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Observations on the variation in volumes of self-collected oral fluid samples submitted for HIV antibody detection

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Saliva is a safe, simple and abundant sample to collect for an ever-increasing number of assays.[1–3] The use of oral fluids for detecting antibodies to human immunodeficiency virus (HIV) has long been suggested as an alternative to the use of blood.[4–6] This could help to eliminate the occupational risks associated with needlestick accidents and injuries from phlebotomy. It could also decrease the patient discomfort and thus improve compliance with repeated testing.[7,8]

Although oral fluid from HIV-1-infected individuals contains antibodies to HIV-1, infectious virus in oral fluid is rare.[9,10] Early studies show that the volume and condition of oral fluid are important factors in successful antibody detection, therefore investigators developed specialised self-collection devices that would enhance the quality obtained and preserve the quality and concentration of antibodies by preventing microbial growth and proteolytic breakdown of the antibodies.[11,12]

Self-collection of samples, however, can lead to variability in the volume or quality of the sample submitted for analysis. Therefore, this study aims to determine the frequency of 'unacceptable' samples submitted by participants being screened for HIV-1 infection in three different settings: i) as part of an insurance application; ii) through an online healthcare company; and iii) at a local hospital under direct supervision of hospital staff.

Until March 2012, Quest Diagnostics provided the pathology services for a number of insurance companies that tested clients for HIV, and for an online medical company (which sent samples to the Quest walk-in clinic at Upper Wimpole Street). All these samples were self-collected

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Table 1. Breakdown of total volumes in collection tubes and relationship with colour indicator

Total volume (sample + buffer)	0 mL	0.5–1.0 mL	1.1–1.5 mL	1.6–2.1 mL
No fluid	27	–	–	–
Clear fluid	–	9	64	–
Blue fluid	–	4	121	3567
Total	27	13	185	3567

using the Omni-SAL collection device (Saliva Diagnostic Systems, Vancouver). Over a two-year period, 3792 oral fluid samples were submitted for HIV antibody testing. Of these, 3542 (93.4%) were self-collected by patients, and the other 250 (6.6%) were collected at the West Middlesex Hospital genitourinary medicine (GUM) clinic under the supervision of professional staff.

The Omni-SAL device consists of a compressed absorbent cotton pad attached to a plastic stem. The pad is placed under the tongue and absorbs fluid from the floor of the mouth. The device incorporates an indicator on the plastic stem that turns blue when an adequate amount of sample has been collected (usually approximately 1.0 mL). The collection pad is then inserted in a stoppered transport tube containing 1.1 mL phosphate-buffered saline (pH 7.0), protease inhibitors, surfactants, antimicrobial agents and 0.2% sodium azide as a preservative. The final optimal total amount of saliva and buffer is 2.1 mL; however, studies have shown that typically self-collected samples can yield 0.5–1.5 mL oral fluid.[13] The collected samples were processed and assayed using a modified HIV enzyme immunoassay (EIA) to detect antibody in the oral fluid.[14]

Over a two-year period, 3792 oral fluid samples were submitted for HIV antibody testing. It was noted that, upon arrival at the laboratory, some of the self-collected samples did not have the optimal 2.1 mL volume of fluid in the specimen tube or were completely empty (apart from the compressed absorbent cotton pad), whereas all of the supervised collected samples contained the correct amount of fluid. Of the samples received, 27 (0.7%) had to be discarded as there was no buffer in the collection tube. Seventy-three (1.9%) samples contained clear fluid; but all had less than the optimal 2.1 mL volume, with some having even less than 1.0 mL. In 125 (3.3%) samples, the buffer was blue but the volume of buffer and oral fluid was lower than expected. The remaining 3567 (94.1%) samples had the expected 2.1 mL blue fluid in the collection tube (Table 1).

Table 2. Breakdown of oral fluid HIV-1 antibody results.

Source	Number of samples	Number (%) positive
Insurance company	1259	9 (0.71)*
Walk-In clinic (for online healthcare company)	2283	29 (1.27)†
Hospital	250	4 (1.60)
Total	3765	42 (1.1%)

*All nine were subsequently determined to be false positives by the more sensitive and specific GACPAT method. The number of false positives was not determined for the other two groups.

†Includes one sample that gave repeatedly equivocal results (10% above the cut-off).

Results of the antibody testing are shown in Table 2. Positive results were highest in the hospital group, followed by the online medical company. Although nine samples from the insurance company were positive, all contained suboptimal sample volumes and were found to be negative by an alternative test method. Thus, in this population, 7.2% (9/125) of samples in which the buffer was blue but the volume was low were presumed to be false positives.

Over the two-year period the laboratory tested more than 3700 oral fluid samples for HIV antibodies. A few were discarded as they were unsuitable for testing due to a lack of buffer – in most of these cases the container arrived with no buffer present. It was assumed that the buffer had leaked in transport or the patient discarded it, not realising its importance.

There were also a number of samples in which the buffer had not turned blue, and the volume in the tube was clearly not the correct amount. In these cases, it was not known if the patient had taken the sample or had not kept the collection device *in situ* for the required amount of time to allow the buffer to turn blue. Some samples were blue but did not contain the correct volume of fluid. Again, in these cases it was assumed that either the patient had collected the correct amount of oral fluid but had poured away some of the buffer, or that the buffer had leaked out before arriving at the laboratory. All of these samples were tested but a caveat was added to the report to indicate that incorrect fluid volume could affect the accuracy of the result.

Of the samples tested, most were reported as negative (98.9%), with only 42 (1.1%) repeatedly positive (Table 2). Interestingly, all positive samples from the insurance company (n=9l) did not contain the correct amount of blue fluid upon receipt. The specimen (if sufficient remained) was returned to the company after testing, and it then sent the oral fluid specimen for confirmatory testing by an alternative method (i.e., immunoglobulin G antibody-capture particle adherence tests [GACPAT]).[15] In all such cases, the confirmatory test yielded negative results. Thus, the assumption was that these were biological false-positives, possibly due to the incorrect volume. Of course, it was very important that these results were handled correctly to prevent unnecessary distress to the patient.[16,17] As is often the case, there was no follow-up second oral fluid sample for these patients, or some of the other positive patients, and thus the laboratory was unable to confirm all of the oral fluid HIV results.

In conclusion, the majority of the samples received indicated compliance with the correct recommended use of the Omni-SAL collection device, containing the correct volume of blue fluid in the collection tube. The stability of the collected specimens at room temperature and the sensitivity and specificity of antibody testing using oral fluids make this an alternative approach for HIV testing outside the confines of the hospital.[18–20] However, the relatively high rate of apparent biologically false-positive results in samples containing low fluid volumes calls into question the utility of testing samples with suboptimal volumes. □

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Misidentification of *Providencia stuartii* as *Serratia fonticola* by Vitek 2

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A 58-year-old male patient suffering from hypertension and receiving angiotensin-converting enzyme inhibitor (ACEI) submitted a midstream urine sample for culture. A non-lactose fermenter (NLF) exceeding 10^5 colony-forming units (cfu)/mL grew on cysteine lactose electrolyte deficient (CLED) agar. It grew as diffuse brown colonies (presence of tryptophan deaminase) on UriSelect 4 medium (Bio-Rad Laboratories). It was also positive for indole production, lysine deamination, and oxidation-fermentation test, but negative for hydrogen sulphide production, motility, ornithine decarboxylation, and lysine decarboxylation. The presumptive identification of this NLF was *Providencia* species. The Vitek 2 Gram-negative (GN) identification card (bioMérieux) was used to identify the NLF. Surprisingly, the Vitek 2 system identified this NLF as *Serratia fonticola* with an excellent confidence level (99% probability). The same result was obtained when the GN card was repeated.

To resolve the discrepancy, the NLF was identified using the API 20E system (bioMérieux) and the Vitek MS system (bioMérieux). Both methods confirmed this NLF as *Providencia stuartii* rather than *S. fonticola*. Confidence levels of the API 20E and the Vitek MS were 97.5% and 99.9% probability, respectively.

In the GN card, only three biochemical test results (i.e., adonitol fermentation, Ellman reaction and urease activity) varied between the current *P. stuartii* strain and previously identified *P. stuartii* strains. Unlike the variable Ellman and urease results among *P. stuartii* strains, the adonitol fermentation is usually negative for *P. stuartii* strains. It was demonstrated that 5% of *P. stuartii* is positive for the adonitol fermentation, whereas 100% of *S. fonticola* is positive for the adonitol fermentation.[1] The infrequent positive result for adonitol fermentation may mislead the GN card to misidentify *P. stuartii* as *S. fonticola*, as in this case.

Although the number of biochemical tests in the API 20E is less than that in the GN card, the API 20E includes key tests that are absent from the GN card, but which are capable of discriminating *P. stuartii* and *S. fonticola* (e.g., fermentation of arabinose, melibiose and rhamnose). It was shown that for *P. stuartii* and *S. fonticola* positive rates were 1% and 100%,

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