A Look Inside: Oral Sampling for Detection of Non-oral Infectious Diseases

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ABSTRACT Efforts to control transmissible infectious diseases rely on the ability to screen large populations, ideally in community settings. These efforts can be limited by the requirement for invasive or logistically difficult collection of patient samples, such as blood, urine, stool, sputum, and nasopharyngeal swabs. Oral sampling is an appealing, noninvasive alternative that could greatly facilitate high-throughput sampling in community settings. Oral sampling has been described for the detection of dozens of human pathogens, including pathogens whose primary sites of infection are outside of the oral cavity, such as the respiratory pathogens *Mycobacterium tuberculosis* and SARS-CoV-2. Oral sampling can demonstrate active infections as well as resolving or previous infections, the latter through the detection of antibodies. Its potential applications are diverse, including improved diagnosis in special populations (e.g., children), population surveillance, and infectious disease screening. In this minireview, we address the use of oral samples for the detection of diseases that primarily manifest outside the oral cavity. Focusing on well-supported examples, we describe applications for such methods and highlight their potential advantages and limitations in medicine, public health, and research.

KEYWORDS diagnosis, screening, saliva, swabs, respiratory diseases, pediatric infectious disease, COVID-19, SARS-CoV-2, tuberculosis, HIV, HCV, Ebola, malaria, *Pneumocystis*, parvovirus, *Mycobacterium tuberculosis*, hepatitis C virus, human immunodeficiency virus, respiratory disease

Transmissible microbial pathogens range from primordial "companions" of humankind such as *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), to newly arisen zoonotic infections such as SARS-CoV-2, the virus that causes COVID-19. Efficient diagnostic methods are needed to rapidly identify infected people, not only for patient care but also to reduce the spread of disease.

Infectious disease case-finding has been greatly facilitated by new molecular pathogen detection methods, some of which are fast and easy to use in the clinic, at point of care (POC), and in community settings. Unfortunately, sample acquisition remains a critical bottleneck. Samples that require invasive procedures, such as blood, bronchioalveolar lavage (BAL) fluid, or induced sputum, cannot be collected with high throughput in settings such as schools and workplaces. Other samples such as urine or stool require privacy and sometimes-unreliable self-sampling protocols.

This article addresses oral sampling, an emerging noninvasive sampling strategy developed to address these challenges. As a portal between the interiors of our bodies and the external world, the mouth is an accessible and logical place to look for pathogens, especially pathogens of the airways. Noninvasive oral sampling refers to methods of sample collection from the oral cavity that are simple, can be performed on almost all patients and in any setting, and produce little or no discomfort. These traits are desirable for screening large populations with high throughput, especially in community

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settings. Because most such methods do not require specialized training, many are easily adapted for self-collection. This increases the capacity for screening of large populations without overburdening public health and health care workers, and helps to facilitate POC testing.

Noninvasive oral sampling can be versatile and is not confined to detection of oral diseases. In many cases, a method used to detect one pathogen can also be applied to others. For example, there is some evidence that a multiplex PCR test to detect several respiratory viruses may be performed similarly using saliva samples compared to nasopharyngeal swabs (1). Because of the nature of this technology, it is also possible that such multiplex PCRs could be used to detect biologically dissimilar pathogens that cause overlapping clinical syndromes.

In this article we discuss non-oral pathogens that have been detected via oral sampling, either in research or clinical context, with an emphasis on distinct applications of noninvasive oral sampling.

NONINVASIVE ORAL SAMPLING TYPES

Examples of noninvasive oral samples include tongue and buccal swabs, saliva, and oral rinses. Many such methods are routine for diagnosing and screening for oral infections; however, this article focuses on the use of oral sampling for detecting infectious agents whose primary site of infection is outside the oral cavity. Exhaled breath (2) is not addressed here because it is designed to be sample derived from the lungs, not the mouth.

The use of tongue swabs has been described for the detection of a variety of pathogens. In addition to its distinctive native flora (3), microbes from other parts of our airways can accumulate on the tongue dorsum due to its position in the mouth and its coarse surface architecture. A typical sample collection process involves firmly scraping the surface of the tongue with a swab for about 10 s followed by transferring the swab to a tube with storage buffer (4, 5).

Different areas of the buccal mucosa have been explored for infectious disease sampling, but most such studies have focused on the inner surface of the cheeks (5, 6). Other studies collected material from the outer gums and mucosa between the gums and the inner lining of the lips. Technically, these swabs come into contact with buccal mucosa as well, but are more often described as collecting oral fluid, which is likely a combination of saliva and gingival crevicular fluid (7–9). Some studies refer to this sample type as oral mucosal transudate, despite nearly identical descriptions of sample collection (10).

Collection of oral fluid with swabs can be contrasted with collection of saliva. For SARS-CoV-2 alone, multiple collection methods have been described, including coughing out, drooling out, spit saliva, collecting saliva with a pipette (for example from a patient requiring endotracheal intubation), and directly sampling from the salivary gland (11–15). The least invasive methods, such as the drooling technique, have been approved as high-yield samples for COVID-19 testing (12).

Oral rinses, sometimes referred to as "gargle lavages," have also been described for the detection of infectious diseases. These typically involve gargling sterile saline solution before spitting out into a sterile container. This material is likely a mixture of sample types, including saliva and oropharyngeal secretions (16–18). However, distinctions have been noted between oral rinses, defined as swishing sterile water without any throat gargling, and samples collected by throat gargling (19).

Once noninvasive oral samples have been obtained, they can be tested in a variety of ways depending on the pathogen and the clinical context. When there is concern for an acute infection, in most cases the direct detection of pathogen cells, antigens, or nucleic acid is most appropriate. Real-time PCR, also known as quantitative PCR (or qPCR), is a laboratory technique that allows for the detection and quantification of specific nucleic acid sequences. The cycle threshold value (C_T value) represents how many cycles of PCR were required in order to detect the nucleic acid sequence; higher C_T

values indicate that more PCR cycles were needed before the sequence was detected and therefore suggests a lower amount of the nucleic acid. There are also applications for antibody testing of noninvasive oral samples, including screening for chronic infectious diseases and population surveillance for immunity.

Oral sampling has been explored for diverse reasons, but there are specific situations that stand out as areas of particular need for noninvasive, high-throughput sampling. The following sections explore several of these.

MINIMIZING HEALTH CARE WORKER EXPOSURE TO INFECTIOUS DISEASES

Many infectious diseases require samples obtained by health care workers for diagnosis. This can pose a significant hazard to health care workers, especially those who are subject to repeated exposures that cumulatively increase the risk to the health care worker over time.

Pulmonary tuberculosis. Oral sampling has been evaluated for the diagnosis of pulmonary TB through the detection of TB DNA by PCR. Sputum production for pulmonary TB diagnosis has several challenges. First, the quality of sputum generation has a significant impact on test performance. Some people with TB infection are not able to produce high quality sputum due to a weak cough or low disease burden with minimal cough symptoms. For those who produce minimal to no sputum on their own, an induced sputum can be obtained, which involves administering a hypertonic solution via nebulizer to the patient. This requires medical equipment, specialized training, and that the health care worker be present when the patient produces sputum, which places the health care worker at increased risk. For these reasons, an alternative sampling method would be useful.

Luabeya et al. (5) conducted a study in South Africa to analyze and describe the performance of oral swab analysis to diagnose pulmonary TB via manual qPCR. One goal was to compare sampling sites, including tongue, buccal, and gum swabs. They found that tongue swabs obtained using Whatman OmniSwabs yielded stronger DNA signals via C_q measurement compared to gum swabs and buccal swabs collected using the same product. These swabs were obtained by firmly brushing the dorsum of the tongue for approximately 10 s. The study also compared the performance of tongue swabs (tested by a manual IS6110-targeted qPCR) to sputum GeneXpert MTB/RIF and found that the sensitivity of at least one of two tongue swabs collected on separate days relative to sputum Xpert was 91.8%. Tongue swabs and sputum Xpert performed identically (sensitivity of 83.1%) when compared to patients with confirmed TB via sputum Xpert or culture. This is because some of the patients with sputum cultures positive for TB had negative Xpert testing from sputum but were positive from oral swab testing, and vice versa (5).

The results of Luabeya et al. (5) offer an important reminder that the oral cavity is internally heterogeneous, such that "oral swabs" collected from tongue dorsa may yield different results from swabs collected from the buccal mucosa. In some cases, this may have delayed the acceptance of oral sampling for infectious diseases. For example, an early assessment of saliva as a specimen for detection of TB by PCR showed very low sensitivity relative to sputum testing (38.5%) (20). The notion of oral sampling for TB was not explored further until surface sampling (buccal mucosa, tongue dorsa) was evaluated a few years later.

Independent studies of oral swabbing for TB have yielded a mixture of results. One study evaluated the performance of buccal swabs obtained from 33 patients in Peru with confirmed pulmonary TB by sputum culture. These swabs were analyzed using the first-generation GeneXpert system rather than home-brew IS6110 PCR, and found a sensitivity of 45% relative to positive culture (21). Relative to the South African study, this one used different analytical methods and relied on buccal swabbing rather than tongue swabbing. Another study conducted in Moldova found a sensitivity of only 36.3% relative to bacteriologically confirmed TB. This study used buccal, not tongue,

swabbing and extended storage of samples in a proprietary storage formulation (22). Such variables may have reduced sensitivity of oral swabbing relative to earlier studies.

A more recent study conducted on a cohort of 103 Chinese patients with suspected TB used tongue swabbing in combination with a loop-mediated isothermal amplification (LAMP) molecular test for TB DNA. This study found low sensitivity (32% to 50%) for single swab specimens relative to sputum bacteriology, but higher sensitivity (82.6%) when three swab specimens were tested (23). As seen in the results of Luabeya et al. (5), tongue swabs collected in the early morning appeared to yield better sensitivity. Importantly, this team also assessed sensitivity relative to an expanded bacteriological definition of TB (either sputum or BAL fluid). LAMP analysis of oral swabs detected three BAL-positive TB patients who were negative by sputum testing (23). Another report, currently in preprint stage, describes a study conducted in Korea on a cohort of 272 patients, including 128 patients with confirmed TB (24). Oral swab specimens were processed with a proprietary microfluidic device ("SLIM") that concentrated bacilli in the swab samples prior to qPCR analysis. Relative to clinically confirmed TB, the sensitivity of the oral swab-based SLIM assay (65.6%) was higher than that of sputum testing with the first-generation GeneXpert (43.4%) (24). These observations illustrate the potential for oral swab-based tests to augment TB case finding relative to sputum testing alone. It is possible that positive sputum production by some patients results in deposition of bacilli on oral surfaces, such that the bacilli can be detected afterward even if the patient does not produce positive sputa during their clinic visits.

SARS-CoV-2. The COVID-19 pandemic has led to significant health care worker exposure that could potentially be mitigated by noninvasive sampling. Although the gold standard for the detection of SARS-CoV-2 in patients is not known, many regard the nasopharyngeal (NP) swab as a reference standard. NP swabbing requires specialized training, causes patients discomfort, and frequently leads to patients sneezing or coughing, which may generate infectious aerosols. Noninvasive sampling reduces these problems and, in many cases, enables self-sampling.

Several noninvasive oral sampling collection methods and samples have been described for the detection of SARS-CoV-2 by PCR. The virus appears to be abundant in the upper airways of symptomatic people, and the primary receptor molecule for the virus, ACE2, is expressed in oral mucosa and epithelia (25, 26). Most evaluations of oral sampling have focused on saliva. Early in the pandemic, Azzi et al. (11) described 25 patients admitted to the hospital with COVID-19 infection based on positive NP swabs. Saliva was collected via a drooling technique, unless the patient was critically ill requiring mechanical ventilation, in which case a clinician obtained saliva intraorally using a pipette. They detected SARS-CoV-2 RNA in 25/25 (100%) patients with COVID-19 infection (10). Another study on 622 patients presenting to an outpatient COVID-19 screening clinic used a spitting out technique, which involved first pooling saliva in the mouth for 1 to 2 min and then spitting into a container. All 622 patients also provided an oropharyngeal (OP) swab, of which 39 were positive for SARS-CoV-2. Of those 39 patients, 33 (84.6%) also had detectable SARS-CoV-2 in their saliva. These researchers also chose a subset of 50 patients with negative OP swabs and tested their saliva, and 1 of them had detectable SARS-CoV-2 in their saliva (27). Hanson et al. collected NP swabs, anterior nasal swabs, and saliva collected by pooling in the mouth and then spitting into a container. There was a very high concordance between NP swabs and saliva (kappa = 0.912); SARS-CoV-2 was detected in 80/354 (22.5%) NP swabs and 81/354 (22.9%) saliva samples (13). The performance of self-collected saliva for the detection of SARS-CoV-2 antigen has also been evaluated, with more limited success. In one study the antigen was detected in only 11.7% of samples from 103 patients with confirmed COVID-19 infection (28), and another study demonstrated only 23.1% positive percent agreement between antigen testing in saliva compared to NP samples (29).

Of note, some studies used saliva obtained through a coughing method. In some cases it may be more appropriate to describe this as sputum. To et al. (15) described a cohort of 23 patients who were asked to cough out saliva in the early morning. Twenty

out of 23 (87%) patients with confirmed SARS-CoV-2 infection by NP swab were also positive by collecting oropharyngeal saliva (15). Similarly, Procop et al. (14) described obtaining "enhanced saliva," which was self-collected by first sniffing strongly to gather nasal secretions, followed by coughing to produce phlegm. NP swabs were collected after this was done. Of 219 patients with both NP swabs and saliva samples, 39 had detectable SARS-CoV-2 by both NP swabs and saliva, yielding a 100% positive predictive agreement. They also reported a very high negative predictive agreement of 99.4% (14). This method appears to yield high-quality samples, but not all patients might be able to produce similar quality samples if a strong cough is required. Furthermore, it is unclear if it is superior to saliva collected simply by drooling or spitting out. Additional studies with paired comparisons of these techniques would be helpful.

Mittal et al. (18) described 50 symptomatic patients with confirmed COVID-19 infection by NP swab. Within 72 h of initial diagnosis, paired sampling was performed with either NP swab or oropharyngeal swab paired with oral rinse obtained by gargling normal saline solution and then spitting into a sterile container. All of the oral rinse samples yielded detectable SARS-CoV-2 RNA. Patients were asked about discomfort with each of the methods and, unsurprisingly, the oral rinse collection procedure was significantly better tolerated than NP or OP swab collection (18). Another study compared oral rinses and saliva to NP or OP swabs in both symptomatic and asymptomatic health care workers, with 285 health care workers participating in the study (224 were symptomatic). Oral rinses were self-collected by swishing sterile water for 15 s, without any throat gargling, and then spitting into a sterile container. Saliva samples were obtained by bringing up saliva from the back of the throat and spitting into a sterile container. Relative to NP swabs, oral rinses detected viral RNA in 64% of participants. Participants who provided saliva samples had either matched OP swabs or NP swabs collected. Relative to OP swabs, saliva had a sensitivity and specificity of 96.7% and 91.4%, respectively. Using NP swabs as the reference, saliva had a sensitivity and specificity of 94.1% and 98.6%, respectively (19).

A meta-analysis published in August 2020 (12) compared SARS-CoV-2 detection in saliva versus NP swabs, relative to confirmed COVID-19 disease. It reported 91% (confidence interval [CI] 80 to 99%) sensitivity for saliva, comparable to but slightly lower than the 98% (CI 89 to 100%) for NP swab. Additionally, these authors identified 18 registered, ongoing clinical trials of saliva-based tests for detection of the virus. As of this writing, several commercial saliva tests have received FDA Emergency Use Authorization.

The timing of sample collection relative to symptom onset may also be an important factor when considering the use of noninvasive oral sampling. Nagura-Ikeda et al. (28) described a cohort that included 88 symptomatic patients, 61 of whom presented during the "early" phase of their infection, defined as within 9 days of symptom onset. The remaining 17 patients had had symptoms for 10 days or more. Saliva samples were self-collected by a spitting out method. They found that samples collected from individuals in this early phase of infection had a sensitivity (relative to NP swabs) of 65.6% to 93.4%, depending on the specific PCR technique used. In contrast, samples from those who presented after 10 days of symptom onset demonstrated a sensitivity of 22.3% to 66.7% (28). Another study prospectively demonstrated that the median viral load from saliva was highest within a week of symptom onset, although a third of their patients had detectable RNA for 20 days or longer (30). This suggests that the performance of some noninvasive samples may depend on when they are collected in relation to symptom onset.

Despite the promise of saliva sampling for SARS-CoV-2, it has limitations, including the potential for aerosol production by some methods and possible heterogeneity of specimen quality. Oral swabbing may help to overcome some of these issues. Some studies have specifically examined self-collected oral swab samples compared to health care provider-obtained samples. Kojima et al. (31) compared "unsupervised"

self-collected oral swabs to clinician-supervised self-collected oral swabs in 45 participants, 29 of whom had SARS-CoV-2 detected in at least one sample (oral, nasal, or NP swab) (31). Patients were provided swabbing instructions, and in the clinician-supervised group, patients were corrected if they forgot a step or did not perform something correctly. In the unsupervised group, no corrections were made if a part of the collection process was incorrect. In this study, participants were asked to first cough deeply to collect secretions in their mouth, then rub a swab on the cheeks, gums, hard palate, and tongue for 20 s. Clinician-observed self-collected oral fluid swabs detected SARS-CoV-2 in 26/29 (90%) SARS-CoV-2-positive participants, compared to 19/29 (66%) in unsupervised self-collected oral swabs, 23/27 (85%) in clinician-observed self-collected nasal swabs, and 23/29 (79%) in clinician-collected NP swabs. As with some of the saliva sampling studies, the preliminary cough may have produced sputum, such that these samples may not have been purely oral. Nonetheless, the results suggested that oral swabs self-collected under clinical supervision can detect SARS-CoV-2 well, perhaps even better than NP swabs. However, the discrepancy between supervised participants who received feedback versus those who did not suggests that optimization of self-collection instructions is important. For example, the authors noted that some of the unsupervised participants forgot to cough prior to collecting the sample (31). Unsupervised samples can fall short in a variety of ways, and the use of sample adequacy controls is critical (32).

Although ACE2, the receptor for SARS-CoV-2, is expressed in diverse oral surface tissues, two studies reported especially high levels of expression in tongue dorsum cells (25, 26). With its relative ease of access, the tongue dorsum may therefore be an interesting site to explore for noninvasive SARS-CoV-2 sampling. Tu et al. compared self-collected tongue, nasal, and midturbinate swabs to provider-collected NP swabs. In this study conducted on 501 symptomatic patients, SARS-CoV-2 RNA was detected from tongue swabs in 90% of patients with confirmed COVID-19 (4). This value was only slightly lower than nasal swabbing (94%); however, signal strength measured by qPCR was also lower in positive oral samples than in positive nasal samples. As a result of this and other considerations, the FDA updated its guidance to recommend nasal swabbing, but not oral swabbing, for noninvasive COVID-19 diagnosis. The study by Tu et al. (4) was conducted in the hectic early days of the COVID-19 pandemic in the United States, and samples were stored in viral transport medium at refrigerator temperatures for up to 4 days before testing. Given the abundance of bacteria in oral cavities, it is possible that some oral swab samples degraded during this storage period, a problem that can potentially be overcome by using alternative storage and transport procedures, or POC testing.

Some recent studies have proposed strategies that combine oral (or oropharyngeal) swabbing with more traditional noninvasive samples such as nasal swabbing for COVID-19 diagnosis. The rationale is that the virus is not always evenly distributed throughout the upper airways, and infected patients who are negative at one site may sometimes be detected by sampling another site. A meta-analysis currently at preprint stage found that while nasal swabbing, oropharyngeal swabbing, and saliva sampling captured lower percent positives than traditional NP swabs (nasal swabs 0.82, 95% CI 0.73 to 0.90; oropharyngeal 0.84, 95% CI 0.57 to 1.0; saliva 0.88, 95% CI 0.81 to 0.93), a combination of nasal and oropharyngeal swabs matched the performance of NP (0.97, 95% CI 0.90 to 1.0). A limitation of this idea is that oropharyngeal swabbing is nearly as invasive as NP swabbing (33). However, another study found that a noninvasive protocol that combined noninvasive oral and nasal swabbing (in a sequential protocol using a single swab) matched the sensitivity of NP swabbing (34).

DIAGNOSIS OF INFECTIOUS DISEASES IN CHILDREN

Diagnosing infectious diseases in children can be challenging if invasive sample collection is required. This has led to the investigation of noninvasive sample collection, including oral sampling.

Tuberculosis. Pediatric pulmonary TB remains difficult to diagnose microbiologically. The gold standard relies on obtaining expectorated sputum of good quality. Most children are unable to provide such a sample without induction, an invasive procedure. For this reason, noninvasive alternatives have been sought. One study enrolled 201 South African children with suspected pulmonary TB. Each child had two buccal swabs and two induced sputum samples collected (35). DNA was extracted from each swab in order to perform quantitative PCR specific for TB DNA and the sputum samples were sent for Xpert MTB/RIF testing and culture. The sensitivity for either oral swab being positive was low, at 43% among patients with TB confirmed by at least one induced sputum culture. This was lower than the 64% sensitivity observed by Xpert testing on one sputum sample. Interestingly, however, oral swabs were positive in 24% of patients with unconfirmed TB (children with signs and symptoms of TB who were started on TB treatment, but were negative by sputum testing) (35). The method correctly excluded 93% of children with no TB. Therefore, oral swabs have the potential to augment pediatric TB screening and care, by detecting cases that were not detected by sputum testing. As noted earlier, it is possible that positive sputum production by some children results in deposition of bacilli on oral surfaces, such that the bacilli can be detected afterward even if the child does not produce positive sputa during their clinic visits. Differing PCR methods may have also contributed to the difference in sensitivity between tongue swabs, which used manual PCR, and sputum, which uses GeneXpert testing. The sensitivity of oral sampling may be further improved in children by using tongue swabs instead of buccal swabs, as demonstrated in another study of adult patients (5).

In contrast, another study conducted in Peru found that oral swabs had significantly lower sensitivity for pediatric TB (6). That study used less sensitive qPCR methods than the South African study (35). Specifically, DNA was not concentrated by ethanol precipitation. Such observations highlight the fact that oral swab samples may have relatively low TB bacillary loads, especially in children.

Human immunodeficiency virus. Many infectious diseases require venipuncture for gold standard testing. In small children, this can be challenging and may lead to avoidance of testing. One example is blood testing for HIV infection. Dziva Chikwari et al. (10) compared blood-based HIV testing to testing using oral mucosal transudate samples obtained with the OraQuick ADVANCE Rapid HIV-1/2 test kit, which detects antibodies against HIV and displays the result on the swab device after 20 min (10). The sample is collected by running the swab between the upper lip and gums, followed by passing the swab between the lower lip and gums. This could be considered a buccal mucosa swab or a swab that collects a mixture of gingival crevicular fluid and saliva, but the manufacturer simply describes it as a device to collect oral fluid. In this study, which looked at 1,776 treatment-naive children 18 months to 18 years of age in Kenya and Zimbabwe, there were 71 children diagnosed with HIV infection by blood-based testing (either by a 4th generation antigen/antibody combination test or a 3rd generation antibody test). The test performed by oral sampling was positive in all 71 of these children (100% sensitivity, 99.9% specificity). One limitation to this strategy, which was pointed out by the authors, is that because it detects antibodies, it cannot be used in newborn children due to circulating maternal antibodies (10).

Parvovirus B19 and SARS-CoV-2. Quantitative PCR testing for other pathogens has been performed on oral samples from children in research contexts. For example, Bodewes et al. (36) used oral sampling with the Oracol device, which uses a sponge to collect oral fluid between the lip and gums, for the detection of parvovirus B19 DNA in 116 children with a compatible rash. This strategy exhibited 68% sensitivity relative to serum IgM-EIA testing, and 61% sensitivity relative to serum DNA testing (36). While oral sampling in this study did not perform as well as serum-based testing, it was capable of detecting IgM antibodies or DNA in a majority of children using a noninvasive approach.

Given the potential role of primary schools and child care in the spread of SARS-CoV-2, there is considerable interest in identifying noninvasive methods for detecting the virus in children, including asymptomatic children, in community settings. To date there are only a couple of peer-reviewed studies that have investigated oral sampling in children specifically (37, 38). Both were small studies ($n = 11$ to 12) with asymptomatic as well as symptomatic participants. The sensitivity of oral swabbing was modest in these studies (73% to 82% relative to NP swabbing, with high C_T values).

These observations established that parvovirus and SARS-CoV-2 can be detected in the oral cavities of at least some children, but larger studies with improved methodologies are needed.

DIAGNOSIS OF INFECTIOUS DISEASES WHEN INVASIVE TESTING IS NOT POSSIBLE OR PRACTICAL

Oral sampling may be especially helpful for patients who are unable to undergo more invasive procedures. For example, the fungal pathogen *Pneumocystis jirovecii* can cause a severe pneumonia, particularly in immunocompromised patients who are susceptible to a variety of infections that can cause a similar presentation. The typical diagnostic modality includes a bronchoscopy with BAL for sample collection. This is invasive, logistically challenging, and may not be safe for patients who are clinically unstable. It is also expensive and it requires multiple staff members with special training. In search of a noninvasive alternative, investigators have evaluated the use of oral washes or oral rinsing for the detection of *P. jirovecii* DNA by PCR, especially in patients with HIV infection or other immunocompromising conditions. Larsen et al. (17) described 108 patients living with HIV who presented with symptoms concerning for *Pneumocystis jirovecii* pneumonia (PJP). Of these, 82% were confirmed to have PJP by the gold standard direct microscopy from a BAL sample. Quantitative-touchdown PCR was performed on oropharyngeal wash samples, which were obtained by the patient gargling 10 ml of sterile saline solution for 60 s, and had a sensitivity of 88% and a specificity of 85% relative to direct microscopy from BAL samples (17). Another study examined 36 immunocompromised patients who presented clinically suspected PJP, 15 of whom were ultimately diagnosed with and treated for PJP. The diagnosis was finalized by the agreement between infectious disease specialists and microbiologists based on review of the patients' symptoms, radiography, and other laboratory findings. Nine out of 15 patients (60%) with PJP infection had malignancies as the cause of their immunosuppression; 3 out of 15 (20%) were people living with HIV. Oropharyngeal wash samples were obtained in a similar method as above (gargling for only 30 s) and PCR was performed on two targets (the mtSSU gene and the DHPS gene), which found a 100% sensitivity and 85.7% specificity for each target, relative to the patients who were diagnosed by consensus between specialists (16). Although this was a small study, their results are encouraging for the use of oral samples to diagnose PJP in situations where BAL samples are not readily obtainable. A caveat to using any PCR-based test for the diagnosis of PJP is that detection of *Pneumocystis jirovecii* DNA may simply represent colonization. However, in one study, only 4% of 100 asymptomatic patients with HIV infection had a positive PCR for *Pneumocystis jirovecii* (39). Clinical judgment is necessary to interpret results in these cases.

NONINVASIVE ORAL SAMPLING FOR TROPICAL INFECTIOUS DISEASES

Noninvasive oral sampling has been described for a variety of tropical infectious diseases. It could be useful in settings in which other sample types are not practical to collect.

Malaria. *Plasmodium* spp. were estimated to infect 228 million people in 2018, with 405,000 deaths. Malaria diagnosis commonly involves light microscopy to examine peripheral blood smears with Giemsa staining and blood-based tests that detect malaria antigens. The performance and ease of use of these methods vary, but the requirement for blood sampling is a limitation in all cases.

Saliva-based testing for malaria antigens has been described. Fung et al. (40) obtained blood and saliva samples concurrently from 8 patients in the Philippines with confirmed malaria infections by smear microscopy. Parasite density ranged from 800/μl to 32,000/μl, in addition to one sample with a "packed field" that was estimated to represent a density of >50,000/μl. Saliva was obtained by rinsing their mouths with water and expectorating into a sterile tube. An antigen called *Pf*HRP2 was detected by enzyme-linked immunosorbent assay (ELISA) in saliva from all 8 patients. It was not detected in any of 16 negative-control patients, indicative of 100% specificity (40). Although this was a small study, the results suggest that saliva could be further explored in malaria diagnostics. Saliva has also been studied in the context of subclinical malaria in children. As with most of the studies reviewed here, it is unknown whether there are temporal differences between the detection of these antigens in saliva specimens versus blood-based testing, because the samples were obtained at the same time. There may be differences in when tests from different sampling sites become positive and this should be investigated further, in malaria as well as in other infectious diseases. Subclinical infections may comprise as much as 80% of malaria's reservoir, making the detection of these infections important to reduce the burden of malaria disease. Tao et al. (41) obtained saliva samples via the drooling technique from 364 children with known subclinical malaria infection in Cameroon and Zambia. Subclinical malaria infection was diagnosed either by light microscopy or rapid antigen detection from a blood sample. The children, some as young as 5 years old, collected the samples themselves and the procedure was acceptable to them. The saliva was analyzed for the presence of several malaria antigens to determine which would perform the best. They identified one antigen, PSSP17, and developed a lateral flow immunoassay rapid test to detect it. The sensitivity of this assay ranged from 91 to 100%, depending on whether light microscopy or molecular testing was used as a reference (41). A saliva-based test such as this could be scaled up more easily than blood-based testing. This could significantly improve case detection and reduce the malaria reservoir.

Ebola virus. Ebola can cause hemorrhagic fever and is frequently fatal. The diagnosis is typically made via reverse transcriptase PCR (RT-PCR) testing for viral RNA in blood. A noninvasive alternative may be helpful for several reasons, including protection of health care workers and greater acceptability in some cultures. Delays in diagnosis associated with the reliance on blood samples have been reported (42).

There were two outbreaks of Ebola viral hemorrhagic fever in the Republic of the Congo in 2003. Formenty et al. (42) describe 24 patients with suspected Ebola associated with those outbreaks. Serum and oral fluid samples, obtained by the Orasure device, were collected from the patients and tested for IgG antibodies, antigen, and RNA. Antibodies were not detected in the oral fluid of seropositive patients; however, testing for viral RNA in oral fluid yielded a sensitivity and specificity of 100%, relative to serum viral RNA testing (42). However, a larger studied conducted in Sierra Leone by Erickson et al. found low sensitivity for oral swabs relative to blood testing. Nonetheless, those authors found the method to be highly sensitive (equivalent to blood) for testing corpses, presumably because of the relatively high viral load in patients who progress (43). Given the risks associated with venipuncture in such settings, oral swabbing may be the preferred sampling method for very sick or deceased patients.

ORAL SAMPLING FOR POPULATION SURVEILLANCE AND SCREENING OF INFECTIOUS DISEASES

Infectious disease screening has many benefits, including earlier diagnosis leading to decreased morbidity and mortality and prevention of spread of communicable diseases. Mass screening can be useful to determine the overall prevalence of disease, and to identify asymptomatic carriers. Oral sampling could simplify infectious disease screening, especially in situations involving large numbers of people, given its noninvasive nature and the ability for patients to collect their own samples.

SARS-CoV-2. Oral sampling for screening purposes has been evaluated in the COVID-19 pandemic, both to detect SARS-CoV-2 RNA directly and to detect SARS-CoV-2 antibodies indicating prior infection. Yokota et al. screened 1,924 asymptomatic individuals with self-collected saliva and provider-collected NP swabs; RT-PCR and/or RT-LAMP were used to detect SARS-CoV-2 RNA. These individuals were either part of a contract tracing initiative, which included people who were exposed to someone with confirmed SARS-CoV-2 infection, or an airport quarantine cohort of asymptomatic travelers arriving in Japan. The sensitivity of saliva samples was estimated to be 92%, compared to an estimated sensitivity of 86% for NP swabs; the specificity for both sample types was >99.9%. These values were obtained by using a Bayesian latent class model, given the lack of a gold standard for COVID-19 diagnosis (44). Another study included 401 people presenting with symptoms of COVID-19, contact with someone with confirmed COVID-19 infection, or general concern for COVID-19 infection. Each person had an NP swab obtained by a health care worker as well as a saliva sample collected by pooling saliva in the mouth for 1 to 2 min followed by spitting into a sterile container. No transport medium was used for the saliva samples. Thirty-five out of 401 participants had detectable SARS-CoV-2 RNA from either the NP swab, the saliva, or both. Most of these individuals (20/35, 57%) were asymptomatic. They found that relative to the NP swabs, saliva had a sensitivity of 73.1%, a specificity of 97.6%, and an accuracy of 96.0% (45). Although the reported sensitivity of saliva was lower in this study, it was calculated using different methods than the study by Yokota et al. and still was able to detect SARS-CoV-2 RNA in a significant proportion of participants. If the sensitivity of saliva-based testing for COVID-19 diagnosis in this patient population is truly as low as this study suggests, it is possible that more frequent testing could be a strategy to improve the overall sensitivity of this test.

Oral sampling has also been used to detect antibodies against SARS-CoV-2, indicative of current or prior infection. Pisanic and Randad et al. (46) described a cohort for which saliva and serum samples were compared to detect IgG antibodies against SARS-CoV-2. They obtained oral samples by using the Oracol collection device, which collects a mixture of saliva and gingival crevicular fluid by brushing the gum line. Thirty-three out of 128 patients (24%) who submitted saliva samples had previously had an illness confirmed to be consistent with COVID-19 infection through detection of SARS-CoV-2 RNA. Of those 33 patients, 28 had samples obtained 10 days after onset of illness, and all 28 had detectable IgG antibodies from oral samples. They also obtained paired serum and saliva samples from a subset of patients, and showed that the detection of IgG was highly correlated between the two sample types (46). These results suggest that oral sampling is a viable option for monitoring evidence of prior COVID-19 infection on a large scale given its high concordance with serologic testing and its ability to be self-collected.

Tuberculosis. Oral sampling has been described in the context of active TB case-finding. Lima et al. (47) describe the use of tongue swabs in 128 individuals who were confirmed to have TB infection by sputum testing, conducted as part of a mass screening study in Brazilian prisons. Two tongue swabs were obtained from each of these individuals; 128 negative controls with sputum negative by GeneXpert testing also had tongue swabs collected. Investigators stratified by level of bacillary load in the sputum and found that 32 out of 39 (82.1%) individuals with medium or high bacillary loads were positive via Xpert testing on at least 1 out of 2 oral swabs. However, the sensitivity of oral swabs was much lower for those with low or very low bacillary loads, with just 34/89 (38.2%) having a positive Xpert test from at least 1 out of 2 oral swabs collected (47). The relatively poor sensitivity observed in this study compared to other studies of oral swabbing for TB (5, 35) may be at least partly explained by the use of GeneXpert to test swab samples. This commercial TB testing system is designed for testing sputum but not swab samples. The use of testing methods specifically designed for oral swabs may make it more feasible to exploit the high throughput of this sampling method in screening programs. It is likely that analytical sensitivity would

need to be increased, given the expectation of low pathogen burden in people with incipient disease. Another possibility to increase the sensitivity of oral swabbing for TB diagnosis in this setting would be to increase the number of swabs collected. Because oral sampling is noninvasive, it would be relatively simple to collect more swabs, although, when scaled up to large populations, this could place an additional burden on the laboratories processing these samples. Further study of the potential increased sensitivity from collecting additional samples would be valuable.

Hepatitis C. Mass screening for hepatitis C (HCV) could significantly improve diagnosis. Because there are several effective treatments available, many long-term complications of the infection could thereby be avoided. Currently, screening is performed by testing for serum HCV antibodies. Oral sampling can be used to increase the capacity for screening and has been demonstrated to be very sensitive.

Tang et al. (9) conducted a meta-analysis and literature review of diagnostic HCV testing, which included several studies focused specifically on oral sampling. The pooled sensitivity and specificity for HCV antibody testing on oral samples were 94% and 100%, respectively. These studies involved various sample collection techniques, but they demonstrated that the pooled sensitivity was highest among studies that used the OraQuick ADVANCE collection kit, which involves swabbing the outer gums to obtain oral fluid, a mixture of gingival crevicular fluid and saliva (9). The performance of a new POC test for the detection of anti-HCV antibodies known as the Well Oral Anti-HCV test was recently described. This test involves a similar procedure as the OraQuick and yields results in 15 to 20 min. Liu et al. (48) enrolled 1,179 patients, 486 of whom were known to have chronic HCV infection, and tested them using the Well Oral test collected by a health care provider. The sensitivity and specificity of this POC test relative to serum testing for HCV antibodies were 92% and 98%, respectively. Some individuals also self-collected and the results of self-collected tests differed from the provider-collected test in only 2 out of 199 participants (48). The use of a point-of-care, self-collected HCV test could substantially improve screening.

HIV. Oral sampling to test for HIV has been studied extensively. A meta-analysis that included studies using the OraQuick point-of-care test demonstrated a pooled sensitivity of 98% and specificity of 99% for the detection of HIV-1/2 antibodies. In comparison, the sensitivity and specificity of blood-based testing were both >99%. The most significant difference between oral sampling and blood-based testing was seen when the data were stratified by settings with high or low HIV prevalence, and pooled positive predictive value (PPV) was calculated. In high-prevalence settings, the PPV was about the same when using oral and blood-based testing (98.65% in oral sampling, 98.50% in blood-based testing). However, in low-prevalence settings, the PPV was significantly lower using OraQuick (88.55% in oral sampling, 97.65% in blood-based testing) (8). A subsequent study compared several different oral sampling kits. The DPP HIV 1/2 Assay, which involves swabbing the outer gums for 15 to 30 s to collect oral fluid, was shown to be 100% sensitive and specific when tested in 507 patients (7). Further study using this assay is warranted to confirm these results.

SUMMARY

The oral cavity is a complex compartment with diverse microenvironments, some of which are highly aerated and continuously washed with saliva, while others are sheltered and anaerobic. It harbors one of the most complex microbial communities in the human body. As a major portal between our bodies and the outside world, it is a useful and very accessible place to look for pathogen biomarkers of infectious disease. As illustrated by the examples in this article (summarized in Table 1), the list includes diseases that are not normally associated with the oral cavity. In some cases, identical methods can potentially be used for multiplex screening for multiple pathogens. With rapid improvements in molecular and point-of-care detection technologies, other examples of oral sampling may be worth visiting or revisiting. By design, oral sampling is an easy idea to evaluate.

TABLE 1 Summary of oral samples and techniques

Reference	Sample type	Sample collection notes	Analyte used
Tuberculosis			
Luabeya et al. (5)	Tongue swab, buccal swab, gum swab	OmniSwab, PurFlock	DNA
Nicol et al. (35)[a]	Buccal swab	OmniSwab, PurFlock	DNA
Flores et al. (6)[a]	Buccal swab	OmniSwab	DNA
Mesman et al. (21)	Buccal swab	OmniSwab	DNA
Lima et al. (47)[b]	Tongue swab		DNA
Shenai et al. (20)	Saliva, exhaled breath condensate	Salivette saliva collector	DNA
Molina-Moya et al. (22)	Buccal swab	PrimeSwab	DNA
Song et al. (23)	Tongue swab		DNA
Kang et al. (24)	Oral mucosal transudated	OMNIgene ORAL OMR-110	DNA
SARS-CoV-2			
Han et al. (37)[a]	Saliva		RNA
Kam et al. (38)[a]	Buccal swab	Mini UTM Kit with flocked swabs	RNA
Azzi et al. (11)	Saliva	Drooling technique	RNA
Williams et al. (27)	Saliva	Spitting out technique	RNA
Hanson et al. (13)	Saliva	Pooling in mouth then spitting	RNA
To et al. (30)	Oropharyngeal saliva	Coughing out early morning saliva	RNA, viral culture
Procop et al. (14)[c]	Enhanced saliva	Sniffing strongly, coughing out	RNA
Mittal et al. (18)[c]	Oral rinse		RNA
Babady et al. (19)	Oral rinse and saliva	Spitting out technique for saliva	RNA
Czumbel et al. (12)	Saliva		RNA
Kojima et al. (31)[c]	Tongue, buccal, gum, hard palate swab	Copan flocked swab	RNA
Tu et al. (4)[c]	Tongue swab	Copan flocked swab	RNA
Yokota et al. (44)[c]	Saliva		RNA
Senok et al. (45)[c]	Saliva	Drooling technique	RNA
Pisanic et al. (46)[c]	Oral mucosal transudate[d]	Oracol device	Antibodies
Nagura-Ikeda et al. (28)[c]	Saliva	Spitting out technique	RNA and antigen
Agulló et al. (29)	Saliva		Antigen
Kandel et al. (34)[c]	Oral rinse, tongue and buccal swab		RNA
HIV			
Dziva Chikwari et al. (10)[a]	Oral mucosal transudate[d]	OraQuick ADVANCE	Antibodies
Pant Pai et al. (8)	Oral mucosal transudate[d]	OraQuick ADVANCE	Antibodies
Beelaert et al. (7)	Oral mucosal transudate[d]	DPP HIV 1/2 Assay	Antibodies
Parvovirus B19			
Bodewes et al. (36)[a]	Oral mucosal transudate[d]	Oracol device	DNA and antibodies
Pneumocystis jirovecii			
Larsen et al. (17)[d]	Oral rinse		DNA
Goterris et al. (16)	Oral rinse		DNA
Fraczek et al. (39)	Oral rinse		DNA
Malaria			
Fung et al. (40)	Saliva	Rinse mouth then expectorate	Antigen
Tao et al. (41)[a]	Saliva	Drooling technique	Antigen
Ebola			
Formenty et al. (42)	Oral mucosal transudate[d]	Orasure device	RNA, antigen, and antibodies
Erickson et al. (43)	Oral swab		RNA
Hepatitis C			
Tang et al. (9)	Oral mucosal transudate[d]	OraQuick ADVANCE	Antibodies
Liu et al. (48)[c]	Oral mucosal transudate[d]	Well Oral Anti-HCV test, OraQuick	Antibodies

[a]Study involving children.
[b]Active TB case finding.
[c]Self-collected samples.
[d]Also referred to as gingival crevicular fluid.

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