



Commercial Saliva Collections Tools

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ABSTRACT Saliva has been used as a specimen for diagnostics purposes for many years, but it has only been in the last 10 years that a number of new tools have been developed that promise to greatly increase the use of oral specimens for broad-based diagnosis and potentially screening applications. This article focuses on tools that are commercially viable or can play a role in whole saliva collection and future testing for critical diseases.

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Conflict of Interest

Disclosure: Paul D. Slowey, PhD is the CEO of Oasis Diagnostic Corporation.

In the literature, the word “saliva” has been used in literal fashion to describe secretions in the oral cavity; however, a number of different sub-components exist in saliva. Various terms may be used to describe fluids collected from the mouth, including the broad term saliva, oral fluids, gingival crevicular fluid and others. Below are brief definitions of the most important terms used when discussing salivary tools with potential diagnostic or investigative applications:

Saliva — a watery substance located in the mouth of organisms, secreted by three main salivary glands – the submandibular, the parotid and sublingual, as well as hundreds of other minor salivary glands and gingival crevicular fluid. Human saliva is composed of 95% water but also electrolytes, mucus, antibacterial compounds and enzymes. It performs many normal functions including food

digestion, lubrication, taste facilitation and bolus formation.

Oral fluids — this term is often used interchangeably with saliva and used frequently in forensic toxicology, particularly in the drug-testing world.

Gingival crevicular fluid — a fluid occurring in minute amounts in the gingival crevice, believed by some authorities to be an inflammatory exudate and by others to cleanse material from the crevice containing sticky plasma proteins, which improve adhesions of the epithelial attachment, have antimicrobial properties and exert antibody activity.¹

It is outside the scope of this manuscript to include a range of good and effective tools on the market that collect various sub-components of whole saliva for a range of research applications. Information on these types of devices has been reported elsewhere.^{2,3}



FIGURE 1: OraSure Oral Specimen Collection Device (OraSure Technologies)



FIGURE 2: Quantisal Oral Fluid Collection Device (Immunoanalysis Corporation)



FIGURE 3: Salivette (Sarstedt)

History

The early pioneers in the commercialization of salivary diagnostic tools were Epitepe, Inc. (Beaverton, Ore.) and Saliva Diagnostics Systems (Vancouver, Wash.). Each developed saliva collection devices that have proved to be possibly the most successful tools on the market today. Now called OraSure Technologies (Bethlehem, Pa., orasure.com), Epitepe, Inc. originally developed the OraSure Oral Specimen Collection Device (**FIGURE 1**) for general-purpose saliva collection, but broadened its use by “marrying” the device to test kits with specific applications in mind. The device consists of a cellulose pad material attached to a plastic stem. The pad material is used to rub the surfaces of the cheeks adjacent to the gumline for a period of time then left in the oral cavity between the teeth and gumline to absorb a salivary sample. OraSure describes this as “oral mucosal transudate.” The pad material is pretreated with proprietary salts that aid the collection process. Collection time is 2 - 5 minutes after which the device is placed into a collection tube containing a buffer and transported to a laboratory. Dilution of the collected sample in buffer requires a centrifugation step upon receipt prior to sample analysis.

The OraSure device was linked to an HIV-1 Enzyme Linked Immunosorbant Assay (ELISA) test from Organon Teknika (Boxtel, Netherlands), which eventually would become the first FDA-approved oral test for detection of the

HIV virus. Applications for the OraSure HIV-1 product include public health screening, surveillance, life insurance risk assessment and outreach programs.

The OraSure device is also used for substance abuse testing for the NIDA-5 (THC, cocaine, opiates, methamphetamines and PCP) and other abused drugs, under the brand name Intercept. This device has widespread application in forensic toxicology, workplace testing and criminal justice settings among others.

Now called StatSure Diagnostic Systems (New York, statsurediagnostics.com), Saliva Diagnostics Systems originally developed the Saliva-Sampler Collection Device, also trademarked in certain parts of the world as Omni-SAL, for standardized saliva collection. The Saliva-Sampler device was used for general-purpose saliva collection and received 510(k) approval from the FDA for such purposes but was never “paired” with any specific diagnostic or abused drug tests until the rights to the product were transferred to California-based Immunoanalysis Corporation (Pomona, Calif., immunoanalysis.com). Immunoanalysis rebranded the product as Quantisal and validated saliva collection to a series of ELISA-based drug tests assays, which have received FDA clearance and are subsequently sold for workplace testing, forensics, criminal justice and other applications.

The Quantisal Oral Fluid Collection Device (**FIGURE 2**) also uses a cellulose material attached to a stem to harvest

saliva. An absorbent pad is placed in the mouth and saliva collected until a sample volume indicator built into the device changes color from white to blue (approximately 2 minutes) indicating sufficient saliva [1.0 mL + or - 10 percent) has been collected to perform any subsequent analysis. The absorbent pad has a series of perforations near the top of the cellulose pad, which allows easy detachment of the pad into a transportation tube containing a stabilizing buffer to ensure safe delivery of the sample to the laboratory for testing.

Another of the early saliva innovators was Sarstedt (Nümbrecht, Germany) which introduced the Salivette tool in 1987. Although Salivette has not received any approvals or clearances from the FDA, the collection system is widely used in the market for research applications ranging from detection of steroid hormones from saliva, HIV - antibody detection, markers of oxidative stress and others.

Salivettes (**FIGURE 3**) are available as cotton or polypropylene rolls/sponges, each with an associated transport tube. Salivette is placed in the mouth and chewed for approximately two minutes then placed into the transport tube for dispatch to a testing laboratory. The device does not incorporate any means of sample sufficiency and the specimen must be centrifuged prior to analysis.

The ORACOL collection kit (**FIGURE 4**) from Malvern Medical Developments (Worcester, U.K., malvernmedical.uk.com) utilizes an absorbent foam material

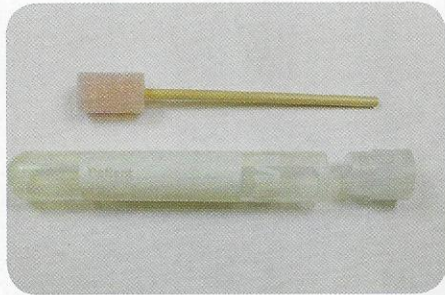


FIGURE 4: ORACOL Saliva Collection Kit (Malvern Medical)



FIGURE 5: UltraSal-2 Split Sample Saliva Collection Device (IDS/Neogen U.S.)

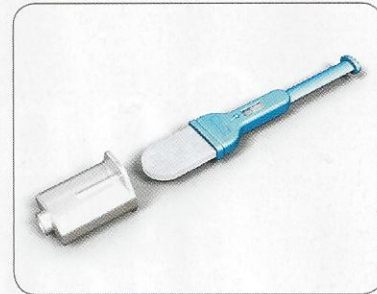


FIGURE 6: Versi-SAL Oral Fluid Collection Device (Oasis Diagnostics)

in a swab format to collect up to 1 mL of saliva. The kit consists of an absorbent foam swab (designed to collect up to 1 mL of saliva), centrifuge tube and cap. Saliva is collected from the oral cavity by absorption then centrifuged in the tube provided. The processed specimen is typically used for infectious disease testing particularly measles, HIV, hepatitis A and B, mumps and rubella.

UltraSal-2 (**FIGURE 5**) is a large-volume saliva collection device featuring two distinct collection tubes that allows "split-sampling" from the same subject. The user expectorates into a mouthpiece connected to two 12 mL tubes and directs saliva into one or the other tube by rotating the device at the appropriate angles to allow sufficient saliva to be collected into each tube. UltraSal-2 is manufactured by International Diagnostic Systems (St Joseph, Mich.), a subsidiary of Neogen Corporation (Lexington, Ky., neogen.com) UltraSal-2 is used mainly for drug testing purposes.

Oasis Diagnostics Corporation (Vancouver, Wash., 4saliva.com) manufactures oral-based tools including Versi-SAL, a device for standardized saliva collection. Versi-SAL uses a non-cellulosic pad material to collect saliva from *under* the tongue. After approximately 1 to 2 minutes, sample sufficiency is indicated by the change in appearance of a sample volume adequacy indicator built into the device. Saliva is subsequently delivered into a standard 2 mL Eppendorf tube by expressing the sample through a plastic compression tube provided with the

device. Various configurations of the device can provide between 0.5 and 1.0 mL of whole saliva, with the possibility of dual samples from the same patient. The Versi-SAL Oral Fluid Collection Device (**FIGURE 6**) is used for general-purpose saliva collection including steroid hormones for wellness testing, abused drug analysis, nicotine testing and others.

There are also a number of "specialized" collection tools for salivary hormone collection developed by manufacturers of microplate ELISA kits as "companion tools" for collection that are sold in conjunction with various test kits. Examples include DiaMetra (Milan, Italy, diametra.com), IBL (Hamburg, Germany, ibl-international.com), Salimetrics (State College, Pa., salimetrics.com) and others. Further, some "emerging" salivary collection tools that will "challenge" today's market-leading products are discussed in the section entitled "What the Future Holds?"

Current Tools for Saliva Collection and Diagnosis

The early success of companies such as OraSure, StatSure, Sarstedt and others paved the way for a much broader array of salivary collection tools that are now available in two specific areas worthy of mention.

The first is in so-called "point-of-care" (POC) tests that marry the capability of standardized saliva collection with functional lateral flow immunochromatographic (LFT) test strips to deliver immediate results from

salivary samples. Areas that have seen the most growth include substance abuse detection and HIV diagnosis. While rapid POC saliva tests are definitely growing in significance, and certain tools have made a clear impact, point-of-care diagnosis using oral samples is still in the embryonic phase.

The other significant growth area for salivary diagnostics is in molecular diagnostics (nucleic acid testing, NAT), particularly PCR, genotyping, sequencing techniques, genome-wide association studies (GWAS) and other molecular techniques where it has been proven that salivary samples are equivalent in performance to blood sampling and are more cost-effective, convenient and simpler to use. This area of salivary diagnostics is one of the most rapidly expanding areas. Within this area, the advent of point-of-care molecular diagnostic platforms offers up the perfect combination of noninvasive sampling with immediate diagnosis for most, if not all diseases or conditions. This is arguably the fastest growth area in oral diagnostics. Some of the tools/devices that have already made an impact in these two areas of the in vitro diagnostic industry are described below.

Of all the salivary diagnostics on the market today there is no doubt that OraSure Technologies' OraQuick Advance HIV 1/2 rapid, oral fluid test for the HIV virus has made the greatest impact. This product launched internationally in 2000. Since then, the device has received FDA



FIGURE 7:
OraQuick Advance
HIV 1/2 Rapid Oral
HIV Test (OraSure
Technologies)

approval and has changed the paradigm for clinical testing for HIV in the U.S. OraQuick Advance HIV 1/2 (FIGURE 7) has been adopted widely by governmental public health organizations including the Centers for Disease Control (CDC), Substance Abuse Mental Health Services Administration (SAMHSA) and the World Health Organization (WHO) overseas as a tool to identify HIV-infected individuals in nontraditional settings including mobile vans, bathhouses, emergency room situations and in publicly funded screening programs.

OraQuick consists of a fairly rigid pad connected to a lateral flow immunochromatographic (LFT) test strip. The user swabs the area under the gumline, collecting a specimen in a few seconds. The sample device is then immersed in a buffer/reagent solution in a tube provided by OraSure. The buffer is allowed to migrate up and onto the LFT test strip embedded in the device. After 20 minutes the results of the qualitative test are read. If a single line is observed the sample is negative. If two lines are observed, the result is classified as a "preliminary positive" result until the result can be confirmed by a more accurate test, usually Western blot analysis. The performance of OraQuick is equivalent or better than many FDA-approved ELISA tests for the HIV virus and has become a standard for diagnosis in the industry.

At the time of writing, the FDA is considering approval of the OraQuick device for over-the-counter use. If



FIGURE 8: DDS Rapid Drugs of Abuse System (Cozart Biosciences)

approved (and approval seems likely), this could open the door for the development and commercialization of many other saliva-based rapid tests.

The area of roadside drug testing is another area where saliva testing has gained a foothold, mainly due to the convenience factor of being able to collect samples noninvasively from would-be "drugged" drivers. Some technological challenges are still to be overcome, but several companies have met with some limited early commercial success. The net result is that a number of other companies are now targeting this area with novel technologies. The most notable successes so far have been Cozart Biosciences (Abingdon, U.K., concateno.com, now Alere, Inc., Waltham, Mass., alere.com), Securetec (Munich, Germany,

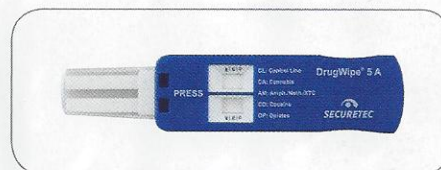


FIGURE 9: Securetec AG Drugwipe 5

securetec.net) and Mavand (Mössingen, Germany, mavand.com).

Cozart developed the RapiScan drug testing system that incorporates saliva collection using the Saliva Diagnostic Systems Omni-SAL device in conjunction with a rapid lateral flow test that could be read by means of a hand-held reading unit (RapiScan). Test results are available in 10-15 minutes from start to finish for a series of six abused drugs. Cozart now markets an upgraded version of the RapiScan device, known as the DDS system (FIGURE 8).

Securetec AG's DrugWipe 5 is a 10-minute test that detects up to six drugs following a very rapid collection of specimen by wiping the tongue until an indicator dye changes color to yellow. Results are read visually on the test strip (FIGURE 9).

TABLE 1

List of Representative Rapid Oral Drugs of Abuse Tests /Manufacturers

Manufacturer	Website	Product Name
American Bio Medica Corporation	abmc.com	OralStat
JAJ Scientific	jajinternational.com	QikTech
Innovacon (Alere)	innovaconinc.com	OrALert
Mavand	mavand.com	Rapid STAT
Envitec	envitec.com	SmartClip
Sun Biomedical	sunbiomed.com	OraLine
Branan Medical	brananmedical.com	Oratect XP
Ulti-med	ultimed.org	SalivaScreen
Varian	varian.com	OraLab 6
Securetec	securetec.net	DrugWipe 5



FIGURE 10: OraGene DNA Salivary DNA Collection Device (DNA Genotek)



FIGURE 11: DNA-SAL Salivary DNA Collection Device (Oasis Diagnostics)



FIGURE 12: SCS Collection System (Greiner Bio-One)

Mavand offers a multi-drug screen known as Rapid STAT that can detect up to seven drugs in 13 minutes or less without any instrumentation. Collection of saliva takes less than 30 seconds and results on the most recent surveys by the European body known as Roadside Testing Assessment or ROSITA (rosita.org) are promising.

This independent group is responsible for evaluation and validation of tools for drug testing at the roadside. For more information on salivary devices with applicability in law enforcement screening, see the ROSITA website, rosita.org. **TABLE 1** is a list of websites that summarize a number of other handheld drug tests that are available for abused drug testing in forensics, employment screening, workplace testing and criminal justice. This list is not comprehensive.

In the molecular diagnostics space, there is one supplier that has pioneered applications for salivary DNA collection. DNA Genotek, now owned by OraSure Technologies, launched the Oragene device (**FIGURE 10**) in two formats for simplified collection and stabilization of DNA from saliva samples. Statistics show that this device has been widely adopted in the personal genomics and research areas. In 2012, the Oragene device became the first salivary collection tool cleared by the FDA for clinical use when Oragene is used in conjunction with the GenMark Diagnostics eSensor test for Warfarin sensitivity.

In order to collect an Oragene specimen, the subject expectorates into a collection tube until a certain volume

of saliva has been collected (2 mL, 2 - 30 minutes). A proprietary buffer solution is released once the attached cap is screwed into place. This buffer solution acts to immediately stabilize the DNA present in saliva for a range of downstream applications. Oragene collects a large quantity of DNA, which is purified using reagents provided by the manufacturer and suitable for multiple diagnostic technologies. High-profile personal genomics companies, including 23andMe (Mountain View, Calif., 23andme.com) and Navigenics (Foster City, Calif., navigenics.com), that provide personal testing services to the general public have embraced the Oragene technology as a means of collecting samples safely and effectively directly from consumers.

The DNA-SAL Salivary DNA Collection device from Oasis Diagnostics (**FIGURE 11**) came to market in 2011 and works via a different mode of action. DNA-SAL is a “raking/scraping” tool that is used to abrade cells from the inside of the cheek by rubbing gently for 30 seconds. Some buccal/epithelial cells remain trapped on the device head, while others remain free flowing in saliva in the mouth. The loose cells are “harvested” using a small quantity of a safe stabilizing solution based upon a mouthwash formulation that is taken in the mouth, “swished” around for 15 seconds, and then expectorated back into the sample tube. DNA is immediately stabilized for long periods of time once the saliva comes in contact with the stabilizing rinse solution. DNA may then be

transported to the laboratory for isolation and downstream analysis, or as an alternative, Oasis provides a method for immediate downstream testing without DNA isolation with a simple sample manipulation.

What the Future Holds?

The future of salivary diagnostics is extremely bright. There are a number of new tools and technologies coming to market simultaneously that are perfectly set up for noninvasive sampling. A number of additional areas hold great promise for salivary testing, but this final section will focus on three areas poised for arguably the greatest growth: saliva collection; point-of-care testing (MDx, proteins, small molecules, etc.); and clinical/research molecular diagnostics (MDx) using RNA/DNA.

Saliva Collection

The increased awareness of saliva as a specimen of choice has spawned a new generation of tools for saliva collection. These new tools increase the opportunities for testing both near the patient and using downstream laboratory technologies. Specific examples of tools likely to have the greatest impact on testing regimens are:

- Greiner Bio-One SCS Saliva Collection System (Monroe, N.C., us.gbo.com)

The SCS Saliva Collection System (**FIGURE 12**) is a series of tubes, reagents and a sample cup for general-purpose saliva collection.



FIGURE 13: Saliva Collection Aid (SalivaBio)

To collect saliva using the SCS system, the user rinses the oral cavity with the first of a number of safe reagents and expectorates the liquid back into the sample cup provided. A separate, unopened sampling tube is immersed in the saliva sample collected, causing the collected sample to run up and into this secondary tube. Once filled, the sample is stable for analysis or for transportation to a laboratory.

■ SalivaBio Saliva Collection Aid (Baltimore, salivabio.com)

This is a brand new device developed by researchers at Johns Hopkins School of Nursing. The Saliva Collection Aid was originally developed for hormonal analysis, but has greater applicability and may be used for most applications where saliva is required. The device works by expectorating saliva into the Saliva Collection Aid, a plastic funnel type device (FIGURE 13). The “plastic funnel” component is connected directly to a transport tube provided by the manufacturer. The tube is capped and sent to a laboratory for processing.

■ Oasis Diagnostics Super-SAL/ iSCPSS device

The Oasis Diagnostics Super-SAL device (FIGURE 14) is a device for the collection of greater than 1.0 mL of saliva in a short time for laboratory testing for small molecules, antibodies and antigens. This device uses a cylindrical pad to collect saliva from in the mouth alongside the tongue for 30 - 45 seconds. Sample sufficiency is indicated by the change in appearance of a Sample Volume Adequacy Indicator (SVAI) built into the device. Once the SVAI is triggered, the sample is

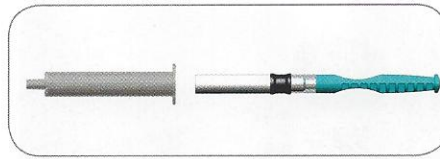


FIGURE 14: Super-SAL (Oasis Diagnostics)

squeezed through a compression tube and into a standard Eppendorf-compatible tube. Pure, whole saliva may then be immediately processed or sent to the laboratory for subsequent testing

Oasis Diagnostics is also collaborating with David Wong's, DMD, DMSc laboratory at the University of California, Los Angeles, to commercialize a device called iSCPSS, the Integrated Saliva Collection Processing Stabilization and Storage System, which integrates the Oasis Super-SAL device with components necessary to separate and independently stabilize both RNA and proteins for downstream research or clinical studies. The iSCPSS device (FIGURE 15) incorporates a proprietary filtration unit, which provides cell-free saliva that is subsequently separated into two distinct fractions. The two fractions are stabilized separately with specified reagents to yield long shelf life fragments that are assay ready. This device became available commercially at the end of 2012.

Point-of-Care Devices

The pending FDA approval for an over-the-counter application for the OraQuick Advance Oral Fluid HIV test is anticipated to lead to the development of a new generation of saliva-based lateral flow (LTF) assays that “piggy-back” on a number of available enabling technology platforms. The author notes already the availability of oral based tests for measles IgM from Microimmune and the Public Health Laboratory Branch at Colindale in the U.K.⁴, CRP from the University of Queensland⁵ and test development in process for cortisol

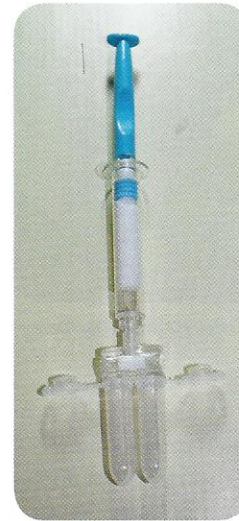


FIGURE 15: iSCPSS RNA/Protein Collection System (Oasis Diagnostics)

from Foresite Diagnostics (York, U.K., foresitediagnostics.com) and Oasis Diagnostics.^{6,7} Areas of growth are likely to be the areas of general wellness, infectious diseases, roadside drug testing and cardiovascular disease diagnosis.

The advent of point-of-care devices for nucleic acid testing (NAT) from companies such as TwistDx (York, U.K., twistdx.co.uk), Biohelix (Beverly, Mass., biohelix.com), Rheonix (Ithaca, N.Y., rheonix.com) and others could offer up new opportunities for oral testing. Currently these devices are based upon blood sampling technologies and would clearly benefit from a noninvasive sample source.

Clinical/Research Diagnostics Using RNA/DNA

Since the discovery of polymerase chain reaction (PCR) and other molecular techniques, the use of DNA as a building block for diagnostics has grown rapidly. Market sources from 2010 estimate that more than 500 million molecular tests are done annually in the United States and that this number will grow to 750 million by 2014.

A fraction of these tests already use saliva as a sample source, but trends indicate that as current studies are published confirming the efficacy

of saliva as an ideal specimen, the proportion of oral based tests will rise sharply. In addition, new research using RNA, including mRNA and miRNA, and proteins (proteomics) as diagnostic tools will only add new markets where saliva will be a specimen of choice.

Conclusion

The future of saliva testing is extremely bright with a number of exciting and functional techniques offering up noninvasive and cost-effective solutions for diagnosis that will find value in disease diagnosis all over the world. ■■■■

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