



CALYPTTE

BIOMEDICAL CORPORATION

Product File

**Aware Messenger™
Oral Fluid Collection Device**

PN 98184

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1 Product Description

The Aware Messenger™ collection device is intended for the collection, stabilization, and transport of an oral fluid specimen to be used for the detection of specific antibodies or analytes. Aware Messenger™ specimens may be tested with conventional laboratory-based immunoassays (e.g. ELISA) enabling high-throughput batch testing, automation, quantitative results, and lower costs. Oral fluid specimens collected with this device are easily obtained and have been shown to yield high quality samples rich with various analytes representative of those found in blood.

Aware Messenger™ was developed by scientists experienced with oral fluid diagnostics. All critical elements of oral fluid collection and diagnostics were considered during the product design including comfort of collection, ease of processing, specimen integrity and stability, and compatibility with traditional assays. By design, the Aware Messenger™ generates a stabilized, high-quality and immunoassay-friendly oral fluid specimen that is simply collected and requires no further processing (e.g. centrifugation).

1.1 Aware Messenger™ Kit Composition

The Aware Messenger™ oral fluid collection kit is available in 50 count boxes (Product Number 98184). Each box contains:

- a. Fifty (50) Aware Messenger™ individually packaged devices. Each packaged device contains:
 - One (1) capped test tube containing Oral Fluid Sample Buffer (1.5 mL)
 - One (1) wrapped Oral Fluid Sample Collection Swab
- b. One (1) Package Insert (English)

1.2 Principles of the Aware Messenger™ Device

The Aware Messenger™ device consists of an expressly designed synthetic swab and collection tube containing a proprietary sample buffer. The buffer has been formulated in appropriate concentrations with specific components (detergents, salts, stabilizing agents, etc.) to optimize specimen recovery and compatibility, allow for optimal sensitivity and specificity, and to promote sample stability.

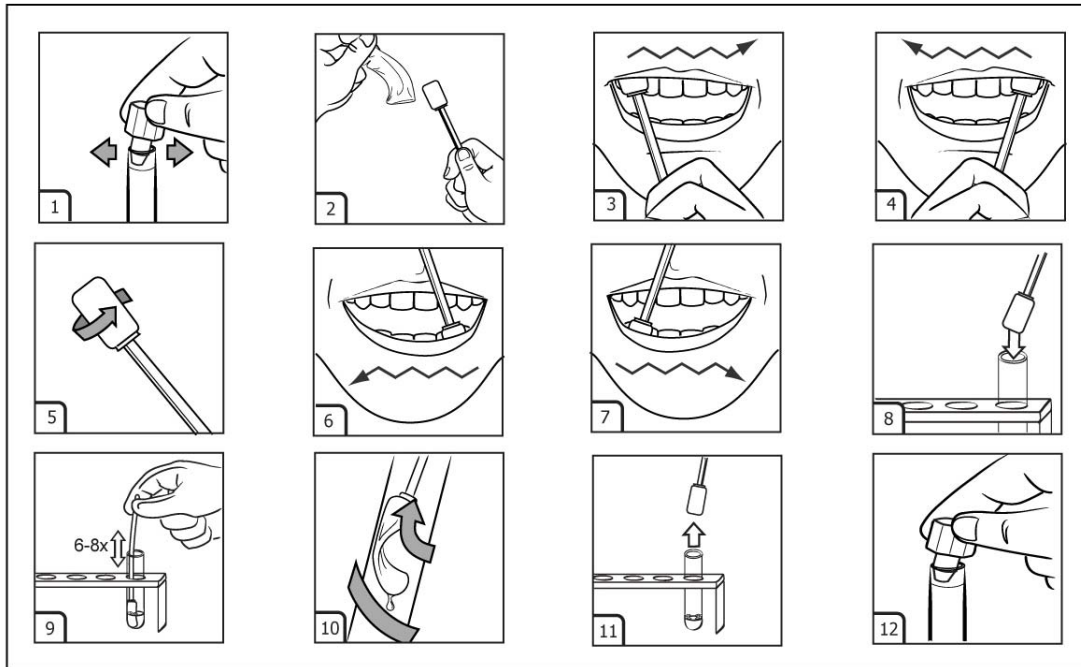


Photo of the Aware Messenger™ Collection Swab and Sample Tube

As is illustrated in the sample collection procedure presented below, the Aware Messenger™ oral fluid sample is collected by wiping the swab across the upper and lower gums. The swab is then placed into the Specimen Collection Tube containing 1.5 mL of Specimen Diluent. After mixing with the sample buffer, the liquid in the swab is expressed out and the swab is discarded. The specimen is now ready for testing,

storage, or transport. No other processing (centrifugation, filtration, etc.) is required. Generally, at least 1.0 mL of specimen is available for use.

Aware Messenger™ Sample Collection Procedure



A key feature of the Aware Messenger™ device is its ability to collect oral mucosal transudate rich with gingival crevicular fluid rather than common saliva. As is discussed in detail below, oral mucosal transudate generally yields a superior specimen to saliva.

1.3 Oral Fluid Specimen Types

A basic understanding of all oral fluid specimen types including oral mucosal transudate and gingival crevicular fluid is necessary to appreciate the advantages of the Aware Messenger™ oral fluid collection device. Note that throughout the literature, the scope of the term “oral fluid” continues to encompass saliva (whole, glandular, “resting”, “stimulated”), gingival crevicular fluid and/or various oral mucosal transudates.

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 blood¹. It is important to note that diagnostic tests for infectious diseases are primarily driven by the detection of IgG as the humoral immune response to an infection. Furthermore, literature reports have demonstrated seroconversion simultaneous to that of oral mucosal transudates^{10,15}. Gingival 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 gingival. The IgG concentration in oral mucosal transudates is less than that found in plasma but higher than in whole saliva².

1.4 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. However, salivary IgE levels primarily reflect response to allergens³ and is therefore of limited value as a disease marker for infectious disease.

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 are identifiable anatomic sites that provide the highest concentration of IgG and other serum proteins 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.

Table 1 compares immunoglobulin concentrations in oral fluids and serum.

Table 1: Comparative Concentrations (mg/ml) of Various Immunoglobulins in Oral Fluids and Serum⁴

Specimen	IgG	IgM	IgA
Serum	14,730.0	1280.0	2860.0
Parotid Saliva	0.36	0.43	39.50
Gingival Crevicular Fluid	3,500.0	250.0	1,110.0
Whole Saliva	14.4	2.1	19.4

2 Oral Fluid as a Diagnostic Medium

With the onset of the Human Immunodeficiency Virus (HIV) epidemic in the mid 1980s and the subsequent realization of the dangers associated with blood collection, there came to be a great interest in the use of alternative diagnostic fluids such as oral fluid. This spike in interest culminated in several publications on the topic of oral fluid diagnosis and a national conference hosted by the New York Academy of Sciences [*Saliva as a Diagnostic Fluid*, Panama City Beach, Florida, October 22-25, 1992].

2.1 Oral Fluid IgG and IgM to Infectious Diseases

The beginning of the 1990s witnessed a flurry of literature reports demonstrating the detection of antigen-specific IgG antibodies in oral fluid specimens. This included reports of IgG to bacterial infections (shigella dysentery⁵, and *Helicobacter pylori*^{6,7}) as well as to viral infections such as Epstein-Barr virus⁸ rubella⁹ and hepatitis C^{10,11,12,13,14}. Thieme et al. further demonstrated a 100% correlation with IgM to hepatitis A in serum and oral fluid¹⁰. 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 A¹⁰ and after vaccination to measles, mumps, and rubella¹⁵.

The use of oral fluid for HIV diagnosis has perhaps garnered the most significant amount of attention and effort. In the late 1980s research showed antibodies to HIV of both the IgA class¹⁶ and the IgG class^{17,18} were detectable in saliva. The first studies using saliva 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 lack of assay sensitivity². Additionally, specimen storage leading to degradation of antibodies by proteases present in saliva may have contributed to these initial reports of low sensitivity. Subsequently, improved immunoassays in addition to a better understanding of the nature of an oral sample in terms of stability^{19,20} sample-handling characteristics (viscosity, bacterial and particulate contamination), and collection methods has led to increased performance and accuracy of the oral fluid based tests for HIV antibodies.

In 1994, the first oral fluid HIV EIA was approved by the United States Food and Drug Administration (FDA) followed by the FDA approval of the first HIV Western Blot confirmatory test using oral fluid in 1996. Today, oral fluid (i.e., oral mucosal transudate) has become the leading candidate a safe testing medium for HIV due to increased ease in sample collection and patient acceptability.

2.2 Oral Fluid IgA and Infectious Diseases

The comparatively high level of secretory IgA (sIgA) in saliva has led to an evaluation of the potential of oral fluid for providing reliable information regarding antigen-specific secretory immunity. Although research is limited, it has been found that protective IgA levels are measurable in saliva after vaccinations against polio virus²¹ cholera²², and after vaccination with a combination vaccine to *Streptococcus mutans* and cholera toxin B²³. Additional studies have shown an antigen-specific response to bacterial infections caused by *Haemophilus influenzae*²⁴, *Bordetella pertussis*²⁵, *Giardia intestinalis*²⁶, and to viral infections caused by rubella²⁷, hepatitis A²⁸, and Epstein-Barr virus²⁹. Although supported by the literature, 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 Infectious Disease Antigens and Oral Fluid

Two studies in the late 70's reported the use of oral fluid collected with swabs for hepatitis B surface antigen and feline leukemia virus, respectively^{30,31}. Concurrent with the increased attention to diagnostic IgG in oral fluid, numerous studies were conducted aimed at the detection of viral antigens in oral fluid. One such viral antigen is Hepatitis B Surface Antigen (HBsAg), a key marker for the disease and infectivity. Thieme et al. demonstrated 100% sensitivity and specificity comparing HBsAg in serum and oral fluid¹⁰. Other antigens have been evaluated for their diagnostic presence in oral fluid. Crowcroft et al. reported the efficacy of a new Epstein-Barr virus (EBV) capsid antigen-antibody capture radioimmunoassay with saliva in epidemiological studies of EBV in school children³². Formentry et al. demonstrated the presence of Ebola virus antigen in a majority of infected test subjects³³.

2.4 Cancer Markers in Oral Fluid

The advantages of oral fluid as a diagnostic medium have led researchers to investigate its use in the field of oncology. Specifically, numerous studies have been conducted to evaluate the potential of testing oral fluid specimens for cancer markers. A vast body of literature exists presenting these efforts including some encouraging results. Nagler et al. measured saliva for the six most studied epithelial serum circulatory tumor markers of oral squamous cell carcinoma (OSCC, tongue) patients³⁴. They found that there were significant increases in cyfra 21-1, tissue polypeptide antigen, and CA 125 in association with the disease. Three other antigens, CA19-9, SCC, and carcinoembryonic antigen, increased but without statistical significance. Chen et al. reported that the use of saliva

may be a new way of screening for malignant ovarian tumors after observing a linear correlation between serum and saliva CA 125 levels with saliva having the overall better diagnostic value³⁵. Concurrent with that study, Di-Xia et al. found greater levels of salivary CA 125 in women with ovarian cancer compared to the levels in healthy women with benign lesions³⁶.

While many other studies have been less successful in demonstrating the ability to measure certain cancer markers in saliva with confidence (e.g. Plante et al. inability reproduce the CA 125 finding³⁷), the influence of inappropriate and/or inadequate methods to collect and stabilize the oral specimen is surely a possible cause for discrepant results.

2.5 Diagnostic Nucleic Acid in Oral Fluid

The development of improved nucleic acid amplification methods have allowed oral fluid specimens to be used for the evaluation of genetic markers and for the diagnosis of diseases where antibody testing would not be suitable. For example, Formenty et al. demonstrated in a study that for all eight seriously ill subjects infected with the Ebola virus, all saliva samples (obtained five to ten days after the onset of symptoms) tested positive for Ebola virus RNA using RT-PCR³³. Amado et al. used both nested RT-PCR and real-time PCR to detect Hepatitis A (HAV) viral RNA in saliva samples from infected patients both with and without anti-HAV IgM and IgG markers for the infection³⁸. The researchers concluded that “oral fluid sample testing could be used as a noninvasive method of detecting HAV RNA during HAV outbreaks”.

2.6 Detection of Illicit Drugs and Other Small Molecules in Oral Fluid

The use of oral fluid to detect and monitor illicit drugs and small molecules has become very popular during the last decade, benefiting from advances in oral fluid collection methods that had hampered oral fluid use in the past^{39,40}. Today, oral fluid is routinely used to detect and/or monitor cotinine, cannabinoids, cocaine, phencyclidine, opiates, barbiturates, diazepam, and amphetamines^{41,42,43}. Literature reports also report the ability to use oral specimens to monitor levels of therapeutic drugs⁴⁴.

2.7 Hormones in Oral Fluid

Today, numerous hormones including estradiol (E2), progesterone (Pg), testosterone (T), DHEA-S, and cortisol are routinely measured in oral fluid specimens. Voss (1999) reported that “saliva can serve as a reliable sample for estradiol determination when coupled with an appropriate assay method”⁴⁵ and Schmidt reported that “salivary cortisol testing may offer a significant measure for pediatric stress, coping, and health research”⁴⁶. Considerable accounts of hormone measurement in oral fluid are available in the literature.

As will be described later in this document, an advantage of the Aware Messenger™ device is that the specimens it collects are much more consistent than the specimens collected under conditions where saliva is stimulated by taste, long collections, or mastication. Consistency between samples is critical in any application that has a quantitative component. The measurement of hormones is such an application. Furthermore, some hormones are notorious for their “sticky” attributes, something that is

minimized in the Aware Messenger™ device by the inclusion of appropriate materials, blocking agents, and detergents.

3 Advantages of Oral Fluid Sampling

3.1 Blood Collection Risks

Whole blood, serum, and plasma are all recognized as potentially infectious for HIV, HCV, and other bloodborne diseases. 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 well as needlestick injuries. 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 phlebotomy and certainly vary by country, the practice of re-using needles, even inadvertently, remains a safety concern for patients⁴⁷.

There is an additional risk to healthcare professionals when needles and lancets are used for the drawing of blood or performing injections. Among the 35 million healthcare workers worldwide, about 3 million receive percutaneous exposures to bloodborne pathogens each year; 2 million of those to HBV, 0.9 million to HCV and 170,000 to HIV. These injuries may result in 15,000 HCV, 70,000 HBV and 500 HIV infections. More than 90% of these infections occur in developing countries. Worldwide, about 40% of HBV and HCV infections and 2.5% of HIV infections in healthcare workers are attributable to occupational sharps exposures⁴⁸.

The elimination of sharps from the HIV testing process eliminates not only the risk of accidental HIV transmission during sampling, but also the risk of accidental transmission of other bloodborne pathogens.

3.2 Safety of Oral Fluid: Infectivity Inhibition, Other Factors

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 examined⁴⁹. Pekovic et al. suggested that HIV-containing leukocytes entering saliva from the blood of dental patients represent an infectious hazard for HIV transmission⁵⁰. 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 contains only noninfectious components of HIV indicating that there may be a breakdown or inactivation of infectious HIV⁵¹. Numerous studies have identified endogenous components that may serve as natural salivary inhibitors of HIV-1^{52,53}. These researchers have identified mechanisms such as viral neutralization and elimination by host antibody immune response⁵⁴ and inhibition of infection utilizing other components such as thrombospondin and high molecular-weight mucins that aggregate the virus into large, insoluble complexes^{55,56}.

The lack of HIV transmission by saliva may be explained by a combination of two general factors; 1) low numbers of infectious particles⁵⁷ 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: 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 transmission⁵⁸. 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 treatment⁵⁹. 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 saliva⁵⁷.

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 leucocyte 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 saliva⁵².

3.3 Patient Appeal of Oral Fluid Testing

It is generally agreed that one of the cardinal elements of any disease intervention, especially HIV, is the identification of those infected so that they 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 services⁶⁰.

Oral fluid collection with the Aware Messenger™ device 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^{61,62}.

3.4 Aware Messenger™: Noninvasive Sampling

Oral fluid samples can be collected with Aware Messenger™ device by minimally trained staff or through self-collection thus eliminating the need for phlebotomists and required supplies. The collection procedure is not technical and can be performed by healthcare, outreach or social workers with limited to no background in laboratory or medical technologies. Furthermore, due to the ease of sampling, specimens are much easier to collect than a blood sample, particularly in children, obese persons, and those with difficult or collapsed venous access. Additionally, oral fluid 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 limited and specimens are required from a large number of people. Lastly, the nature of oral fluid collection using the Aware Messenger™ allows its use in locations where performing phlebotomy is impossible, such as during field research, outreach settings or prevalence surveys in remote locations. The advantages of oral fluid collection makes it highly conducive to 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^{63,64}.

Whereas oral fluid collection in general provides numerous advantages over the collection of blood, the Aware Messenger™ collection device and procedure especially provides for a comfortable sample collection experience compared to other oral fluid collection devices on the market.

4 Design of the Aware Messenger™

Aware Messenger™ was designed by scientists with over 40 combined years of experience in the field of oral fluid diagnostics and who understand the most important elements necessary for an oral fluid collection device. Every aspect of the Aware Messenger™ device, including each detergent, salt, stabilizing agent, and material type, size, and shape was specifically chosen to best accomplish the following objectives:

- a) Collect and release human oral mucosal transudate, a component of oral fluid that is rich with representative entities of the blood (e.g. IgG, other serum proteins, etc.)
- b) Stabilize the specimen to allow for testing at a later time and/or at a site away from where the specimen was collected
- c) Allow for transportation of the specimen to a laboratory for batch testing
- d) Provide a specimen that is easy to work with in a matrix that is compatible with typical immunoassays
- e) Eliminate the need for any sample processing steps at the testing laboratory (e.g. centrifugation, filtering, etc.)
- f) Allow for ease of use
- g) Insure the device is safe

A summary of the rationale and the influence of the Aware Messenger™ attributes upon performance will be described below for each of the two critical device components (swab and sample buffer) as well as for the sample collection procedure.

4.1 The Aware Messenger™ Collection Swab

Based on years of experience in the field, CalypTE scientists recognize the criticality of the collection swab. The design of the Aware Messenger™ collection swab is the result of extensive evaluation and experimentation, with a particular focus on the synthetic material used in the head of the swab.

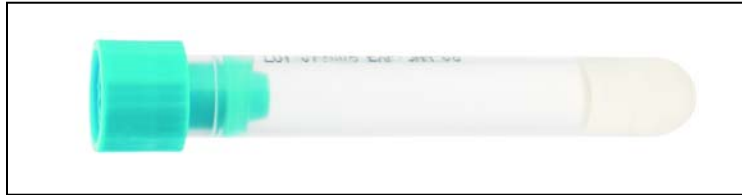


The advantages of the Aware Messenger™ swab are as follows:

- The physical properties of the Aware Messenger™ swab material allow for a high recovery of antibodies, proteins, small molecules, and other entities. Many materials used by other oral fluid collectors are made of high binding materials such as cellulose, polyvinyl alcohol, and certain types of polyesters. The high binding characteristics of these materials result in low recoveries leading to poor sensitivity results and ultimately inaccurate conclusions. CalypTE recommends that past oral fluid studies conducted using alternative materials (e.g. cellulose) be repeated with the Aware Messenger™ prior to making a final claim as to the efficacy for oral fluid testing for a specific application.
- The low binding properties of the material used in the Aware Messenger™ swab eliminates the need for chemical treatment of the swab. Other oral fluid collection devices that utilize more affinitive pad materials typically need chemical treatments to reduce the loss of analytes through binding. For example, the cellulosic OraSure oral fluid collection device is treated with gelatin (a substance originating from the bones, intestines, and organs of cattle and horses). Treatments such as this can create religious, cultural, and philosophical barriers to the use of such products for those uncomfortable with putting animal materials in their mouth.
- The synthetic nature of the Aware Messenger™ swab and its use without treatment allows for excellent lot-to-lot reproducibility. Natural materials such as cellulose have the potential for significant lot-to-lot variability, especially when treated.
- The Aware Messenger™ swab material is laundered after manufacture so it is exceptionally clean. Chemical residues present in other collection materials are not an issue with the Aware Messenger™. Further, the swab material is adhered to the stick through a thermal weld eliminating the presence of adhesives.

- The absence of chemical treatments, adhesives, and manufacturing residues provides for a swab that has no taste. Not only can a taste negatively influence the collection experience (especially during long one to three-minute sample collections), it can also stimulate saliva production thereby diluting the highly desired gingival crevicular fluid.
- In addition to generating a taste, chemical treatments, adhesives, and manufacturing residues present in some oral fluid collection materials are introduced into the oral specimen. For example, the high concentrations of salt present in another commercially available collection pad are transferred to every oral fluid specimen. It is well established that high salt concentrations do not encourage antibody-antigen bonds.
- The Aware Messenger™ collection swab is exceptionally soft providing for a very comfortable collection.
- The loose double ply of the Aware Messenger™ collection swab promotes rapid mixing of the collected oral fluid and the sample buffer. The easy extraction of the sample from the pad is accomplished at the time of collection thereby eliminating the need for centrifugation of the sample at the testing laboratory, a time-consuming practice required by most of the current oral fluid collection devices.
- The thermal bond provides for an exceptionally strong adherence of the swab material to the stick essentially eliminating the risk of the swab head falling off during sample collection. Many collection devices do not have such a bond or even employ a handle thereby increasing the risk of ingestion or choking.
- The handle of the Aware Messenger™ is made of a low binding plastic. Handles used for some other collection devices are made of inappropriate plastics such as polystyrene which is notorious for its high binding properties and brittleness.
- The shape and size of the Aware Messenger™ collection swab was specifically designed to optimize collection of gingival crevicular fluid. The large flat surface area better ensures contact of the collection swab with the tooth-gum interface, a region rich with gingival crevicular fluid. This makes the collection procedure more robust and consistent between test subjects. The narrow side allows for a comfortable fit in the pocket between the gums and cheek. The handle length is optimized to provide a solid grasp and the rounded corners and high quality mold eliminate the possibility of sharp edges.

4.2 The Aware Messenger™ Sample Buffer



Calypte scientists understand the simple but often under-appreciated fact that the sample buffer matrix is one of the most important elements associated with any kind of diagnostic testing. An inappropriate detergent, pH, salt concentration, contaminant, stabilizing agent, or antimicrobial can wreak havoc on the specificity and sensitivity of an assay regardless of the concentration of the target analyte. This is especially true for oral fluid assays considering that larger specimen volumes are generally tested compared to those sample volumes required by serum assays. Similarly, instability of the target analyte in the oral specimen can also adversely impact results. Undoubtedly, attempts at using oral fluid specimens for some applications have been unknowingly thwarted by buffer compatibility issues or the loss of the analyte due to instability. Calypte scientists have utilized their significant experience in the field of immunochemistry and oral fluid diagnostics to develop a sample buffer for the Aware Messenger™ that provides for optimal assay compatibility, sample stability, and test performance with respect to sensitivity and specificity. The Aware Messenger™ sample buffer includes specifically formulated detergents, salts and stabilizing agents.

4.2.1 Detergents

Detergents in an oral sample are perhaps the most important components promoting the sensitivity and specificity of a diagnostic (e.g. ELISA). For blood testing, the issue is moot considering that a relatively small amount of a blood sample is diluted into a kit's sample diluent rich with detergents optimal for the given test. For oral fluid testing, however, detergents are critical considering that greater specimen volumes are tested. It is often the case that for every μL of oral fluid specimen added to the reaction well, a corresponding volume of the kit's sample diluent must be absent. Therefore, the challenge is to supplement the collected oral fluid sample with detergents that will best represent those in a kit's the sample diluent.

Surely no two tests are identical and therefore providing the Aware Messenger™ sample buffer with specific detergents that are optimal for every possible test is impossible. However, through experimentation and experience Calypte scientists have identified particular detergents that consistently increase the specific signal, suppress the nonspecific signal, or do both actions simultaneously. These detergents are present in the Aware Messenger™ sample buffer in optimal concentrations. In contrast, some alternative oral fluid collection devices have unusually high levels of generic detergents or no detergent/sample buffer at all.

4.2.2 Salts

Most other oral fluid collection devices either do not have a sample buffer (the oral sample is simply raw saliva) or include an unbuffered diluent. In either case, the pH of the sample will be dictated entirely by the sample itself and thus promote extreme sample-to-sample variability. This variability with respect to such a key specimen parameter complicates assay optimization efforts and may compromise test results, especially when there is a quantitative element involved. Quite simply, regardless of the absolute amount of the target analyte in an oral specimen and the sensitivity of the test, the assay performance can be greatly compromised and confounded when the pH of the sample is very high or very low. Furthermore, during any analysis, sample-to-sample consistency is extremely critical. For blood testing, not only is there great homology between specimens, but any minor differences are normalized upon dilution of the serum or plasma into the particular assay's sample diluent. This is not the case for oral fluid testing where greater volumes are tested and thus the sample diluent is less influential. To counteract this variability Aware Messenger™ sample buffer is buffered with appropriate salts leading to better homology between specimens. Furthermore, appropriate salinity contributes to optimal antigen-antibody binding in most tests.

4.2.3 Stabilizing agents

A target analyte may be in high concentrations and bathed in an environment of optimal detergents and salinity only to have its instability mask its presence leading to poor sensitivity results. This was a significant problem in the early days of oral fluid diagnostics and remains a major cause of problems when some oral fluid collectors are used. Instability of a target analyte can be attributed to three causes:

- Degradation through thermal energy
- Microbial and enzymatic degradation
- Physical loss due to adherence issues

As is discussed below, Aware Messenger™ employs counter measures to combat each of these three avenues of analyte instability:

Degradation through thermal energy:

Thermal energy affects chemical bonds and eventually can change the presentation of the antibody active site and/or the binding site (epitope) of the target antigen. Such changes can impact both the sensitivity and specificity of a test. Calypte scientists have identified specific stabilizing agents that minimize the affects of thermal energy and included them in the Aware Messenger™ sample buffer. While no set of compounds can completely eliminate the potential for loss over time, Calypte test results have demonstrated the Aware Messenger™ provides for very good stability. Furthermore, the same stabilizing agents have been demonstrated to promote good stability upon the freeze-thaw of the Aware Messenger™ specimen. Please note that Calypte recommends individual stability studies specific to the application be conducted as part of the validation of the device as used with the specific test application.

Microbial and enzymatic degradation:

The growth of bacteria, yeasts, or molds can lead to degradation of the intended analyte as proteases and nucleases act upon their targets. Furthermore, the

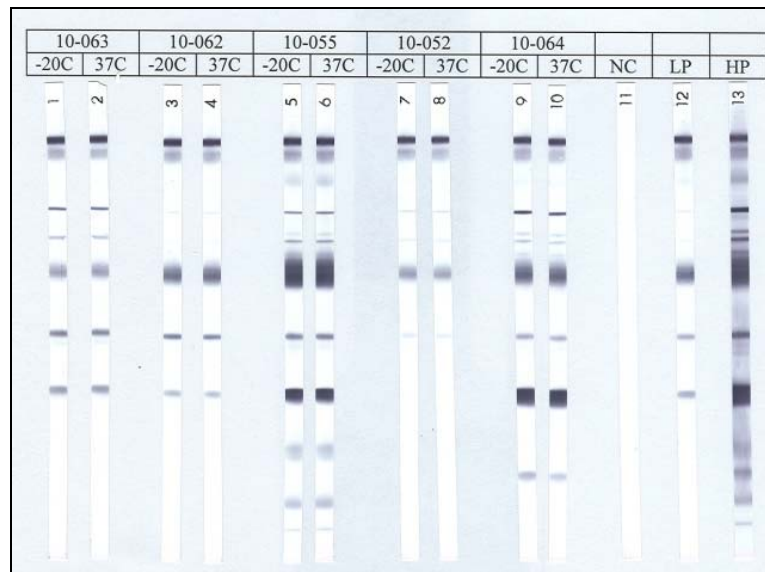
potential for such growth is high considering that the mouth is rich with microbial life. Although the use of antimicrobial agents to combat this problem is not novel, many oral fluid collectors do not utilize antimicrobials or include agents that are inappropriate in type or concentration.

The choice of antimicrobial is far from arbitrary. Many antimicrobial agents are chemically reactive and thus are counterproductive to sample stability and assay performance. Others are plagued by problems such as their instability, sensitivity to light, toxicity, limited range, or disposal and use issues. The antimicrobial agents used in Aware Messenger™ do not have any of these issues and thus provide for a sample buffer with exceptional antimicrobial activity as demonstrated by challenges conducted by independent laboratories⁶⁵.

Physical loss due to adherence issues:

Even the best stabilizing and antimicrobial agents cannot compensate for instability due to loss of an analyte through its gradual adsorption by the collection device materials (e.g. binding to specimen tube and cap). Whereas some oral fluid collection devices utilize inappropriate “sticky” plastics (e.g. polystyrene) or store the collected specimen in the presence of the high-binding cellulose collection swab, the Aware Messenger™ uses a plastic tube and cap composed of materials with extremely low binding characteristics. Furthermore, as mentioned, the swab is not stored with the specimen thus eliminating a site for additional loss through binding.

The result of Calypte’s attention to the above three mechanisms to combat sample instability has resulted in a device that promotes exceptional stability for many applications. For example, in one Calypte study, specimens collected from HIV-infected individuals were tested after 3 weeks at 37°C and then compared to the same specimens that had been immediately frozen upon collection. In both EIA and Western Blot, the specimens did not vary significantly. Below is a photo of the Western Blot results comparing time zero to 3 weeks at 37°C.



Additional EIA studies focusing on assessing the stability of anti-gp41 antibodies have further demonstrated very good antibody stability over several weeks at 37°C.

Minimal loss of activity was observed even after 5 weeks at 37°C. The ability to store the collected oral specimen without refrigeration for several weeks greatly expands the versatility of the collection device. Theoretically, Aware Messenger™ specimens could be collected by non-professionals in the field in remote areas of distant countries then shipped without refrigeration to testing laboratories in the United States or other countries for batch testing. Please note, however, that Calypte recommends stability studies be conducted for each specific application.

4.3 Aware Messenger™ Sample Collection Process

The Aware Messenger™ sample collection process is simple and quick yet delivers a high quality sample that is rich with various analytes representative of those found in blood. As mentioned previously, the pad is made of a soft, highly absorbent polyester fabric that minimizes the possibility of oral abrasion and does not have a taste. The collection process targets the sources where oral IgG and other blood analytes are most concentrated – the oral mucosa and gingival crevices – in order to optimize 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 process does not require any time measurement and takes between 15 and 20 seconds, a much shorter time compared to other oral fluid collection devices (e.g., the OraSure collection device requires time measurement of 2 minutes).

4.3.1 Consistency of the Aware Messenger™ Specimens

Another advantage of the Aware Messenger™ collection procedure is that it results in a much more consistent set of specimens compared to those where saliva is stimulated and collected. As expected, data reveals a tighter distribution of both sample volumes, properties (e.g. pH, salinity), and blood protein concentrations in Aware Messenger™ specimens compared to oral samples collected with other devices. Any sample collection procedure that includes (1) long collection times, (2) mastication of collection materials, or (3) anything material that stimulates saliva (e.g. taste) will result in a diverse set of specimens. Such diversity can significantly confound assay optimization efforts.

Aware Messenger™ specimens can also be self-collected by the test subject. Calypte studies compared the levels of a representative protein (IgG) in specimens that were collected and mixed by test subjects themselves to those mixed by an experienced Calypte scientist⁶⁵. There were no differences between the specimen sets. Self-collection allows for simultaneous collection of specimens from large groups of people. Quite simply, devices can be distributed to a group and in less than one minute later, capped specimens are returned that are ready for testing, storage, or transport.

4.3.2 Robustness of the Aware Messenger™ Collection

A common and often legitimate concern when oral fluid specimens are collected is the influence of other oral factors upon sample integrity. Such factors include (1) beverage ingestion, (2) food ingestion, (3) smoking, (4) dentures, (5) oral care (tooth brushing, etc.), and (6) oral health. Calypte performed several detailed studies demonstrating that none of these factors influence the integrity of the Aware Messenger™ specimen⁶⁵. For example, the integrity of the Aware Messenger™ oral fluid specimen was not influenced by ingestion of beverages that were hot (coffee, tea), cold (cola), acidic (orange juice), sweet, or bitter, even when the beverage was ingested immediately before the sample

was collected. Calypte recommends that similar interference studies be conducted by the user during the validation of the application when using this device. The robustness demonstrated by the Aware Messenger™ provides confidence in its use and eliminates the need for complicated and often confusing device use limitations imposed by other oral fluid collection devices with respect to eating, drinking, and smoking.

4.3.3 Aware Messenger™: Ready-To-Use Specimen

The properties of the Aware Messenger™ collection pad described in section 4.2, coupled with the nature of the collection procedure, allow for the sample to be “processed” upon collection thus eliminating the need for any further processing, such as centrifugation, filtering, etc., at the testing laboratory. This provides for a significant advantage for the Aware Messenger™ compared to most other oral fluid collection devices which require such centrifugation. Preparation of serum or plasma also requires centrifugation. This step-saving feature of the Aware Messenger™ device is especially advantageous in batch testing scenarios where high numbers of specimens are to be evaluated. Testimony from high-volume testing centers confirms that centrifugation steps require the time and effort of laboratory personnel, introduce delays, and very often create ‘bottleneck’ situations. Furthermore, some devices require a second tube be used to collect the specimen from the first tube during centrifugation. This scenario adds the cost for the second tube and cap, labor for the labeling of the second tube, and also introduces an opportunity for sample mix up. In contrast, the Aware Messenger™ specimen always remains in the same labeled tube that it was initially collected in. The several advantages provided for by the Aware Messenger™ make it an ideal candidate for batch testing scenarios where there is a high throughput.

5 Safety of the Aware Messenger™

Attention to safety was strictly followed during the design of the Aware Messenger™ oral fluid collection device. The most critical component of the device with respect to safety is the collection swab, considering that it actually is placed into the mouth. Calypte scientists sourced the most qualified manufacturing site for the Aware Messenger™ swab material. The packaged swab is manufactured under cGMP conditions by an ISO 13485-certified plant. To verify the safety of the swab, Calypte submitted collection swabs to an independent testing laboratory for a series of rigorous biocompatibility studies. The results of the studies demonstrated that the Aware Messenger™ collection swab does not pose a risk to the user and conforms to ISO 10993 standards for biocompatibility.

Inadvertent ingestion of the Aware Messenger™ sample buffer is a recognized, if unlikely, hazard. Calypte has formulated the sample buffer such that there would be no risk to the user upon accidental ingestion.

The Aware Messenger™ collection procedure is safe. The swab material is exceptionally soft and is not chemically treated. The handle is free of flashing and other sharp edges eliminating a source of abrasion. The swab material is tightly adhered to the stick by a thermal bond essentially eliminating the risk of removal and choking during collection. Lastly, exposure to the gum lines is limited to 15-20 seconds.

6 Summary

As presented in the body of this file, the Aware Messenger™ oral fluid collection device was carefully designed by experienced scientists to accomplish the following goals:

Goals

- Collect and release human oral mucosal transudate, a component of oral fluid that is rich with antibodies and analytes as found in the blood (e.g. IgG, other serum proteins, etc.)
- Stabilize the specimen to allow for testing at a later time or at a site remote from where the specimen was collected
- Allow for transport of the specimen to a laboratory for batch testing
- Provide a specimen that is easy to work with in a matrix that is more likely to be compatible with typical immunoassays
- Eliminate the need for any sample processing steps at the testing laboratory (e.g. centrifugation, filtering, etc.)
- Exceptional ease of use
- Eliminate user risk

The result of these efforts has led to the development of a first of its kind oral fluid sampler with many advantages over the competition including:

Advantages

- Simple, rapid, comfortable, and minimally invasive sample collection
- Immunoassay-friendly sample buffer allowing for ease of test optimization and performance
- Improved specimen consistency
- More consistency between collected specimens
- High quality oral specimen rich with the valuable gingival crevicular fluid
- Excellent sample stability
- Ready-to-use specimen that does not require any processing at the laboratory
- Extremely safe

Aware Messenger™ oral fluid collection device allows for the coupling of the benefits inherent to oral fluid sampling and high-volume batch testing. The inclusion of the Aware Messenger™ oral fluid collection device into existing testing schemes provides advantages to the test subject, testing laboratory, and study coordinator.

7 Warnings and Precautions

- a. The performance characteristics of the Aware Messenger™ oral fluid collection device have not been established and validated for any specific assay or test. Use with any specific assay for diagnostic purposes must be properly validated by the laboratory conducting the testing.
- b. The storage condition of collected specimens depends upon the test application.
- c. Although risk of exposure to pathogenic organisms from oral fluid is in most cases lower than from blood, handle specimens and materials contacting specimens as if potentially infectious biological materials in accordance with “Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and Other Bloodborne Pathogens in Health-Care Settings” (CDC, MMWR, June 24, 1988).
- d. Occupational Safety and Health Administration (OSHA) regulations (29 CFR 1910) apply to personnel collecting and handling human clinical specimens.
- e. Federal, state and local regulations for human biologic test specimens apply to the transportation of oral fluid specimens which may contain etiologic agents (39 CFR 111). Other local regulations may also apply.

8 Contact information

Aware Messenger™ oral fluid collection device is available in 50 count boxes (Product Number 98184).

For details or technical questions, please contact:

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