Chapter 13
The Utility of Antibodies in Saliva to Measure Pathogen Exposure and Infection

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Abstract Saliva contains pathogen-specific antibodies that can provide quantitative information on the type and temporality of infection. As such, saliva as a biospecimen can be used to noninvasively assess seroconversion to infectious

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pathogens in the clinical setting as well as for epidemiological surveillance, improving understanding of the epidemiology and natural history of infectious diseases. While saliva is not ideal for analysis of all infectious diseases, it remains a valuable means for data collection across populations varying in age, geographic location, and health status. Developments in the field could allow for rapid testing of exposure to infectious agents through point-of-care assays in areas where blood-based assays or laboratory analysis would be impractical. Here we review the advantages and disadvantages of using salivary assays to assess infectious disease exposure and infection in different settings and explore future directions.

13.1   Introduction

Saliva contains pathogen-specific antibodies that can provide quantitative information on the type and temporality of pathogen exposure and infection. As such, saliva can serve as a noninvasive biospecimen to monitor recent or historical exposure to pathogens in both clinical and epidemiological settings. In clinical settings, saliva-based diagnostic testing can inform clinical decision-making around infectious disease treatment, management, and vaccination. In epidemiological surveys, saliva-based infectious disease detection methods can be used to assess seroprevalence and seroconversion among high-risk populations; gain epidemiological inference on risk factors associated with pathogen exposure, infection, and transmission; and facilitate self-collection and testing in national- and community-level public health infectious disease surveillance programs. Saliva-based methods for infectious disease detection have a unique and critical role in the context of vaccine preventable diseases (VPDs), where they can be used to assess disease burden and improve case detection, estimate population immunity, identify immunity gaps, and assess the impact of vaccination campaigns and routine immunization.

Most of the studies discussed in this chapter utilize "oral fluids" specifically. We thus use the term oral fluids throughout the chapter unless stated otherwise. In this chapter, we discuss the utility of antibodies in oral fluids to measure pathogen exposure and infection in two major infectious disease contexts: (1) clinical diagnostic testing and (2) epidemiological research and population-based surveillance, with a special emphasis on VPDs. We first discuss the utility of oral fluid-based methods in clinical infectious disease diagnostic testing. In this section, we review oral fluid-based HIV testing as an example of a US Food and Drug Administration (FDA) approved and CLIA-waived oral fluid-based test for diagnosis of infection. Second, we discuss the utility of oral fluid-based methods in epidemiological research and the inference that can be gained from using such tools in cross-sectional and longitudinal study designs. We further discuss and provide examples of national- and community-level public health programs for population-based infectious disease surveillance where oral fluid-based self-collection and testing has been implemented. We end this section with a discussion of the utility of oral fluid-based methods in the context of VPDs. Following these two sections, we address the limitations of oral fluid-based methods compared to blood-based methods, such as diagnostic accuracy. We conclude this chapter with a section on

future directions for the field of oral fluid-based infectious disease surveillance and diagnostic testing, highlighting point-of-care (POC) development for VPD-related applications, the identification of asymptomatic subpopulations in population-based studies, and a more nuanced argument on the potential utility of saliva-based secretory IgA (SIgA) biomarkers of infectious disease. Through this chapter, the reader will gain an understanding of the different potential uses of oral fluid-based methods for infectious disease testing in clinical- and population-based settings, the current status of pathogen-specific oral fluid-based methods and assays (Table 13.1), and advantages and disadvantages of oral fluid-based infectious disease testing.

13.2   Utility of Oral Fluid in Clinical Infectious Disease Diagnostic Testing

Serological diagnostic testing has proven to be an indispensable tool in the clinical setting for the diagnosis and management of infectious diseases. Serological diagnostic testing is ideal for the diagnosis of infectious diseases where active infection can be identified by pathogen-specific antibody levels and early clinical identification could initiate treatment interventions and infection control practices. Human immunodeficiency virus (HIV) is one of the few pathogens that has an oral fluid-based clinical diagnostic test that is approved by the US FDA and is Clinical Laboratory Improvement Amendments (CLIA)-waived for diagnosis of infection. In this section, we discuss oral fluid-based diagnostic testing for HIV in the context of its clinical evaluation and FDA approval for diagnostic and point-of-care (POC) purposes.

13.2.1   Oral Fluid-Based Human Immunodeficiency Virus Diagnostic Testing

Identification of infection with HIV is critical to inform clinical decision-making, including initiation of antiretroviral therapy or pre-exposure prophylaxis (PrEP), and to prevent transmission. The Center for Disease Control and Prevention (CDC) recommends that everyone between the ages of 13 and 64 get tested for HIV at least once, and at least once a year for higher risk populations ("HIV/AIDS testing," 2019). A number of studies have shown that IgG antibodies against HIV type 1 (HIV-1) can be detected in the oral fluid of seropositive subjects (Hodinka, Nagashunmugam, & Malamud, 1998). These reports garnered considerable interest and prompted the further development of oral mucosal transudate (OMT)-based methods for serological detection of HIV. Advantages of an OMT-based method to detect HIV-specific antibodies include greater safety when collecting and handling HIV-positive specimens due to low concentrations of HIV antigens in saliva (Major et al., 1991), increased patient compliance (Major et al., 1991; Spielberg et al.,

Table 13.1 Studies regarding salivary pathogen-specific antibody biomarkers of pathogen exposure and/or infection

Pathogen		Antibody biomarker	References
Virus	Dengue virus (DENV)	IgM, IgA, and IgG antibody	Andries et al. (2015), Balmaseda et al. (2008), Spoorthi (2014), Vázquez et al. (2007)
	Ebola	IgM and IgG antibody	Lambe et al. (2016)
	Human immuno-deficiency virus (HIV)	IgG antibody	Delaney et al. (2006), Delaney et al. (2011), Figueroa et al. (2018), Gallo (1997), Hodinka et al. (1998), Wesolowski et al. (2006), Reid et al. (2008)
	Hepatitis A virus (HAV)	IgM and IgG antibody	Amado, Villar, de Paula, de Almeida, and Gaspar (2006), Laufer et al. (1995), Morris-Cunnington et al. (2004), Ochnio, Scheifele, Ho, and Mitchell (1997), Parry, Perry, Panday, and Mortimer (1989), Quoilin et al. (2007), Tourinho et al. (2015)
	Hepatitis B virus (HBV)	IgM, IgG antibody, and viral antigen	Fisker, Georgsen, Stolborg, Khalil, and Christensen (2002), Hutse et al. (2005), Nokes et al. (2001), O'Connell et al. (2000), Piacentini, Thieme, Beller, and Davidson (1993), Quoilin et al. (2007), Simani et al. (2008), Thornton et al. (2000), Weild et al. (2000)
	Hepatitis C virus (HCV)	IgM and IgG antibody	De Cock et al. (2004), Judd et al. (2003), Thornton et al. (2000), Weild et al. (2000)
	Hepatatis E virus (HEV)	IgG and IgA antibody	Pisanic et al. (2017)
	Measles	IgM and IgG antibody	Goyal et al. (2009), Hayford, Al-Emran, et al. (2013), Hübschen et al. (2018), Jani et al. (2008), Kremer and Muller (2005), Nigatu et al. (2008), Ohuma et al. (2009), Sheikh et al. (2011), Shonhai et al. (2015), Thieme et al. (1994), Vainio et al. (2006)
	Mumps	IgM and IgG antibody	Hübschen et al. (2018), Jin et al. (2004), Reid et al. (2008), Thieme et al. (1994), Warrener et al. (2011)
	Norovirus	IgA and IgG antibodies	Griffin et al. (2011, 2015), Lindesmith et al. (2003), Lindesmith et al. (2005), Moe et al. (2004), Pisanic et al. (2018), Wade et al. (2019)
	Pertussis	IgG antibody	Kara et al. (2017), Wang et al. (2014)
	Rotavirus	IgM, IgA, and IgG antibody	Aiyar et al. (1990), Friedman, Entin, Zedaka, and Dagan (1996), Grimwood et al. (1988), Stals, Walther, and Bruggeman (1984)
	Rubella	IgM and IgG antibody	Ben Salah et al. (2003), Hübschen et al. (2018), Nokes et al. (1998), Nokes et al. (2001), Thieme et al. (1994)

Table 13.1 (continued)

Pathogen		Antibody biomarker	References
	Zika virus	IgM, IgA, and IgG antibody	Zhao et al. (2019)
Bacteria	*Escherichia coli* O157	IgM and IgA antibodies	Chart and Jenkins (1998), Chart et al. (2003), Ludwig et al. (2002)
	Salmonella Typhii	IgA antibody	Herath (2003), Zaka-ur-Rab et al. (2012)
	Campylobacter spp.	IgG and IgA antibody	Cawthraw et al. (2002)
	Shigella spp.	IgA antibody	Schultsz et al. (1992)
	Vibrio cholerae	IgA antibody	Jertborn, Svennerholm, and Holmgren (1986)
Parasite	*Plasmodium falciparum*	IgG antibody	Chidi et al. (2011), Estévez et al. (2011)
	Giardia spp.	IgG and IgA (including SIgA) antibody	El-Gebaly et al. (2012), Rodríguez et al. (2004)
	Cryptosporidium spp.	IgG and IgA antibody	Cozon, Biron, Jeannin, Cannella, and Revillard (1994), Egorov et al. (2010); Griffin et al. (2011); Moss et al. (2019)

2000), and the potential to improve access to testing and results (Martin, Williams, Ferguson, & Read, 2018; Nangendo et al., 2017).

Testing for HIV using OMT was enabled by a number of products that have been approved by the US FDA. The OraSure oral-specimen collection device has been licensed by the FDA to use in conjunction with a laboratory-based enzyme immunoassay (EIA) antibody test, the Oral Fluid Vironostika® HIV-1 Microelisa system (approved in 1994) ("Approval of The First U.S. HIV Test System Using Oral Fluid Samples," , 1994). The OraSure® collection apparatus was designed specifically to enhance the collection of OMT rich in IgG antibodies derived from OMT at the gingival crevice (Brandtzaeg, 2013). Two separate large-scale clinical trials determined that OMT-based testing for HIV-1 antibodies using this system was a highly accurate alternative to serum testing. The system performed with a sensitivity and specificity of 99.2% (Granade et al., 1998) to 99.9% (Gallo, 1997). The oral fluid Vironostika HIV-1 Microelisa System has since been replaced and is currently marketed as the Avioq HIV-1 Microelisa System (Food and Drug Administration, 2009). It is important to note that the FDA requires a confirmatory test to rule out false positives (Food and Drug Administration, 2009). The OraSure HIV-1 Western blot kit (Epitope Inc.) has been licensed by the FDA as a more specific method to perform a requisite confirmation of oral fluid specimens positive for HIV-1 specific antibodies (Gallo, 1997), which was approved in 1996. FDA licensure of the OraSure® OMT collection device with an associated EIA method now provides an accurate alternative to routine, blood-based, serological HIV-1 testing.

Rapid, POC tools are critical in meeting national goals to improve coverage of HIV testing and access to HIV status results. The OraQuick® *ADVANCE* Rapid

HIV-1/2 Antibody Test, for the qualitative detection of antibodies to both HIV-1 and HIV-2 in human oral fluids, was approved by the FDA and CLIA-waived in 2004 (Food and Drug Administration, 2004). A direct comparison of six FDA approved rapid HIV antibody tests concluded that this OMT-based rapid test performed similarly to other blood-based rapid tests (Delaney et al., 2011). This same system was repackaged as the OraQuick® In-Home HIV Test, and was approved as an over-the-counter, POC, test in 2012 (Food and Drug Administration, 2012b). Most recently, the FDA approved the Dual Path Platform® (DPP) HIV-1/2 POC test in 2012, which can utilize oral fluid as input specimens (Food and Drug Administration, 2012a). While both blood- and oral fluid-based rapid HIV antibody tests conducted via self-testing provide accurate HIV results compared to when conducted by trained personnel, the sensitivity and specificity have been shown to be higher for self-testing using blood-based rapid tests compared with oral fluid-based rapid tests (Figueroa et al., 2018). Such POC tools can be used in HIV screening programs to identify patients that require intervention, improve access to HIV testing results, and ensure that negative patients are truly negative prior to initiating PrEP.

The CDC continues to strongly advocate for the most sensitive rapid HIV test (Bernard et al., 2014). An evaluation of four separate CDC studies showed that the OraQuick® ADVANCE Rapid HIV-1/2 Antibody test performed with a sensitivity and specificity >99% for HIV antibody in oral fluid specimens in diverse clinical and nonclinical settings (Delaney et al., 2006). Post-marketing surveillance of the rapid OraQuick® test in 368 testing sites affiliated with 17 state and city health departments concluded that oral fluid-based testing performed with a median specificity of 99.89% (99.44–100%) and positive predictive value of 90.00% (range: 50.00–100%) (Wesolowski et al., 2006).

There are several important limitations of OMT-based HIV testing. Because antibody levels are lower in oral fluids, rapid HIV tests using oral fluids are less sensitive when HIV-specific antibody levels are low, i.e., during the early stages of infection (CDC, 2008; Mortimer & Parry, 1991; Pant Pai et al., 2007). These limitations also apply to population-based studies, where OMT-based HIV diagnostic testing was shown to be less sensitive for the early detection of HIV antibodies compared to blood-based methods (Luo et al., 2013). Despite these limitations, a large body of evidence supports the utility of OMT-based testing in widespread screening for HIV infection, including within national-, state-, and community-level public health programs.

13.3 Utility of Oral Fluid in Population-Based Infectious Disease Epidemiology and Surveillance

Saliva can serve as a tool in population-based epidemiological surveillance to improve understanding of the epidemiology and natural history of exposure and infection. Pathogen-specific antibodies are unique biomarkers in their ability to identify both historical and recent exposure to a diversity of pathogens, including

bacteria, viruses, and parasites. Built into epidemiological research, serological methods provide an objective and sensitive method of elucidating population-level seroprevalence, identifying risk factors for exposure and transmission, and monitoring and evaluating preventative intervention strategies.

Blood-based serological methods have several limitations for surveillance and epidemiological studies that can often be mitigated by the use of oral fluid samples. First, the collection of venous blood is invasive and requires phlebotomists. Due to the ease of oral fluid collection, oral fluid-based serology facilitates self-collection and mail-in methods which can be advantageous in both field-based epidemiological studies (Wade et al., 2018) and in national-level population-based infectious disease screening programs (Morris-Cunnington, Edmunds, Miller, & Brown, 2004; Quoilin et al., 2007). Furthermore, self-testing can be reliable and accurate compared to testing performed by trained personnel (Figueroa et al., 2018). Because phlebotomists, transport for researchers and participants, and blood collection consumables are not needed, oral fluid-based testing can also have cost advantages (Morris-Cunnington et al., 2004). Second, blood samples must be centrifuged and frozen relatively quickly, which can be difficult in low-resource settings without electricity and laboratory infrastructure. Provided that oral fluid samples are buffered or stored properly, antibody integrity in oral fluid samples can be maintained for several days, prior to processing. Third, blood is a biohazardous material increasing risk to personnel and adding complexity to shipping. In the case of HIV-positive biospecimens, oral fluids are generally thought to be safer to handle than blood due to lower virus levels (Major et al., 1991). Fourth, social and cultural taboos, religion, and hesitancy toward blood collection can bias participation. Oral fluid-based methods may improve coverage and reach populations not reached by blood collection due to hesitancy or lack of personnel or facilities (Manikkavasagan, Bukasa, Brown, Cohen, & Ramsay, 2010; Wang et al., 2014). By increasing coverage, oral fluid-based methods can reduce case detection biases and improve understanding of the epidemiology of transmission and risk (Manikkavasagan et al., 2010; Wang et al., 2014). Acceptability of oral fluid-based testing is generally high (Krause, Subklew-Sehume, Kenyon, & Colebunders, 2013), often due to a preference for less painful methods that do not require blood collection (Nangendo et al., 2017). Oral fluids also uniquely allow for the study of the secretory or mucosal immune responses. This is especially advantageous for enteric diseases, where salivary IgA has been shown to serve as a noninvasive proxy for intestinal immune induction (Aase et al., 2016). Limitations of oral fluid-based methods compared to blood-based methods are discussed in a later section.

In this section we focus on three aspects of oral fluid-based methods in population-based epidemiological research: (1) development of oral fluid-based methods to evaluate infectious diseases in population-based epidemiological research, including critical parameters to address during each stage of the development process; (2) application of oral fluid-based methods in population-based cross-sectional and longitudinal epidemiological research; and (3) use of oral fluids for epidemiological studies and surveillance of vaccine preventable diseases (VPD).

13.3.1   Development of Oral Fluid-Based Multiplex EIA Tools Intended for Use in Epidemiological Research and Surveillance

While it may not be necessary to seek FDA approval for oral fluid-based methods for epidemiological research, a rigorous assessment of critical assay parameters must be addressed prior to implementing and drawing inference from oral fluid-based infectious disease diagnostic tools in population-based epidemiological studies. The development of oral fluid-based immunoassays can be simplified into two stages, optimization and validation. The US Environmental Protection Agency's (EPA) development of a multiplex EIA to detect salivary IgA and IgG antibodies against potential waterborne pathogens (Augustine et al., 2015; Augustine et al., 2016; Griffin, Chen, Fout, Wafe, & Egorov, 2011), and its subsequent application in population-based studies (Augustine et al., 2017; Griffin et al., 2015; Wade et al., 2018), provides an illustrative example of the rigorous upstream optimization and validation required of an oral fluid-based multiplex EIA intended for use in epidemiological research and surveillance.

During the optimization phase, critical parameters to address include optimizing coupling concentrations, identifying and mitigating protein–reagent interferences, and evaluating cross-reactivity. For the US EPA multiplex EIA for waterborne pathogens, reagent conditions, including pH, protein concentrations, and antibody concentrations, were first optimized for the coupling and confirmation of each unique pathogen-specific antigen to unique Luminex™ xMAP microsphere bead-sets (Augustine et al., 2015; Augustine et al., 2016; Griffin et al., 2011). A Design of Experiments (DOE) approach, a statistical method that can systematically identify factors affecting the output of a process, enabled the group to determine which antigens could be utilized in the multiplex with little to no cross-reactivity, and which ones to exclude from the multiplex EIA pending further studies (Augustine et al., 2015).

During the validation phase, critical parameters to address include establishing the sensitivity and specificity of the oral fluid-based method compared to a gold-standard diagnostic method and defining cutoff values to determine seropositivity and seroconversion. The optimized waterborne pathogen multiplex EIA was validated using matched serum and saliva samples from a longitudinal cohort (Griffin et al., 2011) and plasma samples from a cross-sectional cohort (Augustine et al., 2015) of diagnostically characterized individuals. Defining and validating cutoffs for oral fluid assays to assess seropositivity can be complex, depending on the distribution of immunological response data (discussed briefly in the next section). The US EPA employed finite mixed modeling, a statistical method that can be used to predict exposure subgroup distributions based on antibody levels, to establish cutoff values to differentiate positive and negative individuals in a population for each pathogen included in the optimized multiplex EIA (Augustine et al., 2015; Augustine et al., 2016).

Irrespective of FDA approval, this rigorous process of development, including optimization and validation, provided the proof-of-concept for implementing this oral fluid-based multiplex EIA into population-based studies to investigate swimming-related exposure to waterborne pathogens (discussed in the following sections). This example provides a framework for the development, optimization, and validation of other oral fluid-based multiplex EIAs intended for use in population-based epidemiological research and surveillance.

13.3.2   Application of Oral Fluid-Based Methods in Cross-Sectional Surveys to Estimate Seroprevalence

Most infections result in mild or asymptomatic disease presentation, meaning that surveillance based on clinical manifestation of symptomatic disease represents only the "tip of the iceberg" in terms of the true burden of exposure. Because antibody levels typically reflect historical exposure over a period of months to years, cross-sectional surveys of seroprevalence are rich with information about prior pathogen exposure (Arnold, Scobie, Priest, & Lammie, 2018). Furthermore, multiplex assays create an opportunity to efficiently expand resources beyond single-disease testing (Arnold et al., 2018). Integrating multiplexed serosurveillance using oral fluid samples into field-based cross-sectional surveys could enable an unprecedented evaluation of multi-pathogen seroprevalence and improve estimates on burden of disease and exposure.

The utility of rigorously optimized and validated oral fluid-based multiplex immunoassays in field-based cross-sectional surveys of high-risk populations can be illustrated through the US EPA's use of an oral fluid-based multiplex EIA for waterborne pathogens in an epidemiological study of visitors of Boqueron Beach, Puerto Rico (Augustine et al., 2017). The identification of waterborne pathogen seroprevalence is a necessary first step in linking acute gastrointestinal illness (AGI) to contaminated water, identifying waterborne pathogens associated with AGI, and assessing and managing contaminated water associated with AGI risks in humans (Exum et al., 2016). While blood-based methods can provide these measures, in this study oral fluid-based methods allowed for field-based sample collection in a low-resource setting. Oral fluid samples were collected at the beach site for all participants and probed for pathogen-specific IgG antibodies against *Campylobacter jejuni* (*C. jejuni*), *Helicobacter pylori* (*H. pylori*), *Toxoplasma gondii* (*T. gondii*), hepatitis A virus (HAV), and noroviruses (NoV) GI.1 and GII.4 at a single time point. The study revealed that more than two-thirds of beachgoers were previously infected by at least one of the included waterborne pathogens (Augustine et al., 2017). Over 60% had evidence of prior exposure to NoV, while over 20% had evidence of previous exposure to *H. pylori* and HAV (Augustine et al., 2017). Antibodies against *T. gondii* and *C. jejuni* were less common (Augustine et al., 2017). Interestingly, many of the beachgoers had evidence of detectable antibodies

to two or more pathogen-specific antigens, demonstrating an advantage of simulta-neously multiplexing for multiple pathogens (Augustine et al., 2017).

Immunoassay data from cross-sectional studies can be difficult to interpret due to the wide range of antibody reactivity, including among recently infected, historically infected, previously vaccinated, or unexposed individuals. This complicates the critical step of establishing a cutoff value for discriminating positive and negative individuals in a population-based cross-sectional survey (Augustine et al., 2015). In some cases, cross-sectional studies may allow for cutoff values to be defined for classification of seropositivity. A cutoff can easily be established in the case of a bimodal distribution of serological response data. Data that does not display a bimodal distribution may be more difficult to interpret. Longitudinal studies allow for cutoff values to be defined for classification of seroconversion (i.e., incident infection). Cutoff values for saliva-based serological surveys should be decided upon based on the research question or goal of the cross-sectional survey. For example, less stringent criteria may be applied for population-based screening of saliva samples requiring confirmatory clinical diagnostic testing. In the case of the US EPA study, where the immunological status of subjects was not known or the data were not normally distributed, more stringent criteria may be applied to reduce false positives (Augustine et al., 2017). A conservative cutoff, however, has the limitation of increasing false negatives. One strategy is to employ multiple cutoff values to examine the effects of conservative and liberal cutoff values on the interpretation of results from cross-sectional seroprevalence studies.

13.3.3 Application of Oral Fluid-Based Methods in Longitudinal Studies to Estimate Seroconversion and Incidence of Infection

Integrated multi-pathogen serosurveillance in longitudinal study designs can facili-tate the detection of seroconversion to inform estimates of incident infection in population-based settings. In a time series of two or more saliva samples, a change from an antibody-negative to an antibody-positive sample, or a fourfold increase in pathogen-specific antibody titer between samples, can be employed to measure incident, acute cases of infection or exposure in a defined population over a defined period of time (Exum et al., 2016). Built into longitudinal studies, oral fluid-based methods can inform the incidence of infections, identify risk factors for transmission, and serve as tools in monitoring and evaluating population-based prevention strategies.

The US EPA's application of an optimized multiplex EIA for the detection of salivary IgA and IgG against two NoV genotypes (GI.1 and GII.4) in an NoV challenge trial, and subsequently in a longitudinal population-based study provides a example of the value of oral fluid-based serological methods in longitudinal epidemiological studies of infectious disease (Griffin et al., 2015; Wade et al.,

2018). Because NoV-specific antibodies typically increase after NoV infection (Graham et al., 1994), seroconversion can be used as a serological biomarker of recent infection. To first determine whether NoV-specific IgA and IgG seroconversion could be detected in oral fluids, the optimized NoV EIA was applied to pre- and post-challenge oral fluid samples collected from participants of an NoV (GI.1) challenge study (Griffin et al., 2015). Using a fourfold increase in salivary antibody response as a threshold for seroconversion, NoV-specific IgG in saliva correctly identified all infected and noninfected individuals in the challenge study (Griffin et al., 2015). NoV-specific IgG tests had higher sensitivity than IgA, which displayed weaker fold increases associated with NoV challenge. While participants in a challenge study may not be representative of the general population, these results suggested that IgG antibody responses in oral fluids can be used to detect seroconversion to NoV infection in longitudinal population-based studies.

Subsequently, the optimized multiplex EIA was employed in a field-based longitudinal cohort of asymptomatic beachgoers in Puerto Rico to evaluate the incidence of NoV infection, the risk of NoV transmission, and swimming-related risk factors of NoV infection associated with contaminated recreational waterbodies (Wade et al., 2018). Measuring NoV GI.1- and GII.4-specific IgG levels in a time series of three oral fluid samples the US EPA study determined that 2.6% of asymptomatic participants seroconverted to the two NoV genotypes within 3 weeks of the initial beach visit (Wade et al., 2018). Risk factor analysis revealed that seroconversion rates were approximately five times higher among beachgoers who immersed their heads in beach water compared to non-swimmers and swimmers who did not immerse their heads in the water (Wade et al., 2018). Interestingly, nearly all seroconversion events were unaccompanied by AGI symptoms, supporting an important role for oral fluid-based serological methods in identifying asymptomatic source populations, where clinical symptom presentation would not appropriately capture the incidence of infection (Wade et al., 2018). While a similar inference could have potentially been drawn from blood-based methods, longitudinal blood collection may not have been feasible in this field-based study. By providing beachgoers oral fluid collection kits following the initial beach visit, oral fluid-based self-collection and mail-in testing enabled the collection of longitudinal samples in this field-based study, allowing for the evaluation of incident NoV infection and waterborne transmission of NoV associated with a recreational waterbody.

A separate multiplex EIA developed by investigators at Johns Hopkins Bloomberg School of Public Health was aimed at measuring IgG responses against virus like particles (VLP) of five common NoV genotypes (GI.1, GII.2, GII.4, GII.6, and GII.17) in oral fluid collected from children in an endemic setting (Pisanic et al., 2018). Using a longitudinal cohort of children, under the age of 5 years living in Peru, the study revealed that NoV infections elicit a genotype-specific IgG response that can be measured in oral fluids and correctly determined recent, PCR confirmed, NoV infections (Pisanic et al., 2018). Compared to PCR-diagnosed NoV infection, the oral fluid-based method performed with a sensitivity of 71% and specificity of 96% across the evaluated genotypes (Pisanic et al., 2018). An important observation

in this study was that the median NoV-specific IgG signal in oral fluid was similar among children with or without an NoV positive stool sample, suggesting that most children in this population had been exposed to NoV in the past (Pisanic et al., 2018). Thus, in highly endemic settings it may not be feasible to apply anti-NoV IgG signal cutoff values to discriminate seropositive from seronegative individuals based on a single time point. Although it may be possible to develop a salivary NoV assay to measure the acute phase IgA response at a single time point, without the need for repeat sampling, additional studies are needed.

13.3.4   Oral Fluid-Based Serological Methods Facilitate Self-Collection in Population-Based Postal Surveys for Infectious Disease

Understanding the proportion of a population that is immune or has experienced prior infection with certain pathogens has important epidemiologic applications, including identifying susceptible subpopulations, and monitoring and evaluating preventative intervention strategies (e.g., vaccination programs) (Morris-Cunnington et al., 2004). Blood-based serological surveillance is an accurate method for monitoring population immunity but presents limitations for widespread use in population-based settings as previously described. Oral fluid-based serosurveillance provides tremendous value in population-based surveillance of infectious disease at the national, state, and community level by enabling self-collection (Egorov et al., 2010; Morris-Cunnington et al., 2004; Quoilin et al., 2007), reducing costs (Morris-Cunnington et al., 2004), and potentially increasing response rates compared to blood-based methods.

Pathogen-specific oral fluid-based self-collection and serological testing have been successfully employed in population-based postal surveys of infectious disease screening at the national level, such as HAV in England and Wales (Morris-Cunnington et al., 2004) and HAV, hepatitis B virus (HBV) and hepatitis C virus (HCV) in Belgium (Quoilin et al., 2007), and at the community level, such as *Cryptosporidium hominis* (*C. hominis*) in the USA (Egorov et al., 2010). The HAV study in England and Wales demonstrated three major findings regarding the implementation of oral fluid-based national-level HAV surveillance: (1) oral fluid-based self-collection and serology was logistically feasible; (2) HAV-specific IgG provided accurate estimates of population-level exposure compared to previous population-based studies using blood-based methods; and (3) coupled with extensive demographic and social data, oral fluid-based methods were able to uncover unexpected epidemiological associations with age, ethnicity, and socioeconomic status (Morris-Cunnington et al., 2004). The authors also noted a cost advantage for oral fluid-based methods compared to blood-based methods. Because specially trained personnel were not employed to collect serum sample, no costs were incurred for salaries for trained personnel or for the transport of researchers or participants. In

terms of equipment required for specimen collection, the authors estimated $1.14 per self-collected oral fluid sample compared to $4.00 per blood sample (Morris-Cunnington et al., 2004). Given the success of this program, the Immunisation, Hepatitis and Blood Safety Department of Public Health United Kingdom created and implemented a nationwide hepatitis A outbreak investigation program ("Hepatitis A: Oral Fluid Testing for Household Contacts," 2015). Population-based postal surveillance for HAV, HBV, and HCV in Belgium has also shown value in estimating national-level disease burden and is addressed in the next section where VPD disease surveillance to estimate disease burden is discussed. At the community-level, a study in the USA demonstrated the utility of oral fluid-based self-collection in a longitudinal postal survey of community-acquired *C. hominis* cases (Egorov et al., 2010), but additional studies are needed before incorporating oral fluid-based testing for diarrheal disease into population-based screening programs. Taken together, oral fluid-based self-collection methods have the potential to be incorporated into national- and community- level public health programs as an accurate, inexpensive, and logistically feasible alternative to blood-based serological methods.

13.3.5 Uses of Oral Fluid for VPD Epidemiologic Studies and Surveillance

The acceptability and ease of oral fluid self-collection has facilitated a unique role in the surveillance of vaccine preventable diseases (VPDs). This section reviews uses of oral fluid testing for VPDs, which are defined as all infectious diseases with a licensed vaccine.

Although there are no FDA approved or CLIA-waived oral fluid-based diagnostic tests for VPDs, many studies have reported the development, adaptation, or optimization of commercial or noncommercial assays for use with oral fluid. Diagnostic accuracy studies of oral fluid-based antibody detection have been published on the following VPDs with variable sensitivity and specificity: cholera, dengue, Ebola, hepatitis A, hepatitis B, hepatitis E, Haemophilus influenzae type B (Hib) disease, human papillomavirus (HPV), influenza, measles, meningococcal disease, mumps, pertussis, pneumococcal disease, polio, rubella, tetanus, typhoid, and varicella (Hayford et al., under review; Holroyd et al., under review; Lambe et al., 2016; Pisanic et al., 2017). In addition, POC lateral flow devices for measles and mumps antibody detection using oral fluid are in development (Warrener et al., 2011; Warrener, Slibinskas, Brown, Sasnauskas, & Samuel, 2010).

Despite generally lower diagnostic accuracy for VPDs, oral fluid-based testing is increasingly recognized as an alternative or complementary tool to assess disease burden, exposure to and protection against many VPDs at the population level in order to inform disease control strategies and guide immunization programs. VPD serological surveillance is used primarily in three ways: (1) disease surveillance and

enhanced case detection, (2) assessing population immunity and identifying immunity gaps, and (3) assessing the impact of vaccination campaigns and routine immunization.

13.3.5.1   VPD Disease Surveillance to Estimate Disease Burden and Improve Case Detection

For VPDs, oral fluid testing has been used primarily to: (1) estimate disease burden at the population level or (2) confirm infection among suspected cases. Specific IgM detection is most commonly used for acute disease surveillance but detection of specific IgG, pathogen antigens, or multiple markers may be required for case detection for some VPDs such as hepatitis B or pertussis.

Estimating the burden of VPDs is a core function of public health systems and important for informing disease control strategies and preventing outbreaks. Measuring the prevalence of VPDs can be complicated due to nonspecific clinical case definitions, "tip of the iceberg" case detection, and challenges of conducting high-quality and unbiased representative surveys with biomarker collection (MacNeil, Lee, & Dietz, 2014). Large-scale oral fluid surveys have been used to estimate past exposure and burden of hepatitis A in England and Wales (described above) (Morris-Cunnington et al., 2004) and hepatitis A, B, and C in Flanders, Belgium (Quoilin et al., 2007). Results from the Belgium cross-sectional oral fluid IgG survey in 2003 were compared to a representative serosurvey 10 years earlier. Substantial declines in IgG seroprevalence suggested the burden of hepatitis A and hepatitis C was substantially lower than estimated in 1993. The prevalence of hepatitis B surface antigen (HBsAg), an indicator of active infection, remained stable and below 1% over the 10 year period, indicating that incident infections had not increased. Results affirmed the impact of routine hepatitis B vaccination, which was introduced in 1999 for infants and 12-year-old children, on disease burden. Like other studies discussed above, the study in Belgium showed that population-based surveys with self-collection of oral fluid are acceptable and feasible although achieving high response rates remains a challenge. Other studies have estimated the burden of hepatitis B and C and infections using oral fluid in settings with high disease transmission risk, such as prisons (Weild et al., 2000).

Oral fluid-based testing has also been used to improve measles, mumps, rubella, and pertussis case detection during outbreak investigations and characterize outbreak epidemiology in order to better guide disease control and immunization strategies (Campbell et al., 2014; Manikkavasagan et al., 2010; Ramsay et al., 2003; Reid et al., 2008; Thieme, Piacentini, Davidson, & Steingart, 1994). The public health agency in England and Wales, Public Health England (PHE, formerly HPA) has tested self-collected oral fluid samples from suspected measles, mumps, and rubella cases since 1994 and for pertussis since 2013 as an additional surveillance tool to identify laboratory-confirmed cases (Campbell et al., 2014; Manikkavasagan et al., 2010; Ramsay et al., 2003; Thieme et al., 1994). When a suspected case is identified by a health practitioner, PHE ships an oral fluid

collection kit to the primary care physician or patient. Oral fluid samples are self- or physician-collected with an Oracol swab and returned in the mail at ambient temperature to PHE for testing. The addition of oral fluid-based testing began, in part, to improve case detection as the country moved closer toward elimination of these diseases. The clinical case definition for measles, rubella, and pertussis had poor sensitivity and specificity, which was worsened as disease burden continued to decline. In addition, PHE suspected laboratory confirmation with serum did not identify all cases due to the invasiveness and challenges of blood collection. For all four diseases, oral fluid testing both substantially improved case detection and revealed new information about the epidemiology of outbreaks. For rubella, the incidence of rubella cases increased from 0.5 to 0.77 per 1,000,000 population when oral fluid testing was added. The increase was attributed to improved case ascertainment, particularly among children and adult men, and helped the UK assess progress toward rubella elimination goals. Oral fluid-based surveillance also revealed that the clinical case definition overestimated rubella incidence, especially in children (Manikkavasagan et al., 2010). For measles surveillance, IgM-based oral fluid testing improved the diagnostic accuracy of clinical case detection and significantly increased case detection rates. 56% of confirmed measles cases were identified by an oral fluid sample alone, suggesting blood-based surveillance failed to detect all cases and therefore underestimated the size of outbreaks. For pertussis, case detection with oral fluid was rolled out in England and Wales in 2013 and expanded in 2018 following the same model as measles and rubella outbreak surveillance. Pertussis antitoxin IgG surveillance in oral fluid also improved case detection, particularly among nonhospitalized and milder cases. The age distribution of cases detected in oral fluid revealed a high burden in young infants and that transmission occurred primarily within the household from the mother to infant (Kara et al., 2017; Wang et al., 2014). These findings informed the decision by PHE to vaccinate pregnant women during the 2012 pertussis outbreak.

Another advantage of serological sampling during outbreaks is that DNA or RNA can be detected to improve case detection and genotyped from positive samples to characterize the pathogen strain. Like serum or dried blood spots, genotyping is routinely conducted on oral fluid swabs to identify measles, mumps, and rubella (Abernathy et al., 2009; Chibo, Riddell, Catton, & Birch, 2005; Jin, Brown, Litton, & White, 2004; Jin, Vyse, & Brown, 2002; Ramsay et al., 2003; Reid et al., 2008). Adding nucleic acid testing to serology can improve case detection, particularly if samples are collected quickly after onset of infection prior to a detectable increase of specific IgM in oral fluid. A study of suspected rubella cases in Peru found that IgM serology and viral RNA detection from oral fluid samples detected more cases than blood-based serology alone (Abernathy et al., 2009). After the mass measles vaccination campaign in the UK in 1994, IgM surveillance and viral genotyping with oral fluid and serum samples revealed that the campaign had successfully interrupted endemic measles transmission and new outbreaks were caused primarily by imported cases (Ramsay, Brugha, & Brown, 1997; Ramsay et al., 2003).

13.3.5.2 Estimating Population Immunity and Identifying Immunity Gaps to VPDs

Estimating the proportion of a population exposed to or immune to VPDs enables public health agencies to target disease control and immunization strategies. Because measles, rubella, varicella and, to a lesser degree, mumps viruses are antigenically stable and confer lifelong immunity from natural infection or vaccination, they are good pathogens to assess previous exposure and population immunity across all age groups. For other VPDs like tetanus or hepatitis B where antibody levels wane below detectable levels over time, antibody surveillance can be used among limited age groups or shortly after exposure or vaccination. Many oral fluid-based IgG antibody serosurveys have been conducted to estimate population immunity at national and subnational levels throughout Europe, North America, Africa, and South Asia for tetanus (Tapia et al., 2006), rubella (Ben Salah et al., 2003; Nokes et al., 2001; Thieme et al., 1994), measles (Goyal, Shaikh, Kinikar, & Wairakgar, 2009; Hayford et al., 2013; Kremer & Muller, 2005; Nigatu et al., 2008; Ohuma et al., 2009; Sheikh et al., 2011; Thieme et al., 1994; Vainio et al., 2006), and hepatitis B (Nokes et al., 2001; O'Connell et al., 2000; Simani et al., 2008). A nationally representative postal survey in Ireland revealed exposure to hepatitis B virus was very low based on 0.51% prevalence of antibodies to hepatitis B core antigen in oral fluid (O'Connell et al., 2000). In rural Ethiopia, measles and rubella IgG testing in oral fluid had high sensitivity and specificity and demonstrated that antibody surveillance with oral fluid is feasible and accurate when protocols for collection and testing are rigorous (Nokes et al., 2001). For other VPDs like mumps and HPV, oral fluid testing was not adequately sensitive to accurately estimate population immunity (Buchinsky et al., 2006; Passmore et al., 2007; Rowhani-Rahbar et al., 2009; Thieme et al., 1994; Vainio et al., 2006).

Although the majority of VPD seroprevalence studies are community-based surveys, nonrepresentative study designs have been used to assess population immunity and identify immunity gaps in high-risk settings such as prisons and refugee settlements (Gill, Aston, Vyse, White, & Greenwood, 2002). Oral fluid testing among refugees and asylum seekers in Luxembourg revealed low levels of immunity to measles, mumps, and rubella and guided subsequent vaccination policy (Hübschen, Charpentier, Weicherding, & Muller, 2018). Identifying acceptable and feasible tools like oral fluid sampling to assess population immunity to outbreak prone VPDs is a priority in refugee camps due to high density, elevated disease risk, and the lack of medical records and vaccination history for many displaced people.

13.3.5.3 Assessing Impact of Vaccination Campaigns and Routine Immunization

Vaccination campaigns are conducted to significantly increase population immunity and interrupt disease transmission but they can be expensive and logistically

challenging to roll out. Oral fluid testing enables governments and funders to assess the impact of the campaigns without drawing blood. For example, pre- and post-campaign cross-sectional oral fluid serosurveys were conducted in Ethiopia and Kenya to assess the impact of measles vaccination campaign on population immunity (Nigatu et al., 2008; Ohuma et al., 2009). In Ethiopia, overall seroprevalence increased by 39% but results revealed immunity gaps among children above the target age of the campaign. Age-specific seroprevalence results in the pre-campaign period could have been used to justify increasing the campaign age range to maximize impact. If it is not feasible to collect samples before the campaign, oral fluid studies from the post-campaign period only can provide information on population immunity and remaining immunity gaps. A cross-sectional rubella serosurvey with oral fluid found that 95% of children were seropositive 4 months after a national vaccination campaign (de Azevedo Neto et al., 1995). In contrast, a cross-sectional oral fluid serosurvey of HIV and measles IgG antibodies in Lusaka, Zambia, found that measles population immunity was insufficient to interrupt measles transmission 3 years after a measles vaccination campaign (Lowther et al., 2009). Lastly, to evaluate the impact of a 20-year policy to selectively vaccinate injection drug users (IDUs) in England, an oral fluid survey found vaccination was associated with lower rates of hepatitis B and a continuation of the policy was recommended (Judd et al., 2003). Longitudinal studies with oral fluid collection have demonstrated high seroconversion rates after measles vaccination in Mozambique, Norway, and the USA (Jani et al., 2008; Thieme et al., 1994; Vainio et al., 2006). However, most VPD immunogenicity studies are still conducted with blood.

Although many studies have attempted to validate vaccination history with oral fluid serology, this body of research is often conflicting and can be difficult to interpret, in large part due to expected discrepancies in vaccination status and serostatus caused by primary and secondary vaccine failure, circulation of natural infection, as well as lower diagnostic accuracy of oral fluid assays (Hayford et al., 2013; Jenkins et al., 2009; MacNeil et al., 2014).

13.4 Limitations of Oral Fluid-Based Serological Methods Compared to Blood-Based Methods

Oral fluid-based serological methods have several limitations compared to blood-based methods. First, the diagnostic accuracy of oral fluid-based serology is typically lower than that of blood-based methods but depends on the antigen, assay type, and quality of the collection and testing procedures. Because antibody levels in oral fluid are typically lower than levels observed in sera or plasma, the sensitivity of oral fluid-based testing may be impaired. For example, in studies comparing rubella or measles IgG in serum and oral fluid, many participants with low antibody levels in serum were systematically misclassified as negative by oral fluid (Hayford, Al-Emran, et al., 2013; Helfand et al., 1999; Nokes et al., 2001; Vainio et al.,

2006). In addition, variability in antibody levels in oral fluid related to, for example, time of collection, sample contamination, or hydration and health of participant can increase non-systematic error in oral fluid results. The implications of systematic and non-systematic errors are most significant when a quantitative result is needed or when levels are near the cutoff resulting in qualitative misclassifications. Thus, populations with bimodal antibody distributions of clear positives and negatives are best suited for use with oral fluid testing. Although some reductions in sensitivity can be mitigated by adjusting cutoff values, the tradeoff with specificity must be weighed against the scientific goal. Second, there is currently a lack of standardization in oral fluid collection, storage, and processing methods used across studies and settings. One review of oral fluid-based serological testing for VPDs reported that a majority of studies did not provide details on the collection, transport, storage, or volume of oral fluid samples (Holroyd et al., under review), complicating the ability to compare between studies. Each of these technical considerations is critical for maintaining sample integrity and ensuring high-quality results. Although oral fluid antibodies are not rapidly degraded by enzymes, cold-chain during transport and buffering can reduce degradation during long-term storage. Third, oral fluid antibody levels are highly variable. Along with differences in collection and processing, factors contributing to the variability in IgA levels in oral fluids include age, geography, time of day, and secretion flow rate (Brandtzaeg, 2013). Various stressors and diseases have also been reported to influence oral fluid antibody levels in different ways (Brandtzaeg, 2013). Fourth, similar to blood collection, oral fluid collection is difficult in young children, often resulting in low volumes of diluted saliva. Oral fluid collection methods for young children need to be optimized for size, taste, and texture, while maintaining the ability to recover adequate volumes of oral fluid and the intended analytes. Safety is an especially important issue in young children, due to the risk of choking.

13.5   Future Directions

As we look forward in the field, oral fluid-based methods have the power to not only support individual patient diagnostic testing but also broader applications in population-based epidemiological research. Future directions for oral fluid-based serology include expanding the pathogen panel to comprise emerging infectious diseases of critical priority, developing rapid testing formats such as POC tests, and applying both established and new oral fluid-based EIAs in different clinical and population-based settings. In this section we outline future directions in oral fluid-based serology of infectious diseases that could be of high impact including identifying pathogens for which oral fluid-based detection methods are urgently needed, and new settings where we see the fruitful application of oral fluid-based methods.

13.5.1 Oral Fluid-Based Point-of-Care Diagnostic Tools for Infectious Diseases

It is now well established that oral fluid-based infectious disease diagnostic POC tools add tremendous value in clinical settings, epidemiological research, and national health programs. This has been clearly demonstrated in the case of HIV, described previously. Oral fluid-based POC tools for measles diagnosis are currently being evaluated (Warrener et al., 2010), and in the context of VPDs will provide unprecedented ability to epidemiologically characterize population immunity, identify susceptible populations, and inform vaccination strategies. As more oral fluid-based infectious disease diagnostic tools are advanced into POC development, several critical challenges must be addressed to successfully support this technological transition while maintaining diagnostic accuracy. Three major challenges are: (1) oral fluid matrix effects, (2) interindividual variability in oral fluid composition, and (3) lateral flow platform optimization. Each of these challenges will require methodological advances to develop, optimize, and apply POC infectious disease diagnostic tools in clinical and population-based epidemiological settings.

Oral fluid is a complex matrix, composed of a variety of macromolecules, and exogenous substances such as food particles, many of which can interfere with antibody reactivity in oral fluid-based immunoassays. In laboratory settings, oral fluid samples can be centrifuged to clarify the sample, and diluted to minimize inhibitors of antibody reactivity, prior to application in an ELISA or EIA format. Such laboratory techniques may not be afforded in the case of oral fluid-based POC infectious disease diagnostic tools, intended for rapid identification of infectious diseases in field-based settings. Field-based methods to process oral fluid are urgently needed to support oral fluid-based POC infectious disease diagnostic development and application. One solution may include the addition of portable centrifuge devices to the workflow but this would introduce additional costs and time in the POC protocol. Another solution may involve the use of nanoparticles that can target and concentrate the intended analytes in the oral fluid sample, prior to processing.

While methodological advances are necessary to optimize diagnostic accuracy, oral fluid-based POC infectious disease diagnostic tools are urgently needed in several VPD-related applications. In this section, we identify two examples where oral fluid-based POC tools are needed to inform vaccination decision-making and VPD outbreak investigation.

13.5.1.1 The Role of Oral Fluid-Based Companion Diagnostic Tools to Inform Vaccination Decision-Making

An important takeaway from Sanofi-Pasteur's dengue virus (DENV) vaccine clinical trials was that seronegative vaccine recipients had a higher risk of severe dengue and hospitalization than vaccine recipients who had been previously exposed to DENV

(Aguiar, Stollenwerk, & Halstead, 2016). Secondary infections with a heterologous DENV serotype can lead to hemorrhagic fever or dengue shock syndrome (severe dengue) (Durbin, 2016; Halstead, 2015). As a result, understanding DENV serostatus of any individual prior to receiving a DENV vaccination is critical in endemic regions and for travelers returning from endemic regions. Oral fluid-based diagnostic tests that can reveal an individual's DENV serostatus prior to vaccination could inform vaccination decision-making and avert vaccine-mediated adverse events. For the case of DENV vaccination, rapid POC tools will be needed to support real-time, individual-level, decision-making in both field-based and clinical vaccination settings.

Multiple longitudinal studies, conducted in different patient populations and settings, provide strong support for the utility of oral fluids in discriminating primary and secondary DENV infection. While three separate longitudinal studies demonstrated that oral fluid-based methods for the detection of DENV-specific IgM and IgG were less sensitive than blood-based methods, all of the studies determined that DENV-specific IgG in oral fluids could potentially serve as a biomarker to discriminate primary and secondary infection (Andries et al., 2015; Balmaseda et al., 2008; Vázquez et al., 2007). Considering the fatal outcomes of heterologous DENV infection and the recent licensure of Dengvaxia®, rigorous, FDA approved, oral fluid-based serological methods to determine DENV serostatus prior to vaccine administration are urgently needed. Moreover, oral fluid-based DENV diagnostic testing will need to be developed into rapid POC tools to be effective in field-based applications.

Similarly, oral fluid-based epidemiological tools will be critical in guiding vaccination strategy in endemic regions by informing population-level seroprevalence of DENV exposure and infection. Because of the special considerations around secondary DENV infection, the World Health Organization (WHO) has made the recommendation that the vaccine only be used in endemic areas defined by high seroprevalence (World Health Organization (WHO), 2016). Seroprevalence thresholds are considered the best approach to identify target populations for vaccination (World Health Organization (WHO), 2017). While Dengvaxia® has not been implemented in any countrywide programs to date, its recent licensure demands noninvasive methods for population-based screening to target populations for vaccination.

13.5.1.2 The Role of Oral Fluid-Based POC Tools for VPD Outbreak Investigation

As countries move toward elimination of polio, measles, rubella, and hepatitis B, public health systems need rapid and nimble strategies to confirm suspected cases and identify susceptible populations, both of which can be done with oral fluid-based testing (Ramsay, Brugha, Brown, Cohen, & Miller, 1998). Serological confirmation is needed because clinical case detection becomes less sensitive and specific as

incidence declines but the challenges of blood collection and testing can undermine the intended goals of a comprehensive and nimble case detection system.

The WHO Global Measles and Rubella Laboratory network representing thousands of laboratories in 191 countries has played a crucial role in confirming suspected measles and rubella cases and characterizing viral genotypes and epidemiologic patterns of outbreaks. However, laboratory confirmation with blood samples consistently underestimates the number and size of measles and rubella outbreaks, especially in hard-to-reach, remote, and under-resourced settings (World Health Organization (WHO), 2019). An oral fluid-based POC test for measles and rubella IgM would overcome many of these challenges and potentially improve case detection rate and turnaround time for case confirmation. A POC lateral flow device for measles IgM antibodies had 90% sensitivity and 96% specificity in preliminary studies and 75% and 96% in a field study in Zimbabwe, respectively. Further refinements and field testing of the POC test are needed (Shonhai et al., 2015; Warrener et al., 2011).

Improved assays for VPD-specific IgG detection in oral fluid are also needed to enhance epidemiological surveillance. If diagnostic accuracy can be improved and the impact of variability in specimen quality can be minimized, an oral fluid test on a lab-based or POC device would be an appealing and accessible tool for estimating population immunity to VPDs. Improvements in diagnostic accuracy observed with bead-based or microfluidic assays for other infectious diseases need to be explored for measles, rubella, pertussis, and other VPDs.

13.5.2 Improving Estimates of Asymptomatic Exposure and Infection in Endemic Regions

Understanding asymptomatic exposure and infection is critical for numerous infectious diseases. For example, malaria parasitemia is commonly missed or underreported in passive symptom-based case detection surveillance. It is well established that undetected, clinically immune, individuals serve as reservoirs of ongoing malaria transmission in hypoendemic regions (Bousema, Okell, Felger, & Drakeley, 2014). Because malaria-specific antibodies can persist for months to years after infection, they may have utility as proxy measures of malaria transmission in low-transmission settings (Drakeley et al., 2005). Previous studies have shown that age-specific anti-malaria antibody seroconversion rates are an effective tool to assess endemicity and public health burden of malaria (Corran, Coleman, Riley, & Drakeley, 2007; Kobayashi et al., 2019; Williams et al., 2009). Blood samples are typically used for serological estimation but present challenges in communities with blood-related taboos. Thus, the strategic eradication of malaria in low-transmission regions demands the development and widespread application of noninvasive, low-resource friendly, screening tools for asymptomatic malaria reservoirs.

Two studies have provided a proof-of-concept for the utility of oral fluid in detecting *Plasmodium falciparum* (*P. falciparum*)-specific antibodies in

epidemiological studies in Gambia, Tanzania, and Zambia. (Chidi et al., 2011; Estévez et al., 2011). The Gambia and Tanzania study demonstrated that anti-malarial IgG antibodies against *P. falciparium* antigens merozoite surface protein-1 (MSP-1$_{19}$) and apical membrane antigen (AMA-1) could be detected in oral fluid and correlated strongly with levels in plasma (Estévez et al., 2011). A separate cross-sectional study in Tanzania observed a statistically significant correlation between IgG antibody levels to whole, asexual stage *P. falciparium* antigens in oral fluids and dried blood spots (DBS) (Chidi et al., 2011). In this study, the oral fluid-based assay performed with sensitivity and specificity of 100%, correctly identifying all DBS confirmed positive and negative samples (Chidi et al., 2011).

Two limitations were apparent from these studies. First, IgG antibody titers to *P. falciparum* antigens were significantly lower in oral fluid compared to blood samples. Second, both studies noted a difficulty in assessing whether an adequate sample was collected and a need for standardization in quantity and quality during collection. One potential strategy to evaluate improper collection of oral fluid samples is to test for total IgG concentration. Nevertheless, the results of this study open a pathway for more extensive studies to assess the utility of oral fluid in the detection of antimalarial antibodies, which could be particularly useful for population-based surveys in malaria hypoendemic regions. Further research could also help determine if antimalarial antibodies in oral fluid could be extended to study exposure to other malaria species, such as *Plasmodium vivax*.

13.5.3 Distinguishing Between Secretory IgA and IgA in Population-Based Studies of Enteric Disease

A hallmark of the mucosal immune system is epithelial export of locally produced IgA polymers (mainly dimers) that are transported to the lumen as secretory IgA (SIgA) antibodies (Brandtzaeg, 2009). Pathogen-specific SIgA provides critical immune functions against enteric pathogens at mucosal sites (Brandtzaeg, 2009). Salivary IgA has demonstrated potential as a noninvasive proxy for intestinal immune induction by enteric pathogens (Brandtzaeg, 2007). While pathogen-specific salivary IgA biomarkers have been developed and validated in patient populations for multiple enteric pathogens, including *Campylobacter* spp., *Giardia* spp., *Escherichia coli* O157 (*E. coli* O157), *Salmonella* serotype *Typhii*, *Shigella* spp., *Vibrio cholerae*, Norovirus, and Rotavirus, only a few studies have implemented saliva-based IgA detection into population-based epidemiological studies of enteric disease.

Cross-sectional studies of children with microscopically confirmed *Giardia duodenalis* (*G. duodenalis*) infections have shown that *G. duodenalis*-specific IgA is elevated in the saliva of parasitized children compared to their non-parasitized counterparts (El-Gebaly, Halawa, Moussa, Rabia, & Abu-Zekry, 2012; Rodríguez et al., 2004). While the sensitivity of the *G. duodenalis*-specific IgA ELISAs were

not ideal compared to stool-based microscopic diagnosis, longitudinal studies, incorporating sequential saliva sampling, have shown improved sensitivity in the detection of infection with other enteric pathogens. In a longitudinal study of children with culture confirmed *E. coli* O157 associated hemolytic uremic syndrome (infectious HUS), a saliva-based *E. coli* O157 LPS-specific ELISA performed with a sensitivity of 92% for IgA and 100% for IgM compared to blood-based serology when both initial and follow-up saliva specimens were included in the analysis (Ludwig et al., 2002)—far greater than that observed in cross-sectional studies of children with infectious HUS (Chart & Jenkins, 1998; Chart, Perry, Willshaw, & Cheasty, 2003). Longitudinal studies of children with clinically confirmed typhoid have evaluated *Salmonella* serotype *Typhii* LPS-specific salivary IgA for early diagnosis typhoid infection and determined that diagnostic accuracy is greatest during the second and third weeks of illness (Herath, 2003; Zaka-ur-Rab, Abqari, Shahab, Islam, & Shukla, 2012), reaching a sensitivity of 100% during the second and third week of illness and dropping to 0% by the fifth week (Zaka-ur-Rab et al., 2012) Although limited by a small sample size, longitudinal studies of Shigellosis and Campylobacteriosis have also demonstrated that *Shigella-* and *C. jejuni*-specific IgA are elevated in saliva of clinically confirmed patients compared to healthy controls (Cawthraw, Feldman, Sayers, & Newell, 2002; Schultsz, Qadri, Hossain, Ahmed, & Ciznar, 1992), providing a proof-of-concept for the further development and application of these pathogen-specific saliva-based tools in epidemiological studies. Due to the similar clinical presentation of enteric infections, multiplexing for enteric pathogens would provide additional value in identifying etiologic agents and risk factors associated with enteric disease in population-based studies.

Although studies show promise in the utility of SIgA in epidemiological studies of enteric disease, saliva-based SIgA detection has two important limitations. First, it is challenging to distinguish between polymeric SIgA containing a secretory component (SC) produced locally in the mucosa vs. monomeric IgA devoid of an SC circulating in whole blood. Only one of the studies of enteric disease mentioned above specified a SIgA signal in their ELISA methodology, by using a detect antibody against the IgA SC (Rodríguez et al., 2004). The remaining studies did not discriminate between mucosally produced SIgA and IgA that is devoid of SC, thus conflating the SIgA signal with IgA. The ability to distinguish between SIgA and systemic IgA signal would improve saliva-based infectious disease detection methods, particularly for enteric pathogens whose pathogenicity involves the mucosal immune system.

A second challenge is that the transient and acute nature of the IgA (including SIgA) immune response limits its diagnostic accuracy. In the *C. jejuni* study mentioned above, for example, IgA and IgG levels in saliva declined over time, but IgG levels remained significantly higher in convalescent phases (Cawthraw et al., 2002). This was also the case in a NoV challenge study where NoV-specific salivary IgA peaked at 14 days post-challenge, whereas NoV-specific salivary IgG continued to rise during 21 days post-challenge (Moe, Sair, Lindesmith, Estes, & Jaykus, 2004). Combining NoV-specific IgA and IgG assay results improved the detection of seroconversion to 100% of infected participants, compared to only 83%

when either IgA or IgG was used alone (Moe et al., 2004). While IgA may be advantageous for detecting the acute phase of enteric disease, the larger window of IgG kinetics may allow for increased sensitivity in the detection of historical exposure to enteric pathogens. Salivary IgG has also been used in a *Cryptosporidium parvum* (*C. parvum*) foodborne-outbreak investigation, where *C. parvum*-specific salivary IgG was able to confirm *C. parvum* exposure in all cryptosporidiosis cases (Moss et al., 2019). Additional longitudinal studies of salivary IgA (including SIgA) and IgG responses among patients with clinically confirmed enteric disease, and analytical techniques that combine different salivary antibody isotype results, are needed.

13.6   Conclusions

Throughout this chapter, we have discussed the utility of pathogen-specific anti-bodies in oral fluid to evaluate pathogen exposure and infection. Oral fluid has utility in monitoring recent and/or historical infection in both clinical and epidemiological settings. In clinical settings, oral fluid-based diagnostic testing can inform clinical decision making around infectious disease treatment, management, and vaccination. In epidemiological surveys, oral fluid-based serological methods can be used to assess seroprevalence and seroconversion among high-risk populations and gain epidemiological inference on risk factors associated with pathogen exposure, infec-tion, and transmission. Due to the ease of oral fluid collection, saliva-based methods facilitate self-collection and testing in national- and community-level public health infectious disease surveillance programs. Furthermore, oral fluid-based methods for infectious disease detection have a unique role in the context of VPDs, where they can be used to assess disease burden and improve case detection, measure population immunity and identify immunity gaps, and assess the impact of vaccination cam-paigns and routine immunization. While oral fluid has shown promise as a non-invasive proxy for blood for the detection of pathogen exposure and infectious disease, oral fluid-based methods pose several limitations compared to blood-based serological methods, including reduced sensitivity. Additional research is needed to both improve the diagnostic accuracy of oral fluid-based serological methods and develop oral fluid-based POC tools for infectious disease screening and diagnosis.

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