiped across the upper and lower gum line of the patient, and this swab is then
placed into the Oral Fluid Sample Buffer tube and mixed. The liquid in the swab is expressed out and the swab discarded. The assay test strip is placed vertically into the test tube containing the specimen mixture.

As the diluted specimen migrates up the assay test strip, it rehydrates a reddish Protein A-colloidal gold reagent (“Conjugate”) on the test strip and IgG in the specimen becomes bound to the Protein A/colloidal gold particles (“IgG/conjugate complex”). The specimen/conjugate mixture continues to migrate up the strip and first encounters the Test Zone of the assay test strip containing the HIV antigens. If the specimen contains antibodies to HIV, the IgG/conjugate complex binds to the antigen and becomes immobilized at the antigen line in the Test Zone and a reddish colored line appears indicating a reactive result. The intensity of the line is not proportional to the amount of antibody present in the specimen. In a validly performed test, the absence of a colored line in the Test Zone indicates that the specimen does not contain anti-HIV antibodies.

The specimen/conjugate mixture continues to migrate up the assay test strip until it encounters the Control Zone. The Control Zone contains goat anti-human IgG F(ab′)2 fragments immobilized in a line on the assay test strip. The remaining IgG/conjugate complexes become bound to the immobilized F(ab′)2 fragments and a reddish colored line appears. The appearance of the control line is evidence that the test functioned properly and contained human IgG. A reddish-purple control line will appear in the Control Zone during the performance of all valid tests, whether or not the sample is reactive or negative for antibodies to HIV-1 or -2.

The specimen continues to migrate past the Control Zone into the final absorbent pad, which helps draw the specimen/conjugate mixture through the strip and clear any background color.
2 Oral Fluid Samples as Diagnostic Matrix

2.1 Recent Trends in HIV Testing

For more than a decade, vast amounts of research have occurred into the use of alternative fluids for diagnosis of HIV. Oral fluid (i.e., oral mucosal transudate) has become the leading candidate as a non-invasive and safe testing medium with increased ease in sample collection and patient acceptability.

Similarly, the use of rapid test technologies have upstaged the use of classic ELISA and Western blot systems due to their increased ease of use and applicability over more diverse testing scenarios. ELISA and Western blot tests are feasible only when there is availability of highly trained manpower, instrumentation, and electricity. These tests are not often feasible, particularly in rural areas. If testing is to occur, samples must often be sent to remote cities where there are more advanced laboratories. It has been shown that combinations of highly sensitive and specific rapid test kits with adaptable field conditions can be used to test for the presence of antibodies to HIV-1/2, thus eliminating the need for ELISA, WB, and PCR results.

Calypte Biomedical Corporation has developed the Aware™ line of HIV diagnostic tests combining these technologies to offer oral fluid testing within a rapid test format. It is hoped that the availability of a more patient-acceptable, simple, non-invasive, safe, rapid and accurate alternative to classic blood testing methodologies will promote the identification of greater numbers of HIV-infected persons with the ultimate goals of early identification, early treatment and prevention of disease transmission. The characteristics of this test (i.e. rapid, simple and accurate format with safe, non-invasive collection) enhances the ability of countries to control and monitor HIV infections both in conventional medical settings as well as in remote areas with limited access to conventional medical facilities or trained medical personnel.

2.2 Oral Fluid Specimen Types

A basic understanding of the different types of oral fluid specimens is necessary in choosing what oral fluid type is most appropriate to diagnostic testing. Many diagnostic tests for infectious diseases are driven by the detection of IgG as the humoral immune response to an infection, and this fact is important to keep in mind when deciding upon the specific oral fluid that is most applicable to diagnostics. The scope of oral fluid research encompasses the use of saliva (whole, glandular, “resting”, “stimulated”), gingival crevicular fluid and oral mucosal transudate.
## Definitions:

**Whole Saliva:** Whole saliva consists of salivary secretions of the parotid, submandibular, sublingual, and minor salivary glands. It contains mostly secretory IgA and low levels of IgG. In addition to these glandular sections, whole saliva may contain food particles, red blood cells, leukocytes, bacteria, and sloughed epithelial cells. These may lead to degradation of the available IgG by bacterial and salivary proteases and makes the specimen difficult to process due to the viscosity of the sample. Whole saliva is typically obtained from the mouth by expectoration.

**Pure Saliva:** Pure (or glandular) saliva is obtained directly from the salivary glands via direct sampling from the salivary ducts. Collection of pure saliva is technically difficult and special collection devices must be used. Secretions from the glands are primarily mucous or seromucous and contain high levels of secretory IgA.

**Resting Saliva:** Operationally defined as that which is obtained in the absence of direct stimulation.

**Stimulated Saliva:** Stimulated saliva is obtained by masticatory (e.g., chewing) or gustatory (e.g. citric acid) stimulation. Within the literature, a typical procedure for obtaining stimulated saliva is to have the subject chew on a piece of paraffin. Gustatory stimulation methods include the use of citric acid or sour candy to induce salivation.

**Gingival Crevicular Fluid:** Gingival crevicular fluid is a serum transudate that comes from the continuous seepage of fluid from the gingival capillaries through the crevicular epithelium into the gingival crevice of dentate individuals. Of the oral fluids, this fluid most closely resembles the humoral composition of blood.\(^1\) This crevicular fluid is a specific type of oral mucosal transudate and the two terms have often been used interchangeably. This fluid exists in small quantities in whole saliva but can, with difficulty, be obtained directly from the gingival crevice.

**Oral Mucosal Transudate:** Oral mucosal transudates are fluids from the capillaries beneath the buccal mucosa at the base of the crevice between the teeth and gums (gingival crevicular fluid). These fluids contain secretory IgA, but more importantly on a diagnostic level, contain high levels of IgG and IgM that originate in the plasma and are passively transferred into the mouth across the mucosa and gingiva. The IgG concentration in oral mucosal transudates is less than that found in plasma but higher than in whole saliva.\(^2\)

## 2.3 Immunoglobulins in Oral Fluid

Ellison et al.\(^2\) completed the first studies in 1960 that looked at the presence of immunoglobulin in saliva. Subsequent research has shown that the primary immunoglobulin in saliva is secretory IgA, which is of...
salivary gland origin. Secretory IgM has been detected in the secretions of the minor salivary glands and IgE has been detected in saliva. Salivary IgE levels have been suggested to more accurately reflect response to allergen than those detected in serum\textsuperscript{3}. Table 1 compares immunoglobulin concentrations in oral fluids and serum.

**Table 1: Comparative Concentrations (mglml) of Various Immunoglobulins in Oral Fluids and Serum\textsuperscript{4}**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>14,730.0</td>
<td>1280.0</td>
<td>2860.0</td>
</tr>
<tr>
<td>Parotid Saliva</td>
<td>0.36</td>
<td>0.43</td>
<td>39.5</td>
</tr>
<tr>
<td>Gingival Crevicular Fluid</td>
<td>3,500.0</td>
<td>250.0</td>
<td>1,110.0</td>
</tr>
<tr>
<td>Whole Saliva</td>
<td>14.4</td>
<td>2.1</td>
<td>19.4</td>
</tr>
</tbody>
</table>

Immunoglobulin G (IgG) concentrations in gingival crevicular fluid are significantly higher than those found in whole saliva or other salivary glands. The gingival crevice and oral mucosa is an identifiable anatomic site that provides the highest concentration of IgG within the oral cavity. Consequently, collection of oral fluid that concentrates upon this area of the mouth was identified as the most promising source of fluid as a diagnostic testing medium.

In addition to the determination of the origin and distribution of immunoglobulin isotypes in saliva, investigators have evaluated this matrix for the presence of antigen-specific antibodies.

**2.3.1 Antigen-Specific IgA in Saliva**

The comparatively high level of secretory IgA (sIgA) in saliva has led to an evaluation of the potential of saliva for providing reliable information regarding antigen-specific secretory immunity.

Although research is limited, it has been found that protective IgA levels that are measurable in saliva after vaccinations against polio virus\textsuperscript{5}, cholera\textsuperscript{6}, and after vaccination with a combination vaccine to *Streptococcus mutans* and cholera toxin B\textsuperscript{7}. Additional studies have shown an antigen-specific response to bacterial infections caused by *Haemophilus influenza*\textsuperscript{8}, *Bordetella pertussis*\textsuperscript{9}, *Giardia intestinalis*\textsuperscript{10}, and to viral infections caused by rubella\textsuperscript{11}, hepatitis A\textsuperscript{12}, and Epstein-Barr virus\textsuperscript{13}. However, the diagnostic value of secretory IgA is limited as most assays rely on the detection of IgG as a humoral response to disease challenge.
2.3.2 Antigen-Specific IgG in Saliva

Prior to 1991, various studies showed that detection of antigen-specific IgG was possible through the use of saliva, and these studies formed the foundation for the idea that HIV-specific IgG antibodies may be detectable as well. Antigen-specific IgG antibodies in human saliva were detected in individuals diagnosed with *Shigella* dysentery\(^{14}\). Salivary IgG specific for viral antigens has also been identified in patients with hepatitis A and hepatitis B\(^{15}\), Epstein-Barr virus infections\(^{16}\), and rubella\(^{17}\).

Thieme et al. expanded upon these studies to conclude that simultaneous detection of seroconversion occurs in serum and oral fluid samples for diseases such as Hepatitis A\(^{15}\) and after vaccination to measles, mumps, and rubella\(^{18}\).

2.4 HIV Antibodies in Oral Fluids

At the onset of the Human Immunodeficiency Virus (HIV) epidemic, research showed antibodies of both the IgA class\(^{19}\) and the IgG class\(^{20,21}\) were detectable in saliva. The first studies of oral fluid for the detection of HIV in seropositive patients demonstrated highly variable sensitivities. Problems included the possible removal of antibodies by specimen filtration, over dilution of oral samples, and assay insensitivity\(^{2}\). Additionally, specimen storage leading to degradation of antibodies by proteases present in saliva may have contributed to these initial reports of low sensitivity. Subsequently, tests designed specifically for oral fluid use were developed that overcame the limitations of blood-based test systems adapted for use with oral fluid. Sensitivities of many of these oral fluid assays have improved to levels equivalent to serum. Additionally, much work has gone into understanding the nature of an oral sample in terms of stability\(^{22,23}\), sample-handling characteristics (viscosity, bacterial and particulate contamination), and optimization of collection methods to further realize increased performance and accuracy of the developed tests.

2.5 Detection of HIV Virus and Infectivity Inhibition in Oral Fluid

In 1985, Levy et al. published in the Annals of Internal Medicine that HIV was isolated from the saliva of approximately 10% (3/34) of seropositive subjects examined\(^{24}\). Pekovic et al. suggested that HIV-containing leukocytes entering saliva from the blood of dental patients represent an infectious hazard for HIV transmission\(^{25}\).

While these studies and others demonstrating the recovery of HIV from saliva suggest that HIV transmission via saliva is a genuine concern, a large body of published evidence indicates that oral transmission of the HIV virus by the millions of HIV-infected individuals is a rare event, even when infected blood and exudate is present in the oral fluid. Typically, the saliva of infected individuals usually contains only noninfectious components of HIV indicating that there may be a breakdown or
inactivation of infectious HIV\textsuperscript{26}. Numerous studies have identified endogenous components that may serve as natural salivary inhibitors of HIV-1\textsuperscript{27,28}. These researchers have identified mechanisms such as viral neutralization and elimination by host antibody immune response\textsuperscript{29}, and inhibition of infection utilizing other components such as thrombospondin and high molecular-weight mucins by aggregation of the virus into large, insoluble complexes\textsuperscript{30,31}.

The lack of HIV transmission by saliva may be explained by a combination of two general factors; 1) low numbers of infectious particles\textsuperscript{32} and 2) an inhibitory effect of human saliva on the HIV virus. Baron et al. noted that most of the infectious HIV is shed mucosally by asymptomatic individuals, and is found in, and produced and transmitted by infected mononuclear leukocytes. They formulated a theory about saliva’s effect on HIV. They theorized that saliva, which is hypotonic, may disrupt these infected cells, thereby preventing virus multiplication and cell-to-cell transmission of HIV. As a result of their study, it was concluded that hypotonic disruption may be a major mechanism by which saliva kills infected mononuclear leukocytes and prevents their attachment to mucosal epithelial cells and production of infectious HIV, thereby preventing transmission\textsuperscript{33}. Significant bleeding in the mouth due to dental treatment does not correlate with an increased incidence of detectable infectious virus, further confirming the safety of oral fluid as a testing medium, even in individuals with blood in the saliva due to oral pathologies or recent dental treatment\textsuperscript{34}. Fragments of the nucleic acids of the virus can be recovered which is consistent with disruption of shed infected cells due to the hypotonicity of saliva\textsuperscript{34}.

Further research has been conducted into the protective characteristics of the intraoral environment. Shugars et al. has assessed the inhibitory factors that reduce HIV-1 infectivity in vitro, focusing on secretory leukocyte protease inhibitor (SLPI), a 12-kDa mucosal protein that blocks HIV infection in several cell-culture systems. SLPI appears to interact with a cellular surface molecule to limit viral entry into target cells. Some samples having SLPI well below the concentration required for inhibitory activity in vitro exhibited modest inhibition, suggesting the presence of other anti-HIV-1 components in oral fluids. Thus, SLPI is a major but not sole inhibitor of this virus in saliva\textsuperscript{29}.

### 2.6 Advantages of Oral Fluid Sampling

#### 2.6.1 Blood Collection Risks

The whole blood, serum, and plasma of HIV-positive individuals are all recognized as potentially infectious for HIV. The collection of blood, whether by finger-stick or venipuncture, can pose a potential risk to patients through the potential re-use of non-sterile needles and lancets. As recently as 2000, the WHO estimated that up to 40% of the needles used for injections were previously used, and that roughly 260,000 people were annually infected with HIV as a result. While the numbers may be different for
The issue of patient appeal

The oral health of voluntary authorities. Barriers include the process of testing that may be counseled and referred for therapy. Although a portion of HIV infections may be identified through a variety of mandated, institutionalized testing programs, the identification of infections in the general population relies largely on the public's voluntary participation in testing programs. It logically follows that in order to maximize voluntary participation in testing and counseling programs, the testing process must minimize the barriers that prevent the public from participating.

Barriers to voluntary testing include the universally held perception of pain upon piercing a vein or finger to obtain a blood sample and the possible subsequent risks to the patient of bruising and/or infection. Additional barriers may vary country to country, but can include cultural taboos against blood sampling, suspicion about the government or health authority motives, or the concern regarding ultimate disposition of blood samples collected by the authorities. Finally, testing processes that are difficult or time consuming can provide reasons for people not to take advantage of voluntary HIV testing services.

Oral fluid collection is simple, painless, and minimizes the risk to health care workers and patients. Within the United States where oral fluid testing has been available since the mid-1990s, preference surveys have been conducted by several investigators, the results of which showed that oral fluid testing is the preferred
method of sample collection among various populations screened\textsuperscript{38, 39}.

2.6.3 Ease of Sampling

OMT samples can be collected by appropriately trained staff, without the need for phlebotomists. Training and subsequent use of the OMT collection device and affiliated test is non-technical, and collection of samples can be performed by health-care, outreach or social workers with limited to no background in laboratory or medical technologies. Accordingly, phlebotomist expense and availability do not impact the timely and economical implementation of HIV testing programs.

Due to the ease of sampling for OMT using the Calypte OMT collection device, oral fluid is much easier to collect than a blood sample, particularly in children, obese persons, and those with difficult or collapsed venous access. Additionally, OMT collection is much more logistically feasible in high-throughput testing scenarios such as those within the military, prisons, schools and university settings or in environments where privacy is non-existent or limited. Additionally, the nature of OMT testing is feasible in locations where performing phlebotomy is impossible, such as during field research, outreach settings or prevalence surveys in remote locations.

The non-invasive nature of the collection process has yielded benefits in surveillance programs that target hard-to-reach populations such as intravenous drug users, sex workers, homosexual men and others in whom venipuncture is either impossible or inconvenient\textsuperscript{40, 41}.

2.7 The Calypte OMT Collection Device

The Calypte OMT collection device has been developed in conjunction with the Calypte HIV-1/2 OMT rapid test. Although the collection device design is simple, its development was undertaken in consideration of the available research on physiological differences between oral fluid sources and on material performance, safety and comfort.

The OMT collection process using this device limits collection to sources where oral IgG is most concentrated – the oral mucosa and gingival crevices – in order to optimize the sample collected for best test performance and sensitivity. The pad of the device is gently rubbed along the upper and lower gum lines at the base of the teeth in order to isolate the high IgG content of this physical area while minimizing the diluting effect that saliva collection would cause. The pad is made of a soft, highly absorbent polyester fabric that minimizes the possibility of oral abrasion.

Sample collection can be either performed on a patient by a trained healthcare worker or by the patient himself after instruction and
observation by the trained healthcare worker. After collection of the oral sample, the pad is immersed into a buffered diluent that rinses the pad free of oral antibody. Studies conducted by Calypte Biomedical found that available antibody collected using the OMT device is released into the sample diluent, after removal of the swab, resulting in a sample antibody solution of averaging around 15 pg/ml in HIV-positive subjects, a level approximately 1:1000 that of normal antibody levels in serum or plasma. Although this is a low antibody level, it is still concentrated enough to be detectable in a highly tuned, sensitive immunochromatographic assay, such as Aware™ HIV-1/2 OMT. The diluent is of an appropriate volume and composition as to decrease the viscous nature of an oral fluid sample and contains an antimicrobial agent to minimize microbial degradation. The diluent also renders the sample easily pipettable, and aesthetically non-objectionable to non-laboratory trained personnel. Lastly, the diluent serves to extend sample volume so that sample will remain after initial testing for repeat testing or confirmation testing.

The collection device was designed to be paired with the Calypte HIV-1/2 Rapid OMT test to facilitate testing immediately after collection of the sample. Previous research has showed that the effects of salivary pH, enzymes present (e.g. peroxidase, RNAase, protease), and bacterial proteases that are normal constituents of saliva may affect sample stability parameters. However, company data shows acceptable device performance when the sample is stored several hours after collection. This flexibility facilitates additional, though limited, testing scenarios that are not exclusively restricted to point-of-care use.

2.8 Summary of the Advantages of Oral Fluid Testing for Antibodies to HIV

When considering implementation of oral fluid diagnostic testing, full consideration must be given to the sample source within the oral cavity as well as to various diverse constituents that contribute positively as well as negatively to the composition of the sample matrix. Additionally, oral fluid testing has demonstrated a variety of positive benefits for its use as a diagnostic tool, such as ease of sampling, reduced collection cost, minimization of the infectious nature of the sample and high patient acceptability. Finally, the development of highly sensitive tests for HIV antibodies have allowed for the use of an oral fluid sample without compromising expectations for diagnostic accuracy. By incorporating the use of carefully collected and treated oral fluid specimens with the rapid and accurate result technology present in Aware™ HIV-1/2 OMT assay, the potential applications and sites of HIV diagnostic testing are dramatically expanded over diagnostic technologies previously available.
3 Product Safety

The Aware™ HIV-1/2 OMT and Aware™ HIV-1/2 OMT single pack tests are in-vitro assays, and as such, none of the components of the tests would normally be ingested in the course of running a test. The components of the tests include:

- Test Strip
- Sample Buffer
- Oral Fluid Sample Collection Swab
- Desiccant
- Packaging

The components have been tested for risk of accidental ingestion, hazards of components, and biocompatibility, as determined according to ISO 10993, and were found to comply with international standards of biocompatibility.

4 Disposal of Used Product

As discussed in Sections 2.5 and 2.6, the risk of disease transmission by oral fluids is extremely low, both because viral titers are low and because infectivity is inhibited by substances present in the oral environment. Thus, although the Universal Precautions described by the US Centers for Disease Control for prevention of blood borne pathogen transmission do apply to fluids other than blood, these guidelines do not apply to saliva unless contaminated with visible blood (or in a dental setting, when blood contamination of the oral environment is predictable). Universal Precautions do not provide specific guidelines for waste disposal, deferring instead to local regulations:

"The selection of procedures for disposal of infective waste is determined by the relative risk of disease transmission and application of local regulations, which vary widely."  

Oral fluids present such a low risk of transmission that personal protective equipment (as described in Universal Precautions) is not considered strictly necessary even for sample or patient contact, and the Aware™ HIV-1/2 OMT and Aware™ HIV-1/2 OMT single pack tests contain no sharps and comply with international safety standards (Section 3). Therefore, unless blood contamination is evident or predictable (as in a dental setting), used components of the Aware™ kits need not be considered biohazardous. However, because local conditions are the final determinant of relative risk, used components should be disposed of according to local regulations.
5 Benchmarking Performance of Aware™ Tests

5.1 Similar Products

In addition to Calypte’s Aware™ products, there is at least one other oral fluid rapid HIV test currently on the market in various countries around the world. This product, known as OraQuick® Advance HIV-1/2 (OraSure Technologies, Bethlehem PA, USA), can be used with either blood (including serum and plasma) or oral fluid samples and is approved for professional use in the United States. Like Aware™ oral fluid tests, OraQuick® Advance HIV-1/2 is a colloidal gold immunochromatography test. It differs from Aware™ tests in that the sample is collected with a pad that is integrated into the test device itself (as opposed to a separate swab). After the sample is collected, the pad end of the test device is inserted into a test buffer to drive the immunochromatographic development. Because of these differences, OraQuick® requires a plastic housing, and there is no sample left over after collection for either repeat testing or confirmatory testing. OraQuick® was developed in the late 1990s by a team headed by Calypte Biomedical Corporation’s then Chief Science Officer, Dr. Ronald Mink, who is a co-inventor of the OraQuick® product.

5.2 Performance Comparison

Manufacturers of in vitro diagnostic tests routinely benchmark the performance of their products relative to other products using commercially available sample panels. There are several such panels available for HIV including worldwide, low titer, and seroconverter panels. (Seroconverter panels are frequently used to assess a test’s “window” for how soon an infected individual can be detected once antibodies to HIV start to appear in the subject’s serum, although actual time since exposure is generally not known.)

While such performance panels are commonly available for sera, there are none available for oral fluid specimens. Nonetheless it is still possible to benchmark the performance of oral fluid tests using the commonly available sera panels. One way that this may be accomplished is to synthesize oral fluid specimens by diluting sera specimens to yield antibody levels comparable to what is found in oral fluid. Oral fluid samples obtained with the Aware™ Oral Fluid Collection Swab typically have antibody levels approximately 1:1000 that normal serum or plasma (see section 2.7, above). To mimic how the oral fluid specimens corresponding to sera samples might perform, we diluted sera panel members 1:1000 in the Aware™ Oral Fluid Sample Buffer and ran them with Aware™ HIV-1/2 OMT test strips. This approach has been accepted by government regulatory agencies.

The results given in the tables on the following pages are, in all cases, shown in comparison to commercially available tests run with the serum undiluted (except as may be required by the individual assay for preparation in the assay, according to the manufacturer’s instruction). All tests, except Aware™, were evaluated according to manufacturer’s
instructions by outside parties as indicated. The highly sensitized nature of the Aware™ HIV-1/2 OMT test fares comparably to its serum/plasma HIV assay competitors. Data for both other HIV rapid tests and selected HIV EIAs are provided for comparative purposes.
5.2.1 BBI Worldwide HIV Performance Panel WWRB3O2 (Modified)

The table below presents results of the Aware™ HIV-1/2 OMT on a diverse worldwide panel representing HIV subtypes A,B,C,D,E,F and O, as well as HIV-2.

<table>
<thead>
<tr>
<th>ID</th>
<th>Genotype* (origin)</th>
<th>Rapid HIV Test</th>
<th>U.S. FDA Licensed EIAs (signal1cutoff)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aware™ HIV-112 OMT***</td>
<td>Abbott HIV-1</td>
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<tr>
<td>1</td>
<td>O (Spain)</td>
<td>Pos</td>
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<tr>
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<td>25</td>
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<td>26</td>
<td>B (USA)</td>
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<tr>
<td>27</td>
<td>B/D (USA)</td>
<td>Pos</td>
<td>&gt;11.5</td>
</tr>
<tr>
<td>28</td>
<td>F (Argentina)</td>
<td>Pos</td>
<td>&gt;11.5</td>
</tr>
<tr>
<td>29</td>
<td>B (Argentina)</td>
<td>Pos</td>
<td>&gt;11.5</td>
</tr>
<tr>
<td>30</td>
<td>NEG (Argentina)</td>
<td>Neg</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Number correct: 25125 24125 25125 25125 25125 25125

* Genotype based on sequencing by PCR.
*** Results for all tests except Aware™ as published by BBI in panel package insert.

---

* Omnisense TM Panel members 1:1000 in Oral Fluid Sample Buffer to simulate an oral fluid specimen.
5.2.2 BBI Anti-HIV Low Titer Performance Panel (Modified) PRB1O7

The following table presents results of the Aware™ HIV-1/2 OMT on two commercially available HIV-1 low titer panels compared to other HIV tests.

<table>
<thead>
<tr>
<th>ID</th>
<th>Status</th>
<th>BBI Anti-HIV Low Titer Performance Panel (Modified) PRB1O7*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rapid Tests</td>
</tr>
<tr>
<td>1</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>2</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>3</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>4</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>5</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>6</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>7</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>8</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>9</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>10</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>11</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>12</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>13</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>14</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>15</td>
<td>Pos</td>
<td>Pos</td>
</tr>
</tbody>
</table>

Number correct: 11115 9115 10115 7115 15115 11115 9115 5115

* Results for all tests except Aware™ published by BBI panel package insert except as noted:
  a) per OraQuick® Advance HIV-1/2 package
  b) per UniGold Recombigen package insert
** OMT samples prepared by diluting listed serum panel members 1:1000 in Oral Fluid Sample Buffer to simulate an oral fluid specimen.
5.2.3 **BBI Anti HIV Low Titer Performance Panel PRB1O8**

The following table presents results of the Aware™ HIV-1/2 OMT on several commercially available HIV-1 low titer panels compared to other HIV tests.

<table>
<thead>
<tr>
<th>ID</th>
<th>Status</th>
<th>Rapid HIV Tests</th>
<th>U.S. FDA Licensed EIAs (signal1cutoff)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>2</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>3</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>4</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>6</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>7</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>8</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>9</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>10</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>11</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>12</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>13</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>14</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>15</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
</tr>
</tbody>
</table>

**Number correct**: 14115  14115  8115  13115  11115  11115  11115  14115  8115

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*Results for all tests except Aware™ published by BBI except as indicated a) as per UniGold™ Recombigen® package insert.

**OMT samples prepared by diluting listed serum panel members 1:1000 in Oral Fluid Sample Buffer to simulate an oral fluid specimen.*
### 5.2.4 BBI HIV-1 Seroconversion Panels

The following table presents results of the Aware™ HIV-1/2 OMT on several commercially available HIV-1 seroconversion titer panels compared to other HIV tests.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>First Sequential Seroconverter Sample Detected (# days from first bleed)</th>
<th>HIV Rapid Tests</th>
<th>HIV EIAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF (PRB931)</td>
<td></td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>AQ (PRB941)</td>
<td></td>
<td>18</td>
<td>n/d</td>
</tr>
<tr>
<td>BB (PRB952)</td>
<td></td>
<td>17</td>
<td>n/d</td>
</tr>
<tr>
<td>BG (PRB957)</td>
<td></td>
<td>28</td>
<td>n/d</td>
</tr>
<tr>
<td>BH (PRB958)</td>
<td></td>
<td>17</td>
<td>&gt;17™</td>
</tr>
<tr>
<td>BI (PRB959)</td>
<td></td>
<td>14</td>
<td>14™</td>
</tr>
</tbody>
</table>

* Results for all tests except Aware™ published by BBI except as noted:
  a) per OraQuick® Advance package insert
  c) per Unigold Recombigen package insert
** OMT samples prepared by diluting listed serum panel members 1:1000 in Oral Fluid Sample Buffer to simulate an oral fluid specimen.
References


42 CDC. Recommendations for prevention of HIV transmission in health-care settings. MMWR 1987;36(suppl no. 2S).


44 CDC. Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. MMWR 1989;38(S-6);1-36.