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Efficiency of oral fluid collection devices in extracting antibodies

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Introduction: To facilitate diagnoses, this study determined the efficacy of commercial oral fluid collection devices for their ability to recover three human immunoglobulin isotypes; immunoglobulin A (IgA), IgG, and IgM.
Methods: The sandwich enzyme-linked immunosorbent assay was used to determine antibody recovery from the following devices: (i) OraSure® oral specimen collection device, (ii) saliva•sampler®, (iii) ORALscreen™ collector, (iv) Dri-Angle®, (v) no. 2 cotton roll, (vi) all-gauze sponges device, and (vii) DentaSwabs®. For each isotype tested, the recovered eluate was compared with the concentration applied to the device. The performance of each device was determined at various antibody concentrations.
Results: Recovery of IgA from the saliva•sampler, ORALscreen collector, Dri-Angle and cotton roll was comparable to that seeded onto the device. When compared with the seeded IgG concentration, the mean concentration of antibody recovered by each product differed by approximately ± 9 ng/ml. The average amount of IgM recovered by the cotton roll and all-gauze sponges device was approximately 29 and 39 ng/ml, respectively, less ($P < 0.0001$) than that seeded on the device. For all isotypes tested, the amount of antibody recovered from the device was dependent on the initial seeding concentration.
Conclusion: Collectively, these data suggest that the product used for specimen collection can affect retrieval of antibodies and potentially confound patient diagnosis.

Key words: antibody; collection device; human; oral fluid

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Historically, immunodiagnosis of infectious diseases has relied heavily on the analyses of blood products. As evidenced by the increasing availability of commercial products, the development of diagnostic tests is moving toward rapid, non-invasive assays that can be performed at the point-of-care. This trend has given rise to an increasing number of collection devices. Advantages of non-invasive testing include greater safety in collection and handling of specimens, easier disposal, and improved management of patients with difficult venous access (12). Assessment of client preference revealed that a majority of patients (92%) reported no discomfort with oral fluid collection, whereas 75 and 66% of these patients reported no discomfort for venepuncture-based blood tests and finger-stick tests, respectively (24). Currently, oral fluid-based diagnostics are not being used to the fullest capacity; nonetheless, it is envisioned that saliva will play an increasingly important role in the early diagnosis of disease, the monitoring of disease progression, and the evaluation of patient behavior (i.e. compliance and lifestyle choice) (26).

Oral fluid is a complex body fluid containing a mixture of salivary gland secretions and gingival crevicular fluid. Immunoglobin A (IgA) antibodies are secreted by the salivary glands (8). Gingival fluid contains plasma-derived IgG and IgM antibodies (11). Studies have revealed that oral fluid rich in antibodies may be used to replace blood as a diagnostic fluid for the screening of infectious diseases (6, 9, 24), drugs of abuse (2, 30) and vaccine recipients (5, 13).

Oral fluid harvested with collection devices is reported to enhance the sensitivity, specificity and overall performance of human immunodeficiency virus (HIV) immunoassays compared with the screening of whole saliva specimens (16, 17). Some of the collection devices target the gums of the oral cavity and facilitate passage of the gingival crevicular fluid, resulting in a higher IgG level in the specimen (4, 20).

Oral fluid collection devices have been used successfully to collect oral fluid specimens for the detection of IgA, IgG and IgM antibodies specific against infectious disease pathogens (1, 16, 21–23, 25, 29). Collectively, it appears that the antibody concentration and sensitivity of oral samples depend on the design of the collection device. There is, however, a scarcity of information on the recovery yield of oral fluid IgA and IgM antibodies collected with collection devices because most of the work has been performed on IgG antibodies. In the present study, the recovery yield of human total IgG, IgA and IgM antibodies (Kirkegaard Perry Labs Inc., Gaithersburg, MD) eluted from a panel of commercially available products including OraSure® oral specimen collection device, ORALscreen™ collector (Avitar, Inc., Canton, MA), Dri-Angle® (Dental Health Products, Inc., Youngstown, NY), DentaSwabs® (Kimberly-Clark Corp., Draper, UT), saliva•sampler® (Bamburgh Marsh LLC, Vancouver, WA) and common dentistry items including no. 2 cotton roll (Sullivan-Schein, Melville, NY), and the all-gauze sponges device (Henry Schein Inc., Melville, NY) is quantified. This study evaluates the efficiency of individual collection devices for the collection of human total IgA, IgG and IgM antibodies and their respective use in oral fluid collection.

Materials and methods

Collection devices

The OraSure oral specimen collection device has a flat absorbent cotton pad (3 × 1 cm) pretreated with preservatives and stabilizing reagents supported by a 10-cm plastic stem. The cotton pad was saturated with 1.0 ml immunoglobulin solution, detached from the stem, and centrifuged at 1500 ***g*** for 15 min.

The saliva•sampler has an indicator incorporated into the plastic stem that turns blue when the collection pad (3 × 1.5 cm) is saturated with 1.0 ml immunoglobulin sample. The cotton pad was excised and compressed with a filter sampler (Porex Technologies, Fairburn, GA) in a tube to elute the immunoglobulin sample solution from the device.

The ORALscreen collector consists of a rectangle of foam (0.8 × 0.8 × 1.8 cm) covered with a retractable plastic hood attached to a plastic stem. The plastic hood was drawn back to expose the foam. Thereafter, the foam was saturated with 2.3 ml immunoglobulin sample solution. The plastic hood was then coasted forward until it completely surrounded the foam. By hand, the hood was firmly squeezed over the foam to dispense the immunoglobulin sample.

The Dri-Angle is a triangular, absorbent cellulose pad with 4 × 4.5 × 4.5-cm sides and 0.1-cm thickness. The absorbent pad was saturated with 2.0 ml immunoglobulin sample and centrifuged at 1500 ***g*** for 15 min.

The no. 2 cotton roll is a compressed cylindrical cotton swab (4 × 1 cm), which was absorbed with 2.2 ml immunoglobulin solution. The cotton roll was compressed with a filter sampler and the eluate was collected. From product descriptions, the no. 2 cotton roll is comparable to the commercial untreated Salivette® collection device (Sarstedt Inc., Newton, NC).

The all-gauze sponges device consists of eight layers of cotton sponges (5 × 5 cm), which were soaked with 4 ml immunoglobulin solution. Thereafter, the all-gauze sponges device was compressed with a filter sampler.

The DentaSwabs® consists of a rectangular polystyrene sponge (2.2 × 1.5 × 1.5 cm) attached to a 10-cm plastic stem. The sponge absorbed 1.5 ml immunoglobulin sample solution, was excised from the stem, and was centrifuged at 1500 ***g*** for 15 min.

Sample processing

Immunoglobulin recovery from the above-mentioned devices was determined as follows. A known concentration [250, 125, 16, 2 or 0.5 ng/ml in phosphate-buffered saline (PBS)/Tween-20/5% (weight/volume) skim milk] of purified human IgA, IgG or IgM (Kirkegaard Perry Labs Inc.) was applied to each collection device. For each treatment group, six replicates were conducted. The volume required to saturate the collection device was determined in advance. To standardize the performance of the devices, the maximum saturation volume of antibody solution was allowed to absorb for 2 min. The fluid was extracted from the collection device within 10 min. If available, the procedure described by the manufacturer was used to extract the fluid from the device. Following extraction from individual collection devices, antibody recovery was determined using an 11-point standard curve of the appropriate isotype with an operating range of 0.2–250 ng/ml. Interassay variability was minimized using the standard curve on each enzyme-linked immunosorbent assay (ELISA) plate with a regression coefficient (R^2) of ≥ 0.95.

ELISA

A sandwich ELISA was used to determine the recovery of antibodies extracted from oral fluid collection devices. Plates were coated with 0.1 μg/100 μl/well of goat anti-human IgA, IgG or IgM (Kirkegaard Perry Labs Inc.) in 0.01 M PBS, pH 7.4 and incubated overnight at 4°C. Plates were washed three times with PBS containing 0.5% Tween-20. Human IgA, IgG or IgM diluted two-fold (250–0.02 ng/ml) in buffer [PBS/Tween-20/5% (weight/volume) skim milk] was used to create a standard curve. These preparations were incubated alongside immunoglobulin samples eluted from collection devices at 37°C for 1 h. The plates were washed three times as before and 100 μl biotin-labeled goat anti-human IgA, IgG or IgM (1 : 5000 in dilution buffer, Kirkegaard Perry Labs Inc.) was added for 1 h at 37°C. The plates were washed as before and incubated with 100 μl diluted (1 : 1000) avidin:alkaline phosphatase (Kirkegaard Perry Labs Inc.). The plates were washed and incubated with 100 μl alkaline phosphatase substrate (Kirkegaard Perry Labs Inc.) for 30 min at 37°C. The reaction was stopped by the addition of 100 μl 2 M NaOH. Absorbance values were measured at 405 nm (490-nm reference filter) using a ThermoLabsystems MRX® Revelation microtiter plate reader (Chantilly, VA).

Statistical analyses

The difference between the starting concentration and the recovered value was used to evaluate the performance of the collection devices (i.e. 0 ng/ml = 100% immunoglobulin recovery). By using this difference score, where the expected value was always 0 ng/ml given perfect recovery, data for all concentrations for a collection device could be considered together. The least squares mean concentration (LSMC) of human total IgA, IgG or IgM recovered from starting material was estimated using the generalized linear modeling approach. Data were analysed for the main effect of collection device, without regard to concentration, and then, only secondarily, for the main effect of concentration without regard to device. The collection device by concentration interaction was not addressed. This approach was selected because ‘true’ concentration would not be accessible in routine clinical studies. We were therefore interested in the overall performance across a range of conceivable concentrations rather than changes in device performance across specific ‘true’

concentrations because 'true' concentration would be unknown in clinical studies. Tukey–Kramer was used for pairwise comparisons. Analyses were conducted using STATISTICAL ANALYSIS SYSTEM (SAS 9.1; SAS Institute Inc., Cary, NC) software. For all analyses, $P \leq 0.05$ was considered significant.

Results

IgA

Recovered IgA from the saliva•sampler, ORALscreen collector, Dri-Angle and cotton roll did not differ significantly from that initially seeded onto the collection device (Fig. 1). Conversely, the other three devices yielded a reduced amount of IgA ($P < 0.01$). On average, the OraSure oral specimen collection device, all-gauze sponges device and DentaSwabs yielded approximately 15, 11 and 9 ng/ml less IgA, respectively, compared with the initial seeding concentration.

Comparing devices, the only significant difference ($P < 0.02$) observed was between the OraSure oral specimen collection device and the cotton roll. The yield of IgA from these devices differed by approximately 17 ng/ml (86%).

The amount of IgA recovered from the collection devices was subject to the seeding concentration (Fig. 2). IgA recovery was similar to that seeded on the device at the lower concentrations (0.5 and 2 ng/ml). Devices seeded with ≥ 16 ng/ml yielded a significantly different ($P < 0.002$) amount of IgA. At 250 ng/ml, only ∼20% of the seeded IgA was recovered. This recovery was significantly less ($P < 0.0001$) than that observed for all other lower concentrations. Comparisons between other concentrations of IgA revealed that devices seeded with 16 ng/ml recovered significantly less ($P < 0.05$) antibody than those seeded with 0.5 or 125 ng/ml.

IgG

The mean concentration of IgG that was recovered from each device differed by less than ± 9 ng/ml from the amount used for seeding. It was not significantly different to that seeded onto the device, with the exception of the saliva•sampler ($P < 0.001$), cotton roll ($P < 0.02$), and all-gauze sponges device ($P < 0.01$) (Fig. 1).

In comparing the recovery of IgG between devices, the only significant difference ($P < 0.03$) observed was between the saliva•sampler and the Dri-Angle. On average, the latter yielded approximately 12 ng/ml (71%) more IgG.

Reliability of IgG recovery varied with seeding concentrations (Fig. 2). IgG recovery was comparable to that applied onto the device at concentrations of 0.5 and 2 ng/ml. In contrast, IgG recovery from devices seeded with ≥ 16 ng/ml was significantly different ($P < 0.0001$). The most notable decrease (approximately 15%) in IgG recovery was observed at the 250 ng/ml seeding density. Recovery from devices seeded with 16, 125 and 250 ng/ml IgG differed significantly ($P < 0.01$) from 0.5 and 2 ng/ml concentrations.

IgM

The saliva•sampler, ORALscreen collector and Dri-Angle yielded the best recovery of IgM (Fig. 1). Although significant ($P < 0.03$), IgM recovery from the OraSure oral specimen collection device and DentaSwabs was only moderately less (9 and 5 ng/ml, respectively) than that seeded on the devices. In contrast, the average amount of IgM recovered by the all-gauze sponges device and the cotton roll and was approximately 29 and 39 ng/ml less ($P < 0.0001$) than that seeded on the device, respectively.

The recovery of IgM from the cotton roll and all-gauze sponges device differed ($P < 0.02$) from all other devices. The Dri-Angle differed significantly ($P < 0.02$) from the OraSure oral specimen collection device. The recovery of IgM from the

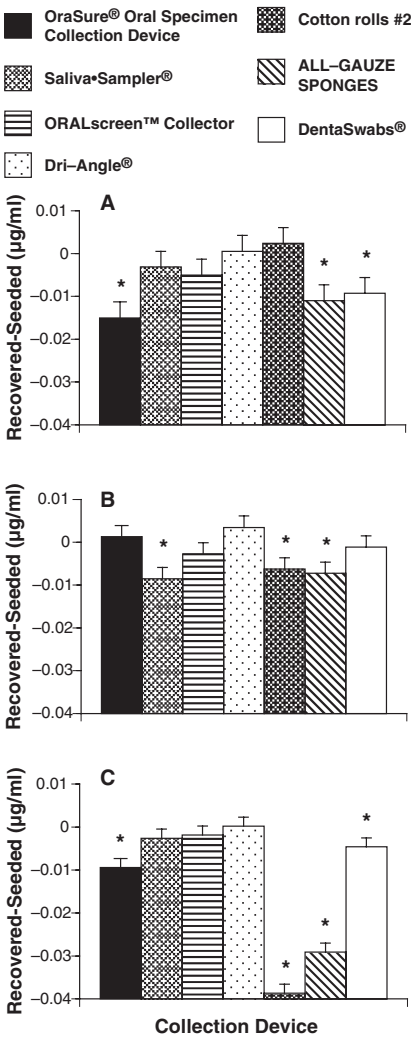

Fig. 1. Recovery of immunoglobulins from various oral fluid collection devices at all concentrations. Recovery of (A) immunoglobulin A (IgA), (B) IgG and (C) IgM, estimated by assessing the magnitude of the difference between the starting concentration and the recovered value. Bar height represents the mean value ± SE of $n \geq 33$. *$P < 0.05$ when compared with 0 µg/ml (i.e. 100% recovery).

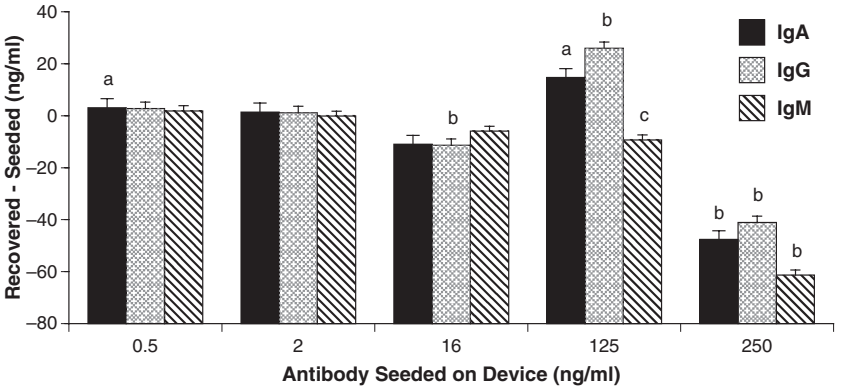

Fig. 2. Recovery of various concentrations of immunoglobulin A (IgA), IgG and IgM from all oral fluid collection devices. Bar height represents the mean value ± SE of $n \geq 40$. [a]$P < 0.05$ when compared with 16 ng/ml within the same isotype. [b]$P < 0.01$ when compared with all other concentrations within the same isotype. [c]$P < 0.01$ when compared with 0.5, 2 and 250 ng/ml within the same isotype.

DentaSwabs, OraSure® oral specimen collection device, saliva•sampler and ORALscreen collector was comparable.

As with the other isotypes, the amount of IgM recovered from the collection devices was subject to the initial antibody concentrations (Fig. 2). At concentrations of 0.5 and 2 ng/ml, recovery of antibody was comparable to that seeded onto the device. IgM recovery was significantly reduced at concentrations ≥ 16 ng/ml, with the greatest discrepancy in the 250 ng/ml seeding concentration group. At this concentration, only ∼25% ($P < 0.0001$) of the IgM was recovered.

Discussion

The development and commercialization of diagnostic tests are moving toward simple, non-invasive assays using point-of-care technologies. Commercial devices have been used successfully for collecting oral fluid for the detection of antibodies directed against infectious disease pathogens (1, 9, 16, 22, 23, 29). These authors measured the salivary antibody concentration after extraction, but did not quantify the recovery yield relative to the initial seeding antibody concentration. Consequently, the findings do not account for the efficiency of individual devices in the extraction of antibodies. To determine the efficiency and quantify the yield of human antibodies by these collection devices, we measured the concentrations of human total IgA, IgG, and IgM before and after extraction with seven oral fluid collection devices.

Recovery of IgA from four of the seven devices did not differ significantly from that which was initially seeded onto the collection pad. Other products, such as the OraSure oral specimen collection device, yielded ∼15 ng/ml less IgA. Depending on the cut-off value that discriminates between infected and uninfected patients, this discrepancy may or may not be clinically relevant. For the detection of IgA against *Streptococcus pneumonia*, Nurkka et al. (23) concluded that the use of four collection methods (including OraSure oral specimen collection device and whole saliva) resulted in minor differences in the recovery of antibody concentrations.

The saliva•sampler, cotton roll and the all-gauge sponges device yielded the lowest LSMC in human IgG. Although these data represent total IgG, the findings are consistent with a report that demonstrated that the untreated Salivette collection device provided specimens with lower sensitivity for hepatitis C IgG detection than the OraSure oral specimen collection device samples (18). Moreover, no significant differences in the sensitivity for anti-hepatitis C IgG in oral fluid specimens collected by Salivette and saliva•sampler were reported (28). Despite their selective performance, oral fluids collected by the OraSure oral specimen collection device and/or saliva•sampler provided sufficient quantities of specific antibody concentrations for the detection of IgG against rubella virus, parvovirus B19 (29), HIV (12, 19), measles virus (27), and *Trypanosoma cruzi* (3).

The level of total IgG subclasses has been reported to be 704-fold lower in oral fluid than in the serum of patients suffering from periodontitis (14). As summarized, the concentration of IgM in oral mucosal transudate and whole saliva can be 200- and 850-fold less, respectively, than that detected in serum (10). Similarly, a significant reduction in the secretion rate of IgM in whole saliva was detected in the elderly population (7). Consequently, for oral fluid-based diagnosis it is crucial to use a collection method that will facilitate maximum IgM recovery, especially when it is necessary to make a presumptive diagnosis during the acute phase of an infection. Our data suggested that the saliva•sampler, ORALscreen collector and Dri-Angle yielded quantities of IgM that did not differ significantly from that seeded on the device. The recovery of IgM by the OraSure oral specimen collection device was less ($P < 0.0001$) than the seeded concentration. Notwithstanding, the ability of this device to harvest IgM from oral fluid may be adequate for some diagnostic applications, as the sensitivity and specificity were 79% and 100% for anti-hepatitis A virus IgM and 100% and 100% for anti-hepatitis B core antigen IgM, respectively (1). The cotton roll and all-gauge sponges device yielded the lowest LSMC of human IgM. While these two devices may be less feasible for the detection of a primary immune response in oral fluid, they may add value to systems that seek to minimize the presence of IgM (i.e. reduce steric hindrance).

As there are advantages and disadvantages associated with each device, we believe that there is not a 'one-size fits all' method. In addition to taking antibody recovery into consideration, it is important to choose the collection device that is best suited for the patient and environment. For instance, specimen collection may be extremely difficult in young children because of lack of cooperation and motor skills (15). The collection method for field applications may be limited to devices that do not require any laboratory equipment. Sample integrity and antibody yield may also be affected by additives and preservatives associated with the collection method (4, 20).

When choosing a collection method, another aspect to consider is the antibody concentration present in the sample. For each isotype, a broad range of concentrations was tested (0.5–250 ng/ml), as these concentrations fall within the spectrum that has been reported within the oral cavity (10). Compared with lower antibody concentrations, samples with 250 ng/ml IgA, IgG or IgM had consistently poor recovery. At times, devices seeded with 125 ng/ml immunoglobulin had recovery slightly > 100%. One possible explanation for this is that the collection devices may concentrate the antibodies in a dilution-dependent manner. Alternatively, it is possible that extrapolation from the standard curve toward the upper asymptote allowed this variability.

In summary, our evaluation of commercial oral fluid collection devices revealed that: (i) recovery of IgA, IgG and IgM in a given sample can vary markedly between devices (ii) initial seeding concentration in the sample can influence the percentage of antibody that is extracted from the device and (iii) devices using manual extraction methods can be as effective in recovering antibodies as those requiring centrifugation.

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