Saliva collection

A complete "how-to" guide for saliva collection and testing

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Introduction

If you're new to the world of saliva biosciences, collection, and testing then you're in luck. The chapters within this e-book promise to be your saliva-tion!

Before we dive into the depths of saliva biology and the nuances of collection and diagnostics, let's take a high-level view of saliva's position as a sample type in the present day. If you dig into the literature on saliva testing (over 14,000 publications in ~60 years), you'll see that today, the study and use of saliva do not have a single research or diagnostic area: researchers studying saliva belong to many scientific disciplines, from infectious diseases and epidemiology, to psychology, sociology, anthropology, and more.

How did the field of salivary biosciences become so widespread and useful to so many different disciplines?

Salivary biosciences reached a number of inflection points that account for its current position as an attractive research and diagnostic sample type. It started in the early 1980s with the finding that hormones such as cortisol (among others) can be measured in saliva and are similar to concentrations found in the blood. In the early 1990s, the collection of oral fluids with the OraQuick™ test became an essential public health tool to help stop the spread of HIV. Later, saliva became the sample of choice for measuring the presence of drugs of abuse. More recently, the scope of saliva use has continued to grow with the application of "omics" techniques and their use in tracking the spread of SARS-CoV-2. These events and many others were major milestones in the growing awareness about the advantages of saliva research and testing.

With the many successes in salivary bioscience and the advantages of its use as a sample type, it's a surprise that saliva is not more widely used for testing. In part, there is a lack of awareness and basic knowledge: with the use of saliva in so many disparate fields, finding best practices and guidelines and applying them to a specific research question or diagnostic problem can be challenging. With the vast collection of literature out there, the time for a "how-to" guide (such as the one you're reading), with essential, "need to know" information, all in one place, is now.

So, let's get on with it. We know you're salivating. Enjoy.

Contents

Saliva research

What is saliva and why is it important for diagnostics research?

Aside from when we lick a stamp, get a whiff of greasy fast food, or hold our mouth open for a routine dental cleaning, we typically don't give saliva more than a fleeting thought. In the diagnostics research world, the same has been historically true: oral sample types, like saliva, have been ignored and undervalued, reserved only for tests for oral infections or cancers[1].

Over the past decade, however, there have been major advances in salivary diagnostic research and saliva has quickly risen as a sample type with huge potential beyond applications in oral health. Look no further than the FDA-approved, direct-to-consumer (DTC) 23andMe™ Personal Genome Service Genetic Health Risk (GHR) tests for proof[2].The test is the first of its kind that assesses an individual's risk of developing 10 medical diseases or conditions, all with DNA extracted from a simple saliva sample.

The advantages of saliva as a diagnostic research sample type

It's easy to see why saliva is entering the fold as an alluring diagnostic research sample type: it's inexpensive to collect, can be sampled without the help of a medical professional and with minimal subject discomfort, it has been successful for human health monitoring and disease surveillance for SARS-CoV-2, and has been developed as a tool for human microbiome analysis[3-6]. Despite its high water content (~99%), it's also an incredibly complex biofluid with interesting physical and chemical properties and plays a critical role in normal human physiology and disease pathophysiology[7].

To gain a better appreciation for saliva and its growing use within the diagnostics research market, we'll take a closer look at some foundational questions about saliva's composition, physiological function, and its underappreciated history in the medical field.

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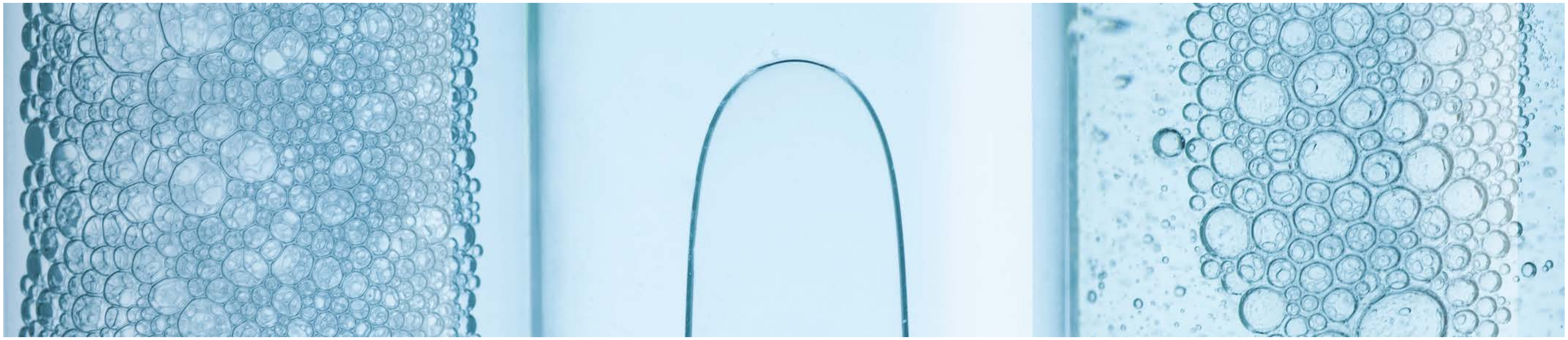

Taking a “lick” at composition: what is saliva made of?

Saliva is a complex mixture derived from the secretions of several different types of salivary glands. The composition and properties of these secretions make many normal, everyday functions possible and vary due to the time of day, diet, age, gender, disease states, or administration of pharmacological agents[8].

90% of the total salivary volume is made of the secretions of 3 glands, collectively called the major salivary glands: the parotid, the submandibular, and the sublingual glands[8]. Whole saliva, which fills our mouths daily at the astounding volume of 1 to 1.5 liters, contains both inorganic and organic components, secreted not only from the major glands, but also from the minor glands, gingival crevicular fluid, and much more[8-10].

Inorganic components of saliva

The inorganic components of whole saliva include Na^+, Cl^-, Ca^{2+}, K^+, HCO_3^-, $H_2PO_4^-$, F^-, I^-, Mg^{2+} and SCN^-[9,10]. Calcium and phosphate help to neutralize acids and, therefore, protect against tooth decay. Bicarbonate also acts as a buffer, maintaining the pH of saliva to be slightly acidic, between 6 and 7[9,10].

Organic components of saliva

There is a much larger and more diverse collection of organic components that make up saliva. Organic compounds include urea, ammonia, glucose, free fatty acids, triglycerides, amino acids, and many others.

There is also a substantial number of key proteins including mucins, amylases, agglutinins, glycoproteins, lysozymes, peroxidases, lactoferrin, immunoglobulins, myeloperoxidase, histatins, cystatins, statherins, and defensins, all of which help perform a variety of salivary functions[9,10].

To give these inorganic and organic electrolytes, proteins, and other compounds more context, let’s take a look at the overall functions of saliva and how each of these components fits in.

What is the function of saliva and what are its key components?

Each salivary component can be categorized as having one of five major functions (though some have multiple functions)[7-11]:

1. Lubrication and chemical/physical protection
2. Buffering and clearance
3. Maintenance of tooth integrity
4. Immune activity and microbial protection
5. Taste and digestion

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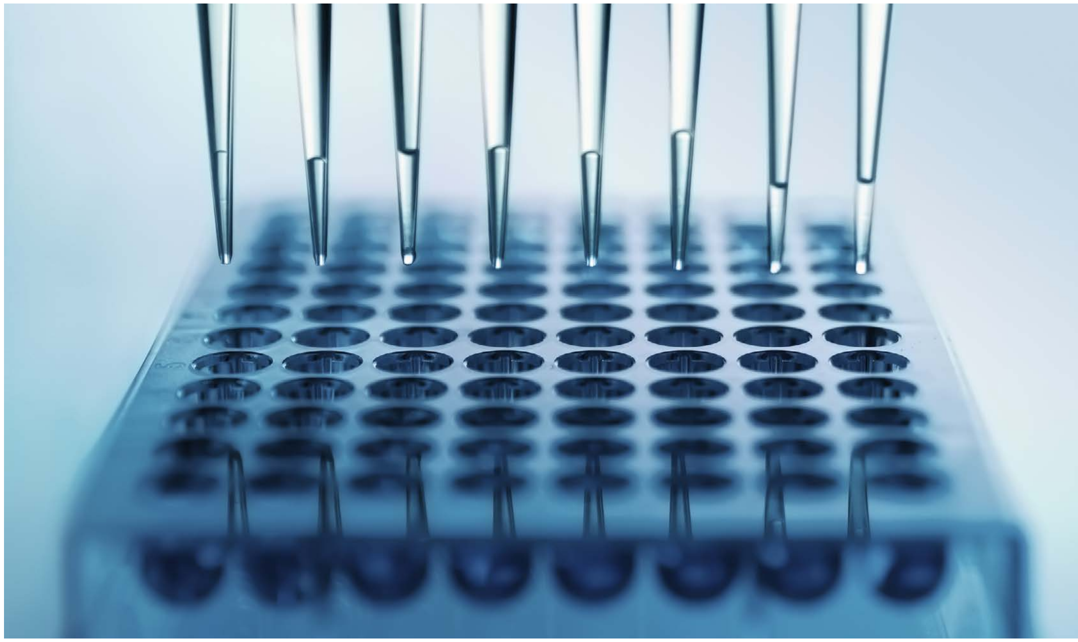

Saliva provides oral lubrication and protection

As oral tissues are open to the exogenous environment, they are under constant assault by toxic chemicals (e.g., carcinogens), microbes (both beneficial and pathogenic), enzymes produced by plaque, and desiccation from constant breathing[9]. Mucins, specifically two highly studied glycoproteins called highly glycosylated mucin (MG1) and single-glycosylated peptide chain mucin (MG2), play an important multi-functional role in the lubrication and protection of teeth and oral tissue.

Physical properties, such as high viscosity/elasticity/adhesiveness and low solubility, enable these and other mucins to lubricate tissue-tissue and tissue-teeth interactions, facilitating mastication, speech, and swallowing[9]. Mucins also bind directly to tooth enamel, promoting the growth of beneficial bacteria, the clearance of caries-causing bacteria (e.g., Streptococcus mutans), acid resistance, and mineral stabilization at the tooth surface[9].

Saliva for buffering and clearance

As mentioned above, bicarbonate plays a major role as a buffer, that neutralizes plaque-promoting acid[9,12]. Histidine-rich proteins (called histatins), urea, and phosphate also help buffer against significant pH change[9,13]. Salivary flow rate, which naturally varies due to many factors, is an additional mechanism by which major pH changes are avoided, aiding in the clearance of plaque-promoting bacteria and compounds[9].

Saliva helps maintain tooth integrity

The inorganic and organic salivary components mentioned above help prevent demineralization of tooth enamel by buffering against large pH changes. Proteins such as statherins, histatins, cystatins, and several others form a protective barrier, called a pellicle, around tooth enamel and actively promote remineralization with calcium and phosphate[9]. This enables long-term maintenance of protective tooth enamel.

Saliva is hypotonic, enhancing the ability to taste salty foods.

Fluoride also helps promote the formation of stronger, more caries-resistant tooth enamel by replacing magnesium and carbonate within enamel crystals[9].

Saliva supports immune activity and microbial protection

The oral cavity can often be the first point of contact and a common route of transmission for pathogenic microbes, including viruses, bacteria, and fungi. Accordingly, one important function of saliva is as the first line of defense against microbial threats.

This defense system consists of lysozyme, peroxidase, lactoferrin, immunoglobulins (e.g., IgA, IgG, and IgM), and a wide array of antimicrobial peptides that can recognize and/or attack specific microbial invaders[7,9]. These salivary components pursue microbes with distinct mechanisms of action[7,9].

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Other proteins, such as mucins, statherins, agglutinins, and histatins, prevent the growth of certain microbes in the oral cavity via a more indirect mechanism of action. These proteins promote aggregation and clearance of viruses, bacteria, and fungi, preventing attachment and colonization[9].

Saliva boosts taste and starts the digestion process

The final function of saliva we'll discuss is perhaps the most top-of-mind for many—facilitating taste and beginning digestion. Saliva is hypotonic (i.e., it contains lower concentrations of certain ions than are present in plasma), enhancing the ability to taste salty foods[7,9]. Saliva also contains amylase and lipase, enabling preliminary digestion of starches and fats from food as a food bolus is formed, swallowed, and delivered to the digestive tract[9].

Over the past century, the composition of saliva and its physiological function have been heavily studied and clearly elucidated. Yet, as stated earlier, saliva is only now emerging as a powerful sample type for diagnostic research, with approved direct-to-consumer (DTC) testing and successful use of salivary diagnostic research as a **"gold standard" for SARS-CoV-2 detection**[2,4].

To better understand where the saliva testing field is going, we need to know where we've been. Let's see how salivary diagnostic research began and how it's evolved over the past century.

The history of saliva research diagnostics: from the Rice Test to today

Blood and urine rapidly **found a place in early medicine**, with uroscopy (the visual examination of urine) dating as far back as the year 1090 in Jerusalem[8,11]. By contrast, saliva's role in medicine came (relative to other biofluids) much later and it was instead used in an unexpected role as an ancient polygraph, called the "Rice Test"[8,11]. Several cultures used the "Rice Test" to assess if a person was guilty of an accused crime, administering a mouthful of dry rice to the accused. If anxiety, presumed to be heightened if one was guilty, prevented significant salivation and subsequent bolus formation, then the accused was deemed guilty[8,11].

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Saliva didn't find its way into medical diagnostic research until the early 1900s, when it was used as a very crude assessment of diathetic diseases (i.e., "abnormal states or conditions"), such as gout or migraine[8,11].

Advancing the promises of salivary diagnostic research with the Thermo Scientific™ SpeciMAX™ Saliva Collection Kits

The promise of saliva as an advantageous sample type is now being widely accepted. To continue its advance, supporting products that promote streamlined, high-throughput sample collection, preparation, assaying, and analysis need to be developed. Two supporting products include the SpeciMAX Saliva Collection Kit and the SpeciMAX Stabilized Saliva Collection Kit.

This article contains product information intended for General Laboratory Use. It is the customer's responsibility to ensure that the performance of the product is suitable for customer's specific use or application.

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Discover SpeciMAX Saliva Collection Kits
Plain/raw and stabilized saliva collection kits

Both kit types provide:

Standardized process

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- Standard cap is compatible with decappers

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- Optimized for saliva collection, barcodes provided (printed on stabilized tubes), individually packaged, 95 kPa–compliant

Specific to the **plain/raw** kit

Cost-effective

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- **Recommended storage: 4°C**

Specific to the **stabilized** kit

Stabilization, safety, and added ease of use

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Saliva collection

How to use the SpeciMAX Saliva Collection Kits

Watch how to use the SpeciMAX Saliva Collection Kits with this short protocol video

SpeciMAX Saliva Collection Kits use 6 mL standardized tubes and caps that are compatible with automation racks and decappers. Paired with a liquid handler, the kits reduce pipetting time for downstream saliva workflows. Designed for safe transport, SpeciMAX Saliva Collection Kits use less refrigeration and incubator space than other supplies. There are no sample swaps to work around, and the kits are available in raw and stabilized versions to help meet your application needs.

Browse collection kits

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Sample accessioning

The future of saliva biomarkers

With saliva research for SARS-CoV-2 growing in popularity and serving as a definitive proof point, salivary diagnostic research is uniquely positioned to continue its ingress into the clinic[1]. Saliva provides improved safety through non-invasive collection.

But what exactly does the future of salivary research look like? Even with so many advantages to using saliva for diagnostic research, it has yet to become a mainstay of clinical practice. The next decade will certainly see new salivary biomarkers being discovered and validated, and testing programs continuing to grow. Even so, there are still many hurdles that need to be overcome to actualize the full potential of saliva as a sample type for research. Let's take a look at some of the barriers that are standing in the way of more widespread analysis of saliva.

Navigating salivaomics

As with so many other biofluids, biomarker discovery efforts have incorporated "omics" and combinations of omics (i.e., multi-omics) techniques for biomarker identification and validation. Saliva is no exception, and "salivaomics" has become a common term to describe the application of omics techniques—including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics—to the analysis of saliva samples[2].

Transcriptomics studies have identified mRNA panels that can distinguish between healthy patients and those with oral squamous cell carcinoma, pancreatic cancer, breast cancer, and others[3-5]. Proteomics techniques have also been applied to saliva samples and many promising cancer-associated biomarkers show clinical potential[6]. Additional proteomics studies and cataloging of salivary proteomics data, as with the Saliva Proteome Knowledge Base,

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have helped to expand the applications of proteomic diagnostics with saliva[7]. Omics studies that use saliva as a sample type are ongoing and a powerful way to identify single or panels of biomarkers that can sensitively and selectively predict various aspects of human disease. In addition, omics techniques can teach researchers much about the basic biology of saliva, which is important for advancing its use as a diagnostic research tool.

Rigorous validation of salivary biomarkers

With salivaomics churning out an incredible amount of data on saliva biomarkers, it is important to remember the urgency for rigorous testing. Precision, accuracy, and sensitivity are essential when making a decision about a patient's prognosis or diagnosis. It is tempting to let the many advantages of salivary research quicken the path to market, but as with the saliva biomarkers that have been discovered through basic research into disease pathophysiology, and salivary bioscience, there is still a desperate need for additional verification and validation in large clinical trials. This is an essential step before salivary testing can enter widespread clinical use for oral and/or systemic diseases.

Increasing basic saliva research

In addition to increasing the number of potential clinical biomarkers, omics techniques are enhancing the amount of basic knowledge about the biomolecules that make up saliva and their physiological function. In this area, saliva is severely lacking compared to other biofluids, such as blood or urine. For this reason, many may be reluctant to adopt the use of saliva for clinical research.

For instance, salivary chemicals and biomolecules can vary depending on sex or age, ethnicity, geographic location, circadian clock, diet, and many other factors[8-11]. Extensive research into how these factors can affect one or many salivary biomarkers and the mechanism by which this occurs will be critical prior to pursuing clinical validation and trial of salivary diagnostics.

Standardizing collection and storage

Standardizing the inputs and processes required to conduct your research is one solution to better understanding the variation in saliva composition. Another is the development of guidelines and standardization. As with any diagnostic research study, creating a standardized protocol under which all testing occurs is essential for accurate results. So far, salivary research has not had such standards put into place, making comparisons across studies and consensus about the utility of specific biomarkers difficult.

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Controllable factors, such as the time of day when saliva is collected or diet, can affect the concentration of specific biomarkers and are not typically controlled for during biomarker studies, which in turn creates variation. The type of **salivary fluid** (e.g., whole saliva, unstimulated whole saliva, stimulated whole saliva, etc.) collection and the **collection device** (e.g., use of an absorbent pad or collection tube) used can also affect the analyte levels present, and therefore, the result of diagnostic testing research[12,13]. In addition, analytes have different stabilities at **different temperatures**. How samples are stored, at what temperature, and the number of freeze-thaw cycles they go through are other factors that need to be standardized in order to minimize analyte degradation[14].

Balancing sensitivity with affordability

A major challenge to developing accurate salivary diagnostic research is developing detection methods that can sensitively and accurately identify low-abundance biomarkers in saliva. To capitalize on the advantage of a non-invasive, easy-to-collect sample type, such as saliva, diagnostic research systems have to be small, at the point of care, and still be easy for patients to comply with.

Balancing all of these factors can increase costs, as a diagnostic research device may require the use of microfluidics or chips for detection. While that is not always the case, as with the recent report of a paper-capture, CRISPR-based, **point-of-care SARS-CoV-2 test** using saliva samples, this is a major limitation. Technologies with adequate sensitivity and low cost are essential to broaden the implementation of salivary research[15].

SpeciMAX collection kits are driving the future of saliva

Salivary diagnostic research has an incredible amount of potential for medicine and health care. Thermo Fisher is transforming this potential into reality by solving some of the hurdles that face salivary research.

Our **SpeciMAX Saliva Collection Kits** are one such solution. They are automation-ready, making the development and implementation of standardized testing protocols simple, straightforward, and efficient. The SpeciMAX Saliva Collection Kits also make the advantages of saliva collection real, with an easy-to-use design that is preferred by most users. For saliva research programs, our collection kits are designed to help you save on refrigeration and freezer space and optimized for sample accessioning.

Contact us to see how the SpeciMAX Saliva Collection Kits are bringing the future of saliva collection and research today.

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Automation

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5 benefits of saliva testing: why it's the next big thing for *in vitro* diagnostics

For a multitude of different diseases, from diabetes to cancer, early intervention is key to limiting morbidity and mortality. *In vitro* diagnostics (IVDs) that sensitively detect disease-specific biomarkers are central to timely diagnosis and successful treatment.

While IVDs have been powerful for health care providers, there are still significant limitations preventing the rapid detection of disease for the global population. These challenges, outlined by Lee and Wong, fall into three major categories, including a lack of[1]:

1. Definitive molecular biomarkers for specific diseases
2. Easy and inexpensive sampling methods with minimal discomfort
3. Accurate, easy-to-use, and portable platforms to facilitate early disease detection

Salivary diagnostic tests: a solution to IVD's shortcomings

There is no magical, one-size-fits-all solution for the issues IVDs face. However, the emergence of salivary diagnostic tests—which use whole saliva and other oral sample types for biomarker detection address the latter two issues outlined above[1-3]. Saliva collection and testing can be an easy and inexpensive sampling method with minimal discomfort to the end user, and is both accurate and easy to use as a portable and scalable platform for disease detection.

As a diagnostic tool, the use of salivary sample types has several major advantages over traditional and historically favored biofluids, such as blood.

Let's take a look at the major advantages of saliva collection and the overall benefits of saliva testing.

Saliva provides improved safety through non-invasive collection

The collection of blood, or phlebotomy, is necessary for a huge range of diagnostic tests, making it one of the most common

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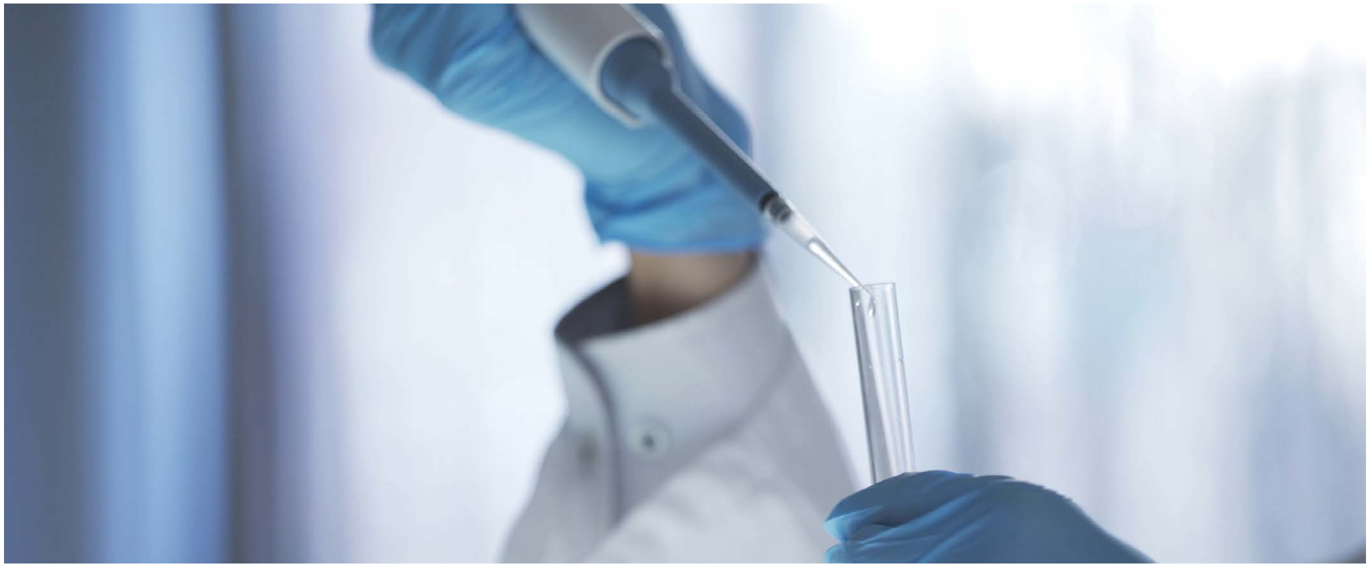
invasive procedures in health care[4]. Though commonly accepted as a necessary part of diagnostics, when done without the proper procedure, the invasive nature of phlebotomy can lead to the exposure of health care professionals to blood-borne pathogens, such as HIV, hepatitis viruses, bacteria, and parasites[2,4]. Compared to other biofluids, there are numerous benefits to using saliva for diagnostic testing. Saliva collection is non-invasive, biomarker testing is low cost, and diagnostics can be as sensitive as tests that rely on traditionally-favored biofluids such as blood.



For patients, the use of needles poses several complications. They may be uncomfortable with needle use or the sight of blood, which can lead to a variety of vasovagal reactions (such as fainting) in 0.9% to 3.4% of patients[5]. More commonly, 14% to 45% of patients experience pain and bruising[5]. Lastly, insufficient sterilization before venipuncture can lead to the introduction of microbes directly into the patient's bloodstream[4].

Nasopharyngeal swabbing has been the “go-to” sampling method for SARS-CoV-2 testing, but one major advantage to saliva collection and testing is that it is a non-invasive collection method. This fact alone is capturing the interest of both health care providers and end users. As saliva diagnostics have started to be used for SARS-CoV-2 testing, advantages over nasopharyngeal swabbing have become apparent. The latter triggers sneezing and coughing for patients, which increases the risk of viral transmission and causes mild to severe discomfort in patients[6].

Saliva is an easier sample type to self-administer in the presence of a health care provider

Nasopharyngeal swabbing and phlebotomy both require the collection of samples by a trained health care professional, foundational training of personnel on how to safely perform collection, a scheduled location and timing for collection, and the use of PPE to limit exposure to pathogens[4].

The collection of saliva is much easier, more flexible, and introduces the option of convenient self-collection by a patient while in the presence of a health care provider. Decentralized collection and expanding test sites enable the sampling of a wider and more diverse population. Recently, a variety of saliva collection devices and testing methods has proven effective for facilitating scalable, routine testing for a broad population, an important practice for consistent monitoring of ongoing viral spread[12].

Better stability and transportability

As mentioned above, using saliva as a sample type can allow decentralized self-collection, even in areas without access to a medical system. As long as there is a way to transport samples from a patient’s residence

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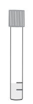
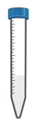
Save precious space with SpeciMAX Dx Saliva Collection Kits

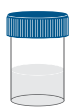


SpeciMAX Dx Saliva Collection Kit
6 mL tubes
One shelf: 216 tubes*

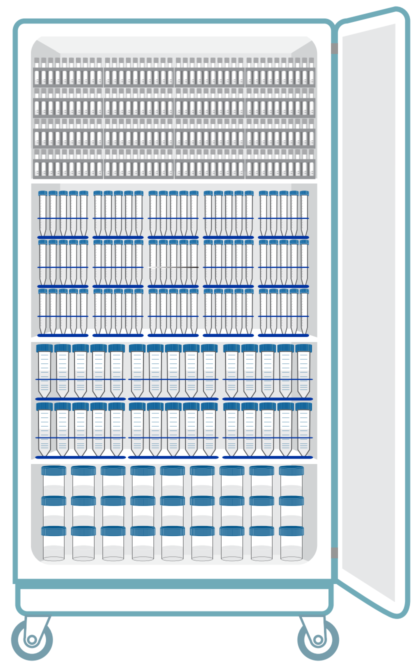


15 mL conical tubes
One shelf: 80 tubes*

50 mL conical tubes
One shelf: 36 tubes*



60 mL containers
One shelf: 26 containers*



Heratherm incubator

SpeciMAX Dx Stabilized Saliva Collection Kit
6 mL tubes
No incubator needed—stabilization solution deactivates the virus

* Calculation made on Thermo Scientific™ Heratherm™ IMH100-S incubator size.

to a testing facility, a decentralized testing system provides significant advantages. With SARS-CoV-2 testing, many emergency authorized tests have implemented at-home kits that use saliva collection methods and several studies have demonstrated the stability of SARS-CoV-2 RNA in saliva, under a variety of storage conditions[13-15].

This type of decentralized, widespread testing would be much more challenging to implement using blood. Transportation of blood samples and excessive handling can lead to lysis of red blood cells, which can skew lab results[16]. Fluctuations in temperature, prolonged transport times, and overall transport quality can also alter diagnostic results[17].

SpeciMAX Dx Collection Kits can be stored effectively in standard 6 mL test tubes, 15 and 50 mL conical tubes, and 60 mL containers. Save space while keeping samples safe in the Thermo Scientific™ Heratherm™ incubator.

Saliva sampling is an affordable solution compared to blood or nasopharyngeal sampling
Several studies have reported the cost-effective nature of saliva sampling compared to blood sampling[8,18]. The price of extraction and purification of

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select salivary biomarkers is also lower: for commercial extraction of DNA from saliva, the cost is $6.93 per sample versus $10.88 per sample for blood[8].

In the case of SARS-CoV-2 testing, saliva testing is equally as sensitive as nasopharyngeal testing, yet much less costly, saving up to $636,105 per 100,000 persons sampled[19-21].

Accurate detection of oral and systemic disease biomarkers

Saliva is a complex biofluid with a vast array of diverse biomarkers present[22]. One disadvantage to its use as a diagnostic sample type is that many potential analytes are present in 100- to 1,000-fold lower concentrations than in blood, putting significant pressure on the sensitivity of the IVD technology for accurate detection and diagnosis[23,24].

Recently the number of salivary biomarkers for oral and systemic diseases has increased, and sensitive, selective methodologies have aided in their detection. DNA, RNA, and protein biomarkers associated with cancer, periodontal disease, autoimmune diseases, viral and bacterial diseases, neurodegenerative diseases, and cardiovascular diseases have been identified in saliva. With the growing use of genomics, transcriptomics, proteomics, and other "omics" techniques to study saliva, the discovery of other biomarkers is on the horizon[1,22].

While regulatory agencies haven't yet approved tests in many of these disease areas, commercially available, saliva-based diagnostics are available for substance abuse, HIV, and HPV-related oral cancers. These widely used tests act as important validation for ongoing development and commercialization efforts[22-24]. In addition, several studies have demonstrated that saliva diagnostic tests for SARS-CoV-2 are highly concordant with tests that use nasopharyngeal swabs[19-21].

Bringing the benefits of saliva testing to broader populations

Thermo Fisher Scientific is working toward streamlining saliva sample collection and creating kits that make saliva testing as efficient, rapid, reliable, and secure as possible.

Thermo Fisher has launched SpeciMAX Dx Saliva Collection kits to make sample collection convenient and downstream analysis fully scalable through automated instruments. The collection of saliva specimens compared to nasopharyngeal swabs can save $636,105 per 100,000 patients sampled by reducing the need for manual labor[19,21]. This helps reduce cost and touch-time by using saliva specimens for SARS-CoV-2 testing.

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Using saliva as a diagnostic fluid: 5 triumphs for salivary biomarkers

For most people, saliva is written off as an inert fluid, necessary for food digestion. But for researchers, it's increasingly being appreciated for its molecular complexity and the potential for the DNA, RNA, proteins, and small molecules present to be used as biomarkers for oral and systemic diseases. While the diagnostics community has long been hesitant, over the past two decades, acceptance of saliva as a diagnostic fluid has grown.

SARS-CoV-2 has helped further the cause, raising additional awareness about the diagnostic uses of saliva, serving as a proof point for diagnostics developers considering saliva as a sample type. While blood, urine, and nasopharyngeal swabbing still remain favorites in the clinical setting, there are many other diagnostic areas where saliva testing has been heavily researched and incredibly helpful for detecting oral and systemic health issues.

Let's take a look at some of the application areas where researchers are making the most headway into the successful use of saliva as a diagnostic fluid.

Infectious diseases

For systemic infectious diseases, sensitive and selective detection, early in an infection, can be critical to preventing morbidity and mortality. The use of salivary diagnostics for the detection of infectious diseases, along with the many advantages of saliva sample types, has made it a major focus for the development of diagnostics.

One of the early uses of saliva as a diagnostic fluid **was for HIV detection**. A number of different assays, with high sensitivity and selectivity, were developed to detect IgG or IgA antibodies against specific HIV protein epitopes[1-3].

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An FDA-approved, commercially available HIV test, OraSure™, detects salivary antibodies against the p24 antigen of HIV[4]. Rapid tests for the detection of hepatitis C virus and other viral saliva biomarkers, including anti-virus antibodies (e.g., IgG, IgA, and IgM) or RNA, have been developed for hepatitis A, hepatitis B, dengue, Ebola, Zika, measles, mumps, and rubella[6]. Recently, SARS-CoV-2 RNA tests using saliva have joined the list of successes for infectious disease detection and several studies have indicated a high concordance with the "go-to" sample type, nasopharyngeal swabs[7-9].

Oral microbiome

As "omics" techniques have gained traction in nearly every facet of biomedical (and other) sciences, saliva, as a sample type has not been spared. Accordingly, the Salivaomics Knowledge Base was established to act as a data repository for human salivary "omics" data[10]. A growing appreciation for the microbiome in human health has enticed researchers to focus on the oral microbiome. In these studies, saliva is a sample type that can provide a snapshot of the microbial species present, in order to inform the search for microbial biomarkers for oral and systemic diseases.

Despite some analytical challenges, since 90% of the DNA in a saliva sample is host (human) DNA, there have been a number of promising discoveries that may hold promise for the future. For instance, children living with autism spectrum disorders were found to have a significantly higher abundance of bacterial pathogens (such as *Haemophilus*) and reduced abundance of commensals (including *Prevotella, Fusobacterium,* and others), compared to children without autism[12]. As with other "omics" techniques, these results have potential but oral microbiome analysis doesn't yet have the precision, accuracy, or sensitivity required for the clinical spotlight[13].

Oncology

Cancer is a major cause of morbidity and mortality and is on the rise worldwide[14].

Early cancer diagnosis and treatment monitoring are still evolving.

Developing diagnostics that can detect cancer early, predict clinical progression, or track therapeutic response is a major focus for researchers and a strategy that can limit the impacts of the disease. Molecular diagnostics for cancer biomarkers have long been an integral part of disease detection, treatment selection, disease monitoring, and tracking metastasis[15].

Because saliva holds many advantages over liquid or tissue biopsies, which are commonly used in cancer diagnostics, there has been significant effort to apply genomics, proteomics, and metabolomics to detect DNA, RNA, protein, and other biomarkers[16]. Thus far, salivary biomarker studies have focused mainly on brain, esophageal, gastric, head and neck, lung, ovarian, pancreas, prostate, and breast cancers[16]. While there have been many promising findings, the possibility of salivary biomarkers as an alternative to liquid biopsy for early cancer diagnosis and treatment monitoring is still evolving. Promising salivary biomarkers have been identified, yet the mechanism by which they enter the saliva and their role in oncogenesis are still in the discovery phase[16].

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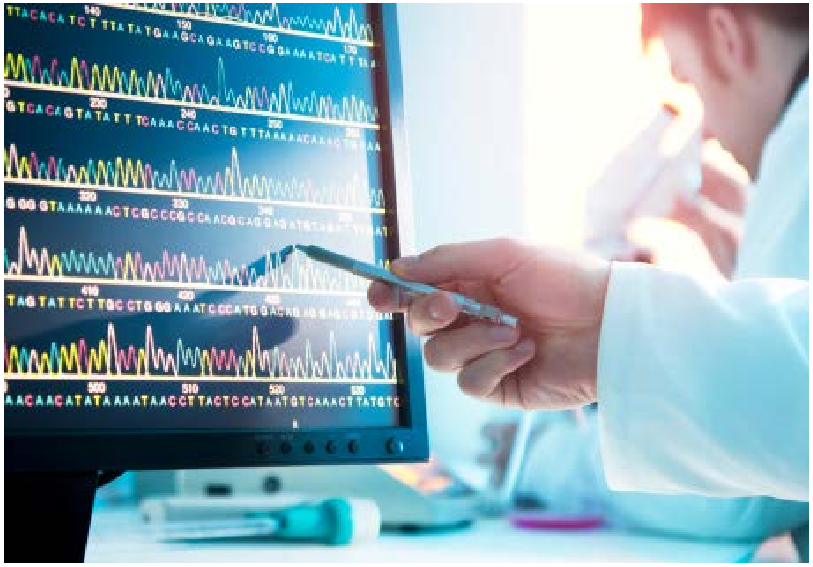

Drug abuse and therapeutic monitoring

Unbound or free drugs—both illicit and therapeutic—are excreted into oral fluids and concentrations in saliva correlate closely with free drug concentrations in plasma. For this reason, saliva collection and several assessment methods have been used for the detection of drugs of abuse as well as therapeutic monitoring for psychotropic medication[17,18].

Testing for drugs of abuse

The simplicity and rapid nature of saliva testing make it particularly applicable to workplace and roadside drug testing. Several commercially available collection devices are available for cannabinoids, opioids, cocaine, amphetamine, and benzodiazepines along with LC- or GC-MS–based methods of detection. While salivary testing for drugs of abuse has provided reliable alternatives to blood and urine testing, there is still a need for additional research into confounding factors, such as time since sample collection and since last drug use, and their effects on diagnostic test results[17].

Therapeutic monitoring

Saliva testing has also been particularly useful for treatment monitoring for psychiatric medication. For patients living with bipolar disorder and other psychiatric diseases, noncompliance with prescribed treatment regimens is common and is correlated with poor outcomes, such as increased ER visits and worse functioning[18]. In addition, therapeutic windows may vary from patient to patient or be very narrow, as is the case with lithium and valproic acid.

Monitoring drug concentrations through saliva is particularly helpful and has become more accurate, reliable, and highly sensitive[18]. Studies on salivary detection of lithium, valproic acid, carbamazepine, lamotrigine, antidepressants, and antipsychotics have been done with varying degrees of success and correlations with serum levels have demonstrated significant promise. However, there are still several challenges and considerations, such as a lack of standardization and a large variation in other confounding variables (e.g., polypharmacy, collection and processing protocols, dehydration, etc.)[18].

Hormone monitoring

It is well accepted that hormones such as cortisol or testosterone move from plasma into the saliva and can be produced directly by salivary glands, as is the case with salivary melatonin or cortisone, a precursor to cortisol[19].

Cortisol is the most researched and used test in the salivary biomarker field and several studies correlate salivary cortisol with pain or stress in both humans and animals[19]. Testosterone is another hormone that has been well studied and found to correlate with the presence of depression, anxiety disorders, and aggressive behavior[19]. On the other side of the emotional spectrum, salivary oxytocin has been found to be a reliable biomarker for human well-being[19].

More triumphs for saliva as a diagnostic fluid

Over the next decade, researchers will continue to discover more accurate and reliable salivary biomarkers and develop diagnostic tests to sensitively and selectively detect them. For more rapid innovation, products that streamline workflows and save diagnostics labs time and money are needed.

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Thermo Fisher developed the **SpeciMAX Dx Saliva Collection Kit** for simplified self-collection of saliva samples for SARS-CoV-2 testing and many other application areas. The collection tubes are designed so that they can be paired with an automated liquid handler to eliminate messy manual transfer steps and use incubator, storage, or refrigeration space efficiently.

This article contains product information intended for General Laboratory Use. It is the customer's responsibility to ensure that the performance of the product is suitable for customer's specific use or application.

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How to get more out of your saliva program: powerful considerations for saliva collection and handling

Compared to traditional biofluids (e.g., blood), oral biofluids such as saliva hold a lot of potential for diagnostic testing. Saliva collection is minimally invasive, safe, and can be done quickly and in a variety of settings, giving it a big upper hand over (often) painful blood draws, which need to be done in a laboratory setting by a medical professional[1-3].

Yet much of saliva's potential in diagnostics remains untapped. Because saliva has not been as widely used as other biofluids, there is less awareness among scientists and researchers about the infrastructure, reagents, and best sample collection and handling practices required to implement an effective saliva testing program or research study. In addition, there is a great need for standardization to remove variability in workflows and ensure consistent data collection and analysis[4-6].

To ensure that your saliva diagnostics program gets off on the right foot, we've collected some best practices and common considerations—from study design to sample collection and handling—for setting up a workflow and getting the most out of your efforts.

Designing your study or program

Regardless of the scope of your testing program or research study, the primary goal is to generate sensitive and selective detection of a specific salivary biomarker. This means eliminating any elements of your study design that can lead to sample contamination, participant noncompliance, or other confounding factors that could have unexpected downstream effects.

Location of sample collection

The guidance for where sample collection takes place should be based on compliance to the product specifications and proximity to testing labs. Sample collection for diagnostics should be administered by, or in the presence of, a professional health care provider, and in a location near the testing facility. researchers can then directly oversee sample collection to analysis. Across participants, standardization to a pre-approved protocol is more easily

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achieved and verified. Locations that require further transport risk possible specimen degradation, cross-contamination, or mishandling.

Conducting saliva collection in settings such as a person's home or "in the field" has its appeal compared to approved centralized collection facilities (i.e., achieve a more rapid, decentralized sample collection) but there is a greater risk of human error when samples are collected outside the presence of a professional health care provider. This includes contamination or deviation from protocols, as individuals without experience in sample collection may be responsible for sterile technique or (relatively) complex procedures. While procedurally challenging to account for errors, decentralized collection for surveillance of SARS-CoV-2 has been beneficial and will likely continue to be used in cases of **infectious disease** monitoring[7,8].

Managing noncompliance

Noncompliance can be a major issue with decentralized sample collection. In research areas where saliva testing has been more routinely performed, such as in salivary cortisol testing, experts recommend **several strategies for ensuring participant adherence**, including[9]:

- Explain the importance and the "why" behind protocol adherence around saliva collection and handling
- Outline the detailed protocol and do one-on-one practice with participants, allowing them to ask questions
- Provide clear, detailed instructions (e.g., written, video) and contact information for the research team
- Make collection kits so all required materials are available on-demand
- Remind participants at or before key sample collection times and of key steps in the protocol, including handling and freezing of samples post-collection

Using these strategies can help ensure the consistency of sample collection and adherence to the study protocol or diagnostic workflow.

Collecting samples from patients

Some biological factors can affect the concentration of specific salivary analytes and their effective collection. For instance, many salivary hormones follow a **diurnal pattern of expression**, and therefore, a sample collection scheme must include standardized timing for saliva collection[10]. Other pivotal factors include the method of saliva collection and sample volume.

"For best results, saliva donors should be instructed to refrain from eating, drinking, using tobacco products, or chewing gum for at least 30 minutes." See SpeciMAX™ Dx Instructions for Use for more details.

Variations in salivary glands secretion

Not all oral samples are created equal. Whole saliva is a complex mixture of biomolecules and contains oral fluids from various salivary glands, including the parotid, submandibular, sublingual, and other minor secretory glands. The secretions from these glands differ in composition and thus, sampling them individually versus sampling whole saliva can provide different diagnostic results.

In addition, saliva **composition changes** in response to taste, smell, and even the stimulation of chewing[11]. Another factor to consider is blood contamination in the saliva; blood contains certain biomarkers at much higher concentrations than are present in saliva. This can be particularly concerning in populations where **oral health is impacted** by gingivitis, periodontal disease, HIV, tobacco use, and other factors and can be

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controlled by incorporating careful screening methodologies for saliva samples [12-14]. Taken together, understanding the presence or absence of a specific salivary biomarker in whole saliva or secretions from a particular gland is a critical prerequisite to establishing a reliable testing scheme.

Sample collection methods

A standard practice for saliva collection is the collection of unstimulated whole saliva, using the passive drool method. Whole saliva is most easily collected using collection tubes with funnels to prevent spillover. Saliva donors should allow saliva to pool in their mouths and then drool the saliva into the top of the funnel affixed to the saliva collection device. Consider the liquid portion of the saliva, not the bubbles, when determining if enough saliva has been collected.

SpeciMAX Dx Stabilized Saliva Collection Kit for collection of preserved saliva in nonhazardous and virus-inactivated solution.

Thermo Fisher recently launched its line of **SpeciMAX Dx Saliva Collection Kits** that addresses sample collection concerns with a funnel top into a standardized test tube. Depending on the application, raw and stabilized variations of the tube can be purchased.

When an analyte from a specific gland is required or populations are being studied that are unable to do passive drool (e.g., infants or geriatric populations with xerostomia), a variety of swabs are available for different age groups and analytes. If self-administration is required for your testing scheme, the swab method may be technically challenging and suffer from noncompliance issues.

Choosing which collection method to use depends on a combination of the age of participants, target analyte, number of required samples, sample volume, sample storage, and whether or not assistance will be provided for sampling.

Sample handling for saliva

Maintaining sample integrity for the short or long term is critical to running a successful saliva testing program. The choices made about cold chain management and workflow manipulation can affect analyte concentration and, therefore, the reliability of diagnostic results.

Cold chain management

Though some analytes are **stable at room temperature**, many are not[15]. For example, the Thermo Scientific **SpeciMAX Dx Stabilized Saliva Collection Kit** is to be stored at 15–30°C. In general, some analytes still require storage with **inhibitors to prevent degradation**[19].

Temperature sensitivity can severely limit the ability to implement an at-home or decentralized testing system. Transporting saliva samples after collection can require dry ice, increasing the complexity and cost of testing, ultimately limiting the scope of participation.

Post-collection handling

Many biomolecules are sensitive to freeze-thaw cycles. Accordingly, minimizing freeze-thaw cycles can help maintain sample integrity. Certain hormones, such as progesterone and estradiol, are sensitive

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to freeze-thaw cycles while other biomolecules—like DNA—are more robust, though there is still a decrease in quality the more a sample is refrozen[20].

To avoid freeze-thaw cycles, it may be necessary to pipet an individual sample into multiple cryovials and use one aliquot at a time for analysis. This may create a labor-intensive workflow and be challenging with viscous whole saliva samples, but necessary to ensure reliable results.

Implementing best practices using saliva collection with SpeciMAX Dx Saliva Collection Kits

There's a lot of planning that goes into establishing a trusted saliva collection and testing workflow. The **SpeciMAX Dx Saliva Collection Kits** help address many of the considerations raised above: they have a simple design with an easy-to-use collection funnel to minimize mess during passive drool collection, pre-marked lines to determine when sampling is complete, and compatibility with automated liquid handlers to minimize manual pipetting steps. The SpeciMAX Dx collection tubes also enable efficient use of refrigeration and freezer space, allowing labs to fit 6 times as many SpeciMAX tubes as 50 mL conical tubes in the same space.

Learn more about **SpeciMAX Dx Saliva Collection Kits for *in vitro* diagnostics** and see how Thermo Fisher can help you establish a powerful high-throughput saliva testing program from sample collection and preparation to PCR analysis and results.

Want to learn more about saliva as a sample type? Start from the beginning.

What is saliva and why is it important for diagnostic research?

5 benefits of saliva testing: why it's the next big thing for in vitro diagnostics

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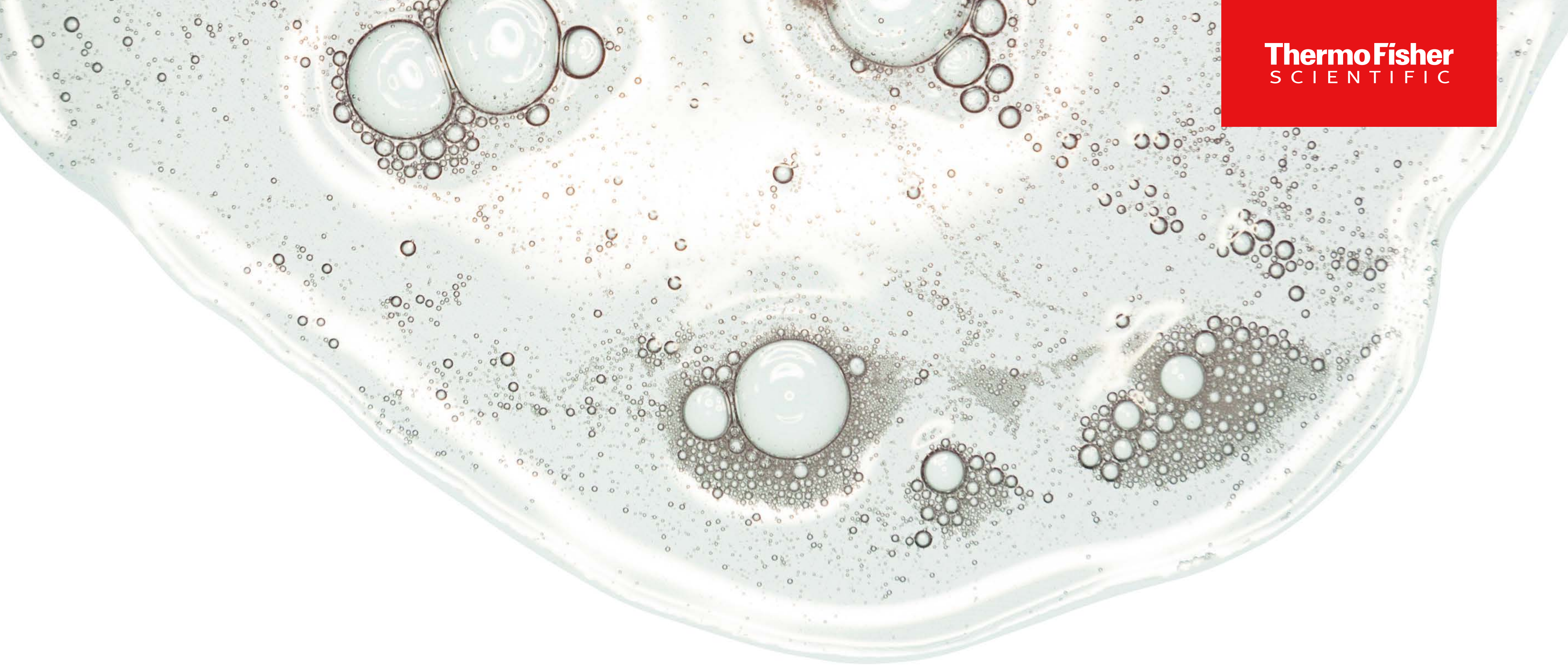

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