

# Comparison of Oral Fluid Collectors for Use in a Rapid Point-of-Care Diagnostic Device

Carol Holm-Hansen,<sup>1</sup> Gary Tong,<sup>2</sup> Cheryl Davis,<sup>2</sup> William R. Abrams,<sup>3</sup> and Daniel Malamud<sup>2\*</sup>

Division for Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway,<sup>1</sup> and Department of Biochemistry<sup>2</sup> and School of Dental Medicine, Department of Anatomy and Cell Biology,<sup>3</sup> University of Pennsylvania, Philadelphia, Pennsylvania

Received 24 February 2004/Returned for modification 2 April 2004/Accepted 13 May 2004

**Orally based diagnostic testing is emerging as an alternative, noninvasive method for analyzing a variety of analytes. These analytes include pathogens, antibodies, drugs, and nucleic acids. In the present study we developed a protocol for evaluation of collectors that could be used in orally based, point-of-care diagnostics. A performance comparison was carried out with a number of commercially available collectors, and their ability to deliver fluid, proteins, bacteria, and nucleic acid from pathogens compatible with PCR was assessed. The collectors were all capable of picking up and delivering test materials, albeit at various levels.**

Over the past several years, there has been increasing interest in orally based diagnostics, which provide a noninvasive technique for sampling (2, 3, 4, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16). This interest has been accelerated as more reports demonstrate the reliability, sensitivity, and specificity of saliva tests and other oral fluid-based tests (3, 4, 5, 6, 9). In addition, several commercial entities have designed and marketed collection devices, facilitating the development of new saliva tests or orally based tests (6, 14, 16). While it is feasible to collect pure saliva directly from the salivary ducts, from practical considerations, tests based on this type of sampling could not be widely marketed because of the degree of training required for the specialist collecting the specimen. Similarly, gingival crevicular fluid can be collected on paper points (8, 10) and provides an excellent sampling of a serum transudate, but it is unlikely that commercial tests would be developed for this fluid due to the difficulty in applying the collector and the relatively small volume of fluid collected. Instead, oral mucosal transudate (1) collected between the gum and cheek (Orasure) or from under the tongue (Saliva Diagnostics) has been demonstrated to be valuable for monitoring specific antibodies by oral sampling.

In the present study, we used a number of commercially available collection devices and established a procedure for evaluating their ability to collect and release fluid, proteins, or bacteria, and we also evaluated a performance standard for subsequent PCR. The ease and aesthetics of the collections were subjectively considered, since the success of any diagnostic will also be based on the acceptability of the method to both the subject and the investigator. Thus, the overall aim of this study was to compare the abilities of several collectors to absorb and release fluids, bacteria, DNA, and protein within the context of an orally based diagnostic protocol.

## MATERIALS AND METHODS

Eight commercially available specimen collectors were initially evaluated for fluid absorption and release, including the OraSure HIV-1 oral fluid specimen device (OraSure Technologies Inc., Bethlehem, Pa.), UpLink (OraSure Technologies Inc.), Salivette (Sarstedt, Newton, N.C.), Toothette-Plus swabs (Sage Products Inc., Crystal Lake, Ill.), BBL CultureSwab orange cap (220129), white cap (220093), and red cap ("EZ") (Becton Dickinson and Co., Sparks, Md.), and TRANSORB wicks (Filtrona Richmond, Inc., Colonial Heights, Va.).

OraSure and UpLink are specially designed devices for the collection of oral fluid. The OraSure device consists of an adsorbent pad on a plastic stick, while UpLink is a unique sample collector that absorbs a metered dose that can be easily transferred from the plastic handle. The Salivette collector is an adsorbent cotton roll that collects a mixed sample of whole saliva and other oral fluids. Toothette-Plus is an oral hygiene product designed to moisten and clean the oral cavity and consists of a sponge-like pad on a plastic stick. The BBL CultureSwab collection swabs are designed to collect aerobic organisms from throat, vagina, skin, or wound specimens (white cap; 22129), male urethral samples and ear, nose, throat, and eye specimens (orange cap; 220129) or general specimen laboratory use (red cap; "EZ"). The BBL CultureSwab collectors resemble single-ended Q-tips. TRANSORB wicks are composed of bonded cellulose acetate fiber and are used in diagnostic test devices and systems such as membrane enzyme immunoassays, fluorescence polarization immunoassays, (and microparticle immunoassays). The Transorb wicks serve as reservoirs to absorb and retain excess test sample and liquid reagents. Wicks used in this study were supplied by the manufacturer as 1.2-mm-thick by 14.7-mm-wide by 97-mm-long strips and then cut to approximately 20 mm in length.

Whole saliva was stimulated by chewing neutral gum-based pellets (provided by the Wm. Wrigley Jr. Co., Chicago, Ill.) and collected into iced 15-ml Falcon tubes. Saliva samples were pooled and clarified by centrifugation (Sorval RCSC) (1,935 × g) for 15 min. The supernatant was divided into aliquots and transferred to new tubes.

To evaluate their absorptive properties, collectors were submerged in water or clarified saliva for 3 min and centrifuged at 4,000 rpm for 10 min to remove absorbed fluid from the collector. The weights of the dry, wet, and centrifuged collectors and the volumes of water or saliva absorbed and released after centrifugation were recorded and compared.

The ability of four collectors (OraSure, UpLink, TRANSORB, and BBL white cap) to adsorb and release pathogens was investigated with *Bacillus cereus*, a spore-forming gram-positive soil bacterium associated with food-borne diseases. These four collectors were chosen based on a subjective evaluation of the suitability for use with large populations of individuals for a point-of-care detection system.

*B. cereus* was grown overnight at 37°C with shaking. The bacteria were washed three times in sterile phosphate-buffered saline (PBS), and the final pellet was suspended in 10 ml of sterile PBS. The optical density at 600 nm was recorded, and the volume was adjusted to yield a concentration of approximately  $5 \times 10^9$  bacteria per ml. A 1:10 serial dilution was prepared, and 100  $\mu$ l of each dilution from  $10^{-1}$  to  $10^{-7}$  was streaked on Luria-Bertani agar plates. The plates were incubated at 37°C overnight, and colonies were counted. The dilution that

\* Corresponding author. Mailing address: Department of Biochemistry, University of Pennsylvania School of Dental Medicine, 240 South 40th St., Philadelphia, PA 19104-6030. Phone: (215) 898-6576. Fax: (215) 898-3695. E-mail: malamud@pobox.upenn.edu.

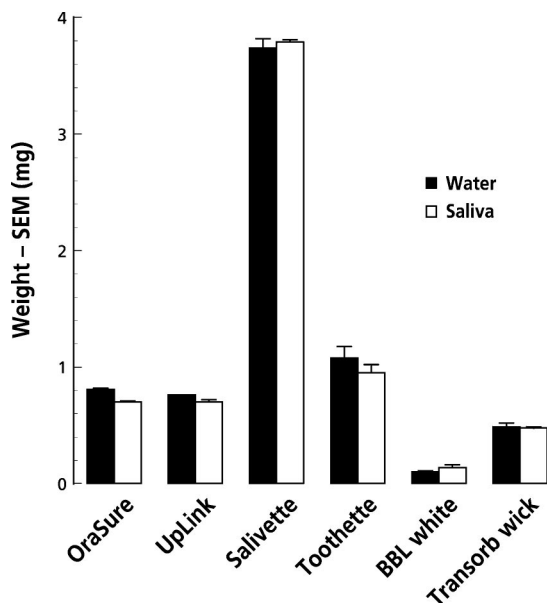


FIG. 1. Comparison of the absolute fluid transfer capacity of six different collectors, using either water or clarified whole saliva. Each collector was soaked in water or saliva for 3 min and then centrifuged to collect the fluid in a tarred vessel, and the transferred mass was determined in triplicate. The values shown are the averages  $\pm$  standard errors for triplicate determinations.

yielded approximately 100 colonies per plate, in addition to one dilution higher and lower ( $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$ ), were used to determine the efficiencies of the collectors in adsorbing and releasing *B. cereus*. The collectors were soaked in 2 ml of *B. cereus* dilutions ( $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$ ) for 2 min. To release bacteria, the collectors were either centrifuged at 2,500 rpm for 10 min or soaked in 2 ml of PBS for 2 min, and 100- $\mu$ l aliquots of the released material were plated onto LB agar.

The abilities of four collectors (OraSure, UpLink, TRANSORB, and BBL white cap) to absorb and release protein were investigated by monitoring immunoreactive amylase in saliva samples. Whole saliva was collected as described above. Serocluster "U" vinyl 96-well microtiter plates (Costar, Cambridge, Mass.) were coated with saliva samples released from the collectors in a carbonate-bicarbonate buffer (pH 9.6) (dilutions of 1:8000, 1:16,000, and 1:32,000), blocked with 1% bovine serum albumin in 20 mM Tris, and incubated with 1:500 rabbit anti-human  $\alpha$ -amylase (Sigma, St. Louis, Mo.) followed by 1:500-diluted goat anti-rabbit alkaline phosphatase conjugate (Zymed, San Francisco, Calif.). The control was 5  $\mu$ g of  $\alpha$ -amylase/ml from *Bacillus licheniformis* (Sigma). Incubations were performed at 37°C for 60 min, and plates were washed four times between incubations with PBS containing 0.05% Tween 20. The reaction was developed with a *p*-nitrophenyl phosphate substrate (Sigma) for 30 min, and optical density was measured at 410 nm.

Four collectors (OraSure, UpLink, TRANSORB, and BBL white cap) were examined for their ability to be used to carry out PCR with released bacteria. OraSure collector pads were formulated with a hypertonic solution to stimulate the flow of transudate; however, untreated pads are also capable of collecting oral mucosal fluid. Because we anticipated that the salt component of the OraSure collector might interfere with subsequent PCR analyses, these studies were carried out with treated OraSure pads, as well as the same pad without added salt.

The collectors were soaked for 2 min in a solution of *B. cereus* (1:25 dilution;  $2 \times 10^8$  cells) and then rinsed in 2 ml of buffer soaking solution (10 mM Tris [pH 8.5]) for 5 min to elute the absorbed bacteria. Aliquots of these samples, along with positive and negative controls, were prepared using the QIAGEN blood and cell culture DNA mini-kit (QIAGEN Inc., Valencia Calif.). Aliquots (10  $\mu$ l) were then added to PCR master mix, as described in Materials and Methods, and PCR products were visualized after gel electrophoresis (1% agarose, 0.2- $\mu$ g/ml ethidium bromide).

The presence of possible PCR inhibitors was examined in four collectors (OraSure, UpLink, TRANSORB, and BBL white cap). The collectors were soaked in 2 ml of water for 2 min and then centrifuged to remove retained fluid. The presence of potential PCR inhibitors was examined both in the water remaining after the 2-min soak and in the water expressed from the collectors. Aliquots of

the water samples from the collectors and a control water sample that had not been in contact with the collectors were added to a PCR master mix containing 25  $\mu$ l of 2 $\times$  PCR premix containing 100 mM Tris-HCl (pH 8.3), 100 mM KCl, MgCl<sub>2</sub> (concentration not provided by manufacturer), and 400  $\mu$ M (each) deoxynucleoside triphosphates (MasterAmp 2 $\times$  PCR PreMix; Epicentre, Madison, Wis.), 1  $\mu$ l (each) primer (forward primer, 17.0  $\mu$ mol/ml; reverse primer, 11.9  $\mu$ mol/ml); 3  $\mu$ l of *B. cereus* genomic DNA (35 ng/ $\mu$ l), and 0.5  $\mu$ l of *Taq* polymerase (5 U/ $\mu$ l, Eppendorf *Taq* DNA polymerase; Brinkmann Instruments, Inc., Westbury, N.Y.). Primer sequences for a 305-bp DNA product were AAGGT TCAAAGATGGTATTCAGG and TCTCGCTTCACTATTCCAAGT. The DNA was prepared by using QIAGEN midi-preps (QIAGEN GmbH, Hilden, Germany), following the manufacturer's instructions. PCR products were visualized after gel electrophoresis (1% agarose, 0.2- $\mu$ g/ml ethidium bromide).

## RESULTS

To compare the abilities of the collectors to deliver a fluid sample for diagnostic testing, each of the collectors was immersed in distilled water or clarified whole saliva, and then the fluid was removed from the collector by centrifugation. The collectors were weighed before and after collection, and the fluid released was also weighed. As shown in Fig. 1, all of the collectors were capable of picking up and releasing either water or saliva. There were no significant differences among any of the collectors in terms of their abilities to absorb and deliver

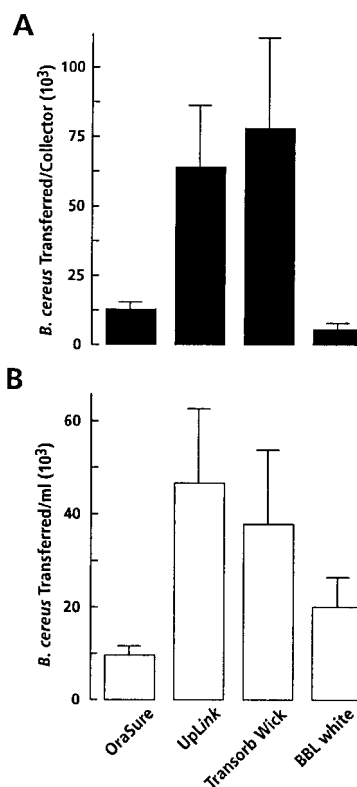


FIG. 2. The abilities to absorb and transfer live *B. cereus* by using different collectors by soaking were compared (A). Collectors were allowed to absorb bacteria from a 2-ml suspension for 2 min and then moved to a transfer solution, where they were allowed to soak for 3 min, after which the collectors were removed. Aliquots of the transferred solution were plated at three different dilutions, and the resulting colonies were counted. Each value represents the average number of bacteria  $\pm$  standard error of the mean for the three dilutions. (B) Comparison of bacterial transfer capacity. The fluid volume capacity of each collector was used to normalize the number of bacteria transferred.

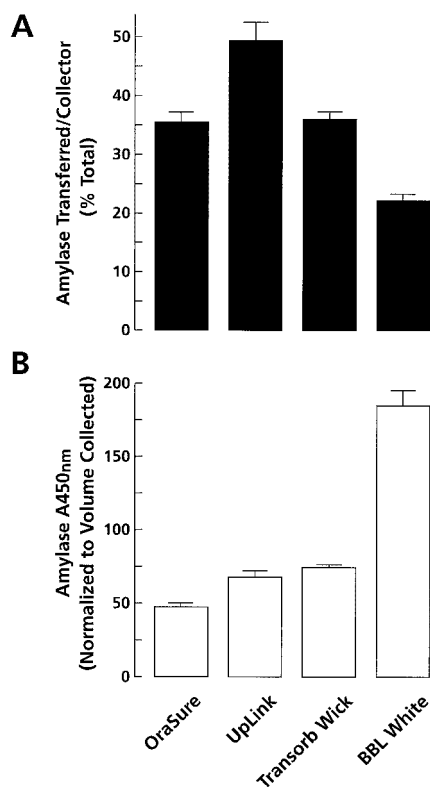


FIG. 3. Comparison of the abilities of collectors to transfer immunoreactive amylase (A). Collectors were soaked in 2 ml of clarified whole saliva for 2 min and then transferred to PBS for 2 min. Micro-titer plates were then coated with aliquots of the soaking solution, and the amount of amylase transferred was determined by enzyme-linked immunosorbent assay as described in Materials and Methods. Results shown are averages  $\pm$  standard errors for triplicate determinations. (B) Comparison of protein transfer capacity. The fluid volume capacity of each collector was used to normalize the amylase transferred. A450nm, absorbance at 450 nm.

the two fluids. There were, however, significant differences in terms of the volumes collected (Salivette collected the most, followed by Toothette, followed by OraSure and UpLink, followed by Transorb, and then BBL white).

To determine the amounts of bacteria that could be obtained with the collectors, each was immersed in a solution of *B. cereus*, and bacteria were then eluted either by soaking in buffer or by centrifugation. Plating and colony counting quantitated the bacteria released. The data presented in Fig. 2, represent the results from the soaking protocol; however, similar data were obtained by centrifuging the collectors. Note that in terms of their ability to pick up and release live bacteria, there was a wide range of values, and the low level for BBL white probably reflects the smaller volume collected, as seen in Fig. 1. Results indicated that the order of collector performance from most to least bacteria delivered was as follows: Transorb and UpLink were similar, followed by OraSure and then BBL White. With normalization for collector capacity, no significant alteration of the order is observed (UpLink and Transorb were similar, followed by BBL White and then OraSure).

To assess the abilities of the collectors to pick up and release protein from an oral sample, each of the collectors was soaked

in whole, clarified saliva for 3 min, and then the absorbed protein was removed from the pad by either soaking or centrifugation. Released amylase was measured in an enzyme-linked immunosorbent assay, as described in Materials and Methods (see Fig. 3). When normalized for volume capacity, the BBL white collector demonstrates a notably high transfer capacity for its size.

The collectors were all able to pick up and release the soluble protein amylase, whether removed by soaking (see below) or centrifugation (data not shown), using the same protocol as described in Fig. 1 and 2. Once again, similar data were obtained with soaking and centrifugation.

In a diagnostic test, it is often desirable to quantitate the number of bacteria by using PCR rather than colony counting because of the sensitivity and reproducibility of the technique. In order to determine if the material released from the collectors would be suitable for PCR analysis, experiments were carried out similar to those shown in Fig. 2, except that the amount of bacteria released from the collectors was determined by using PCR, as described in Materials and Methods. As can be seen (Fig. 4), PCR analysis of released bacteria was successful with all of the collectors. The apparent low level of recovery with BBL white is consistent with the smaller volume of fluid collected. However, this device might be ideal for diagnostic sampling when the site in the oral cavity is known, for

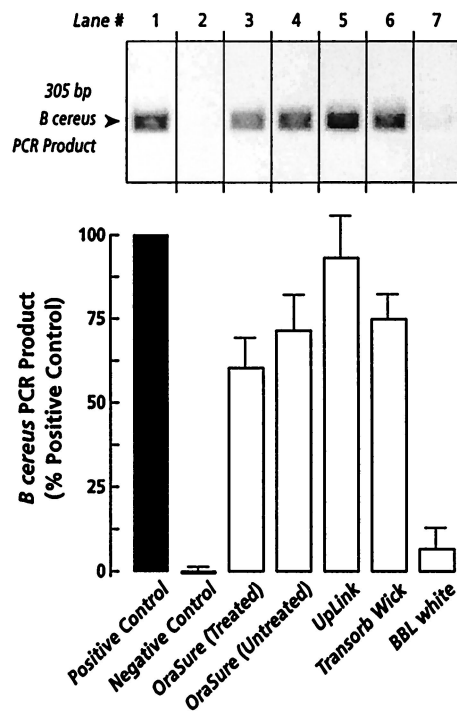


FIG. 4. To determine the ability of the collectors to pick up bacteria, release them, and then use the solution to detect *B. cereus* by PCR, each of the collectors was immersed in a solution of *B. cereus* for 2 min, and then the collectors were soaked in buffer for 3 min and that buffer was used as a source of DNA (QIAGEN blood and cell culture Mini kit). PCR As shown, all of the collectors were able to pick up and release *B. cereus* that could subsequently be detected by PCR. A representative gel demonstrating the amplification of the 305-bp product is shown above. The graph presents the digitized data (average  $\pm$  standard error) for triplicate determinations.

TABLE 1. Performance of fluid collectors

Collector	Transfer test result <sup>a</sup>			
	Fluid	Bacteria	Protein	Nucleic acid
Salivette	++++	—	—	—
Toothette	+++	—	—	—
OraSure (untreated)	++	++	++++	++++
UpLink	++	++++	+++	++++
Transorb wick	++	+++	+++	++++
BBL white cap	+	+	++	+

<sup>a</sup> Symbols: —, not done; +, hit (semiquantitative four-point scale).

example, in obtaining a swab for group A Strep or a herpetic lesion. Thus, it is clear that the type of collector to be used depends on the nature of the diagnostic test, as has already been reported for drugs of abuse (16).

### DISCUSSION

A semiquantitative summary of all of the data that compares the relative merits of each collector according to the criteria examined is shown in Table 1. All of the collectors are capable of adsorbing and delivering to a sample recovery tube either water or whole stimulated saliva. There are major differences, however, in terms of the absolute volumes collected, and the collectors can be ranked from large to small volumes as follows: Salivette followed by Toothette, followed by Orasure and UpLink, followed by Transorb, and then BBL white cap. However, the Salivette was not included in the remaining comparisons because it was felt to be an aesthetically incompatible design for a large noninvasive testing program. In terms of delivering bacteria, the OraSure, UpLink, and Transorb filters were equally efficient, while the BBL white cap, which picks up considerably less fluid, also delivers less bacteria and proteins.

In summary, we have presented a protocol for evaluating commercially available collectors suitable for orally based diagnostic tests. All of the evaluated collectors efficiently absorbed and released fluid, albeit at various levels. Four collectors were further studied. From these experiments, it was observed the OraSure, UpLink, and Transorb wicks are well suited for collection and release of fluid, protein, and bacteria

and as a source of nucleic acid for amplification. Other considerations, such as the ideal sites or times for collection in the oral cavity, have not yet been determined.

### ACKNOWLEDGMENTS

This research was supported by NIH grant UO1-DE-014964. We greatly appreciate the technical assistance of Rola Alkhatib.

### REFERENCES

- Atkinson, J. C., C. Dawes, T. Ericson, P. C. Fox, K. Gandara, D. Malamud, I. D. Mandel, M. Navazesh, and L. A. Tabak. 1993. Guidelines for saliva nomenclature and collection. *Ann. N. Y. Acad. Sci.* **694**:xi-xii.
- Bellini, W. J., and R. F. Helfand. 2003. The challenges and strategies for laboratory diagnosis of measles in an international setting. *J. Infect. Dis.* **187**(Suppl. 1):S283-S290.
- Cone, E. J., L. Presley, M. Lehrer, W. Seiter, M. Smith, K. W. Kardos, E. Fritch, S. Salamone, and R. S. Niedbala. 2002. Oral fluid testing for drugs of abuse: positive prevalence rates by Intercept immunoassay screening and GC-MS-MS confirmation and suggested cutoff concentrations. *J. Anal. Toxicol.* **26**:541-546.
- George, R. J. 1997. Future applications of oral fluid specimen technology. *In* R. Sadovskey (ed.), *Oral specimen testing for human immunodeficiency virus*. *Am. J. Med.* **102**(Suppl. 4A):21-25.
- Hodinka, R. L., T. Nagashunmugam, and D. Malamud. 1998. Detection of human immunodeficiency virus antibodies in oral fluids. *Clin. Diagn. Lab. Immunol.* **5**:419-426.
- Kefalides, P. T. 1999. Saliva research leads to new diagnostic tools and therapeutic options. *Ann. Intern. Med.* **131**:991-992.
- Lawrence, H. P. 2002. Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. *J. Can. Dent. Assoc.* **68**:170-174.
- Malamud, D., and L. Tabak (ed.). 1993. *Ann. N. Y. Acad. Sci.*, vol. 694. Saliva as a diagnostic fluid. New York Academy of Sciences, New York, N.Y.
- McKie, A., A. Vyse, and C. Maple. 2002. Novel methods for the detection of microbial antibodies in oral fluid. *Lancet Infect. Dis.* **ii**:18-24.
- Nakashima, K., C. Demeurisse, and G. Cimasoni. 1994. The recovery efficiency of various materials for sampling enzymes and polymorphonuclear leukocytes from gingival crevices. *J. Clin. Periodontol.* **21**:479-483.
- Sadovskey, R. (ed.). 1997. Oral specimen testing for human immunodeficiency virus. *Proceedings of a symposium*. *Am. J. Med.* **102**(Suppl. 4A).
- Slavkin, H. C. 1998. Toward molecularly based diagnostics for the oral cavity. *J. Am. Dent. Assoc.* **129**:1138-1143.
- Streckfus, C. F., and L. R. Bigler. 2002. Saliva as a diagnostic fluid. *Oral Dis.* **8**:69-76.
- Tabak, L. A. 2001. A revolution in biomedical assessment: the development of salivary diagnostics. *J. Dent. Educ.* **65**:1335-1339.
- Van der Eijk, A. A., H. G. Niesters, H. M. Gotz, H. L. Janssen, S. W. Schalm, A. D. Osterhaus, and R. A. de Man. 2004. Paired measurements of quantitative hepatitis B virus DNA in saliva and serum of chronic hepatitis B patients: implications for saliva as infectious agent. *J. Clin. Virol.* **29**:92-94.
- Walsh, J. M., R. Flegel, D. J. Crouch, L. Cangianelli, and J. Baudys. 2003. An evaluation of rapid point-of-collection oral fluid drug-testing devices. *J. Anal. Toxicol.* **27**:429-439.