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Detecting viruses by using salivary diagnostics

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Abstract

Background—Diagnostics that involve the use of oral fluids have become increasingly available commercially in recent years and are of particular interest because of their relative ease of use, low cost and noninvasive collection of oral fluid for testing.

Types of Studies Reviewed—The authors discuss the use of salivary diagnostics for virus detection with an emphasis on rapid detection of infection by using point-of-care devices. In particular, they review salivary diagnostics for human immunodeficiency virus, hepatitis C virus and human papillomavirus. Oral mucosal transudate contains secretory immunoglobulin (Ig) A, as well as IgM and IgG, which makes it a good source for immunodiagnostic-based devices.

Clinical Implications—Because patients often visit a dentist more regularly than they do a physician, there is increased discussion in the dental community regarding the need for practitioners to be aware of salivary diagnostics and to be willing and able to administer these tests to their patients.

Keywords

Human immunodeficiency virus; human papillomavirus; hepatitis C virus; saliva; diagnostic; point of care; oral fluid

Although research projects on the development of saliva-based diagnostic testing are progressing rapidly and several commercial tests are available, use of these tests by dentists is modest. Point-of-care (POC) salivary tests can be used in the field, in emergency departments, in medical and dental clinics and, eventually, at home. In this review, we highlight the existing screening tests for viral infectious diseases with the hope that dental professionals will play a greater role in this field because the oral cavity and its fluids are in the domain of dentistry.

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One key advantage in developing diagnostic tests for viruses and bacteria rather than for systemic diseases is that a single target (that is, analyte) is sufficient to identify the pathogen. In the case of systemic diseases (for example, diabetes, cancer, Alzheimer disease and cardiovascular diseases), multiple biomarkers—or a “signature” profile—typically are required and these can provide a clue, but rarely a definitive diagnosis.

A major clinical issue with respect to infectious diseases is distinguishing a bacterial infection from a viral infection. In the case of upper-respiratory diseases and meningitis or pneumonia, the identification of a bacterial etiology allows appropriate and immediate antibiotic treatment. Misdiagnosis as a viral disease, particularly in the case of meningitis, can lead to death when the infection actually is of bacterial origin. Likewise, the inappropriate use of broad-range antibiotics in cases in which a viral infection is mistaken for a bacterial infection, besides being ineffective, may lead to allergic reactions, toxicity and a deterioration of the patient's condition. Furthermore, the excessive use of broad-range antibiotics is regarded as a major cause of multidrug-resistant bacteria. Although investigators have devoted much attention to novel techniques that allow differentiation between acute viral and bacterial infections[1] (or to addressing biomarker-based disease by means of microarray technology[2]), no simple test currently is available to distinguish between a bacterial and a viral infection.

Another key issue is to ascertain the need for a POC test to obtain an immediate result compared with a laboratory test, which may require days or weeks to obtain results. Diagnosis of microbial infections currently involves blood analysis (sedimentation and white blood cell count), quantification of common biomarkers (for example, C-reactive protein) and, to a lesser extent, the more time-consuming microbial cultivation. When suspecting a viral infection, clinicians may use nucleic acid–based amplification technologies.

The use of widely available rapid antibody detection tests is of value to screen for infections such as human immunodeficiency virus (HIV) in which the presence of antibodies against the pathogen is suggestive of an active infection. For infections with human papillomavirus (HPV) or herpes simplex virus (HSV), antibody detection is not informative because the presence of antibodies may indicate a previous or latent infection. However, some currently available HPV nucleic acid–based tests detect the virus and are specific for types of oral HPV.

In some cases, clinicians can use the ratio of immunoglobulin (Ig) M to IgG to distinguish acute disease from chronic disease. In saliva, a similar approach appears feasible, because salivary IgM is present in cases of acute hepatitis but not in cases of chronic disease.[3-5] Treating any infectious disease at the point of diagnosis will speed up recovery and decrease the opportunity for spread of the disease. In addition, if the patient does not return to the clinician's office for follow-up or obtain test results that are available only after days or weeks, access to therapy will be compromised.

When developing a new diagnostic test, investigators must consider the test sample's source. Most commonly, disease diagnosis involving a physical examination includes obtaining a

blood sample, which, for a wide variety of analytes, has become routine. Currently, many infections can be detected with a full-scale blood analysis that includes blood cell counts, antibodies to common conditions and a variety of metabolic markers. Blood tests may be supplemented with, or replaced by, a urine sample in some cases.[6] Salivary tests, although rapidly increasing in use, still constitute a minority of all diagnostic tests performed.

One issue in using a saliva-based test is the nature of the target analyte. If testing for an antibody to a specific virus (for example, HIV, HPV or influenza) in which the antibodies are known to be detectable in blood, they also will be found in saliva, albeit at a somewhat lower concentration. On the other hand, if one is looking for an antigen or nucleic acid associated with a specific pathogen, those targets may or may not be detectable in saliva. Many investigators have conducted studies involving pathogen-derived nucleic acids and antigens, as well as antibodies to viral pathogens found in saliva.[4,5,7-33] Saliva remains an attractive biological matrix for POC diagnosis, especially when focusing on applications in remote settings or home-care situations and, as we propose, in the dental setting. Although diagnostic tests that involve the use of finger-stick blood are well accepted, the advantage of using saliva is that the collection is completely noninvasive and when patients are given a choice, they prefer saliva testing to tests requiring blood.[34,35]

Viruses Detected By Using Oral-Based Clinical Samples

Oral samples

Clinicians can use a number of oral samples to diagnose viruses, including whole saliva, gingival crevicular fluid, oral swabs of mucosal tissue, dental plaque, oral biopsy specimens and volatiles in breath. Studies reported in the literature typically involved the use of whole saliva or another oral fluid obtained by means of an adsorbent collector. Many of the tests on the market involve the use of oral mucosal transudate (OMT) obtained by swabbing the buccal mucosa and tongue. This sample is rich in antibodies and contains surface pathogens. Saliva, as collected from the salivary glands, consists primarily of secretory IgA (sIgA), whereas OMT contains a mixture of sIgA, IgG and IgM. Thus, OMT provides a richer source of antibodies, including those directed against bacterial and viral pathogens, and it is relatively more concentrated in oral surface pathogens and antigens derived from those pathogens. We should note that investigators in many research studies performed analyses on whole saliva clarified by centrifugation, and these results may differ from those for fluid collected with an oral swab.

Investigators have detected a large number of viruses in oral samples by using an antigen, an antibody or nucleic acid targets. Reports in the literature[4,5,7-33] focus on the diagnosis of viral infections that require immediate therapeutic intervention and those that pose a threat to blood transfusion safety (blood donor screening). In this review, we included only HSV in the large group of herpesviruses; it resembles HIV in that it is a sexually transmitted virus for which there are therapeutic options to decrease the viral load but no cure, as the virus can become latent and reappear when drug therapy is discontinued. Thus, currently available treatments reduce the chance of transmission, but a latent infection remains.

The literature regarding salivary-based antibody tests for detection of viral infections is extensive. For diagnosis of severe and high-mortality infections such as Ebola or rabies, antibody assays are less useful because the infected patient may not survive long enough to go through seroconversion. Researchers have not demonstrated the presence of antibodies to Ebola and rabies in saliva, but they have isolated the virus from various bodily fluids including saliva, which supports the concept that a salivary antigen or nucleic acid diagnostic test could be developed for these viruses.

Diagnosis of pathogens

Diagnosis of bacterial and viral pathogens is based increasingly on a combination assay that measures both antibody and antigen or antibody and nucleic acid. Two decades ago, nucleic acid–based detection often involved use of hybridization (such as Southern blot or dot blot analysis). However, we have observed that investigators in more recent studies used one of the nucleic acid amplification technologies. Because most of these technologies require concentration and purification of the nucleic acid targets, they are applicable to any biological matrix, including oral samples. Several automated, laboratory-based commercial systems are available to perform these assays, not only for diagnosis of the disease but also for monitoring the effect of antiviral drugs on the viral load (for example, Cobas Amplicor, Roche Molecular Diagnostics, Pleasanton, Calif.). Nucleic acid–based diagnosis is attractive because it allows for simultaneous detection of multiple targets (that is, multiplexing); a current research focus is the development of amplification technologies that minimize the need for nucleic acid purification.

The first definitive proof of the presence of HIV antibodies in saliva demonstrated the potential of oral fluid for screening purposes and saliva-based antibody assays for almost any known pathogen, including those in the veterinary sector.[36] When performing specific antibody tests, one needs to consider the vaccination policies used in various countries; for example, people who received the Bacillus Calmette-Guérin vaccine may have false-positive test results on the Mantoux tuberculin skin test.[37] Likewise, participants in HIV vaccine trials may demonstrate positive reactivity in subsequently administered antibody tests.

Major Viral Infections

In this review, we focus on HIV, hepatitis C virus (HCV) and HPV because these three major viruses are responsible for a series of worldwide epidemics that have had an enormous effect on morbidity and mortality. Most people now understand the impact and risk of HIV infection, but the risk and sequelae of HPV and HCV infections are much less recognized. Recently, the Centers for Disease Control and Prevention (CDC), Atlanta, reported that HCV is responsible for more deaths in the United States than is HIV.[38] Furthermore, HPV, originally associated only with cervical cancer, now is linked to an increasing incidence of oral cancer.

Salivary diagnostics for HIV

HIV is the cause of AIDS. The infection of immune system cells eventually leads to the loss of cell-mediated immunity. If the infection is left untreated, opportunistic infections and

cancers develop, which eventually lead to death. HIV infection is detected easily with an antibody-based screening test after seroconversion; however, early infections are difficult to recognize because they are accompanied only by mild flulike symptoms, and an antigen or nucleic acid assay is required in the weeks before seroconversion. Diagnosis according to a reactive antibody assay requires a confirmatory test with either a Western blot (via blood or saliva) or a polymerase chain reaction (PCR) (via blood). Well-managed drug therapy is required to keep viral propagation at close to undetectable levels. Currently, no cure exists for HIV infection, and once it is integrated into the human genome, it remains and can replicate unless suppressed by medication. Although some investigators have reported the isolation of infective viral particles from oral samples and demonstrated the presence of viral particles in epithelial cells of the buccal mucosa,[39-41] the chance of transmitting HIV through saliva remains extremely low.[42] Moreover, a large body of literature supports the presence of effective anti-infective activity of human salivary secretions by a variety of salivary proteins, including defensins, lysozymes, lactoferrin secretory leukocyte protease inhibitor and *DMBT1* (glycoprotein-340/salivary agglutinin),[43] as well as lysis of HIV in the oral cavity owing to the hypotonicity of saliva.[44]

All of the existing oral-based diagnostic tests for HIV infection are screening tests, detecting antibodies to HIV-1 or both HIV-1 and HIV-2. In general, these tests involve the use of nitrocellulose lateral flow strips that contain two capture zones: a control line that detects the presence of all antibodies in the sample and a test line that specifically reacts with HIV-1 or, ideally, with both HIV-1 and HIV-2. A reactive result needs to be confirmed with a second test. This confirmatory test can be a Western blot that involves the use of saliva or blood and that detects antibodies to multiple HIV antigens, or it could be a blood-based PCR test that detects HIV RNA.

Although many oral tests are on the market, the U.S. Food and Drug Administration (FDA) has approved only one test. The test, which was approved in 2004,[45] involves use of a POC device (OraQuick ADVANCE Rapid HIV-1/2 Antibody Test, OraSure Technologies, Bethlehem, Pa.). The clinician collects oral fluid with a swab and places it directly into a developing solution in the device; after 20 minutes, he or she can visualize the resulting lines.

Results from multiple studies demonstrated that the sensitivity and specificity of these oral tests are comparable to those of tests for antibody detection that involve the use of plasma or finger-stick blood.[46.47]

Several investigators have conducted studies pertaining to the development and application of technologies used to detect HIV antibodies, HIV-derived antigen and nucleic acids in oral samples.[3,15,48-58] These include technologies used for high-throughput tests conducted in clinical laboratories, as well as rapid, single-sample tests for POC or home-testing devices. As is seen for other infectious diseases, salivary antibody diagnostics for HIV are as effective as blood-based diagnostics. However, because of differences in concentration and stability, other pathogen-specific targets (antigen, nucleic acid) are not always detectable in saliva.

For example, the fourth-generation immuno-assays detect p24 antigen and antibodies against HIV, allowing earlier detection of HIV infection with blood-based samples.[59,60] However, investigators have not yet demonstrated that these tests work with saliva samples. Similarly, detection of viral RNA in saliva is more difficult than is detection in a blood sample owing to decreased viral load. Researchers have reported higher loads of HIV in saliva than in serum in some patients,[61] and these patients are referred to as hypersecretors. Detection of HIV RNA in saliva is possible because current technologies include concentration and purification steps to attain the required sensitivity.

Salivary diagnostics for HCV

The common hepatitis viruses are named with the letters A through E. Vaccines are available for hepatitis A virus and hepatitis B virus (HBV); vaccines are in development for hepatitis E virus, but the FDA has not yet approved them. Blood safety procedures for donor blood for transfusion-transmissible infectious diseases include various tests for HBV (screening for the presence of antibody and antigen) and HCV (screening for the presence of antibody and nucleic acid targets). No vaccine currently is available for HCV.

HCV, like HIV, is an RNA virus. Chronic infection causes liver cirrhosis, which may lead to liver failure, cancer or extremely dilated sub-mucosal veins in the stomach and esophagus. Acute infections generally are accompanied by mild symptoms and are not recognized easily. In contrast to HIV, HCV infections can resolve spontaneously; however, like HIV, the virus may remain latent and can be activated at a later time. The first step in screening is to test for the presence of antibodies; if the test result is positive, then a confirmatory test is required. Typically, the confirmatory test, as for HIV, is a Western (immunoblot) assay combined with a nucleic acid–based viral load assay.[62]

Recently, there has been a great deal of interest in saliva-based rapid tests for HCV, which has been referred to as the “silent epidemic.”[63] As mentioned earlier, the CDC recently reported that more people in the United States die each year of HCV than they do of HIV.[38] Because a number of drugs are available to treat HCV, and many more are under development, diagnostic testing for the presence of the virus can lead to timely therapeutic intervention. Screening tests for HCV, similar to those for HIV, typically rely on detecting specific anti-HCV antibodies. Although an Internet search reveals several such tests, none of these saliva-based tests, to date, has received FDA approval.

OraSure Technologies markets an FDA-approved test for HCV that uses finger-stick blood; in addition, the company has a salivary test that is used widely in Europe but has not been approved for sale in the United States. Drobnik and colleagues[64] compared screening test results of the OraQuick HCV Rapid Antibody Test (OraSure Technologies) with those of the criterion standard enzyme immunoassay (by means of a blood draw) (Abbott HCV EIA 2.0, Abbott Laboratories, Abbott Park, Ill.). The researchers confirmed reactive samples with use of a Western blot (Chiron RIBA HCV, Bio-Rad Laboratories, Hercules, Calif.). The authors reported that the test results of the OraQuick HCV Rapid Antibody Test matched those of the Abbott HCV EIA 2.0 immunoassay 97.5 percent of the time.[64]

Salivary diagnostics for HPV

HPV is a DNA virus; investigators have identified more than 100 HPV types,[65] approximately 20 of which are considered to be high-risk HPVs with the possibility of leading to malignant neoplasia. About 70 percent of cervical cancers are attributed to HPV types 16 and 18 and are part of a national prevention and screening program that includes use of an anti-HPV vaccine. Most HPV infections are self-cleared but antibodies to the virus remain; thus, antibody tests to screen for HPV infection are not considered useful.

Since 1990, there has been a striking increase in oropharyngeal squamous cell carcinoma (OSCC), and approximately 60 percent of these tumors are associated with HPV.[66] These tumors largely are seen in white men with no history of tobacco or alcohol use, in contrast to oral cancer not associated with HPV, in which tobacco and alcohol use are major factors in disease development. Chaturvedi and colleagues[67] reported that although the incidence of HPV-negative OSCC decreased from two cases per 100,000 to one case per 100,000 from 1988 to 2004, HPV-associated OSCC increased from 0.8 case per 100,000 to 2.6 cases per 100,000 during the same period.

Gillison and colleagues[68] conducted a large study of the prevalence of oral HPV infection in the United States in 2009-2010. They reported that oral HPV infection in men and women aged 14 through 69 years was 6.9 percent, with a 1 percent incidence of high-risk HPV 16. An intriguing HPV genome sequencing report by Andrews and colleagues[69] pertaining to two couples who developed HPV-associated tonsillar carcinoma revealed the identical HPV 16 in the partners of both couples, which suggests the potentially infectious nature of this cancer.

Salivary diagnostic tests are available for HPV, and essentially they involve the use of PCR; thus, they are not POC tests. Kits containing a salivary collector are placed in transport media and sent to a central laboratory for analysis. Investigators in the field have used oral swabs, expectorated saliva or an expectorated oral rinse with mouthwash (OraRisk HPV test, OralDNA Labs, Brentwood, Tenn., which, to our knowledge, is the only salivary diagnostic test for HPV commercially available in the United States).[70,71] The latter collection technique probably has the highest sensitivity, because it samples the entire oral cavity and the swishing of the solution dislodges mucosal cells. Investigators in the laboratory use a variety of primers to detect as many HPV types as possible. Early diagnosis is critical for survival of patients with OSCC, and, thus, it is likely that use of salivary HPV analyses will continue to increase.

Conclusions

Although several salivary tests are available for the detection of viral infections, and many others are being developed, the use of these tests by dental professionals has been limited. Although investigators have reported dentists' and patients' acceptance of oral tests for the diagnosis of systemic diseases,[72,73] the use of available salivary tests in dental settings is modest. Such issues as scope of the profession, time required for testing and third-party reimbursements have been advanced as obstacles, and these issues need to be addressed. The CDC has been active in expanding the number of sites at which health care professionals can

carry out HIV testing. Strauss and colleagues[74] reported that in 2008, 19.5 million people did not visit a general health care provider but did visit a dental care provider. Thus, by conducting salivary HIV tests in a dental setting, practitioners would be able to identify infections in a cohort that might not otherwise be detected.

Recently, the New York State Department of Health AIDS Institute enlisted the five dental schools in the state to begin carrying out oral screening for HIV antibodies in 2012. Any reactive test results will require that a sample be sent for a confirmatory test, and if the results are positive, the patient will be referred for care (Howard Lavigne, deputy director-clinical education, New York State Department of Health-AIDS Institute, Central New York Regional Office, Syracuse, N.Y., written communication, Aug. 17, 2012). We hope that other salivary tests (for example, HCV, HPV and influenza) can be added and that the program can be extended to include community health centers and, ultimately, dental offices.

As soon as specific guidelines and approaches for infectious disease screening in the dental setting are defined and the new rapid salivary tests (for example, HCV) are approved by the FDA, testing during dental visits seems an appropriate and cost-efficient way to screen patients for infection.

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Abbreviation Key

CDC	Centers for Disease Control and Prevention
FDA	Food and Drug Administration
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV	Herpes simplex virus
Ig	Immunoglobulin
OMT	Oral mucosal transudate
OSCC	Oropharyngeal squamous cell carcinoma
PCR	Polymerase chain reaction
POC	Point of care
sIgA	Secretory immunoglobulin A