based disinfectants, spores are more resistant and therefore require either increased concentration of free chlorine or greater contact time, or both. Previous studies on the effect of chlorine-based disinfectants on *Clostridium difficile* spores have produced highly variable data. Wheeldon *et al*.[2] showed a 2.76–2.96 $\log_{10}$ spore reduction when treated with 1000 ppm available chlorine over a 15–30 min contact time in the presence of soil. Perez *et al*.[3] reported that in order to achieve >6 $\log_{10}$ reduction in viable spores on stainless steel surfaces in the presence of soil, a 1000 ppm free-chlorine solution needed to be applied for 15–20 min. More recently, Ungurs *et al*.[4] reported that precleaning with detergent followed by sufficient exposure time with at least a 1000 ppm free-chlorine solution resulted in a 4 $\log_{10}$ reduction. In a study by Speight *et al*.,[5] 32 sanitising agents were examined for their sporicidal activity under clean and dirty conditions and showed that three products failed to reduce the viability of spores by 10^3 under any test condition.

Overall, what is apparent from these studies is that 1000 ppm free-chlorine is the pivotal concentration. Hence, in the context of the current study, any application of sanitising solution prior to achieving this optimal (1000 ppm) concentration would result in a compromised ability to kill spores and a vulnerability relating to their survival and potential to infect new hosts.

What is currently lacking with the use of chlorine-based sanitising agents in healthcare cleaning regimes is a simple and effective means to aid domestic staff in their real-time assessment of RFC concentration in sanitising solutions. Simple real-time estimation methodology of approximate RFC concentration should be developed in the form of a rapid colorimetric determination, as is used for quality control purposes for RFC determination in swimming pools, or by using RFC probes as part of hand-held electronic devices. Such easy-to-perform and real-time adoption of these devices should be included as part of the cleaning regimes within healthcare and domestic staff trained and educated to ensure optimal maintenance of RFC concentrations.

In conclusion, this study emphasises the importance of water temperature in dissolving chlorine-based NaDCC tablets in order to reach the optimum free-chlorine concentration as quickly as possible, and highlights the deactivating ability of clinical soil. Therefore, it is important that these simple messages are conveyed to domestic staff in order to optimise chlorine-based disinfection protocols employed in healthcare environments and ensure the effectiveness of this critical control in infection prevention. □

References

1 Clasen T, Edmondson P. Sodium dichloroisocyanurate (NaDCC) tablets as an alternative to sodium hypochlorite for the routine treatment of drinking water at the household level. *Int J Hyg Environ Health* 2006; **209** (2): 173–81.

2 Wheeldon LJ, Worthington T, Hilton AC, Lambert PA, Elliott TS. Sporicidal activity of two disinfectants against *Clostridium difficile* spores. *Br J Nurs* 2008; **17** (5): 316–20.

3 Perez J, Springthorpe VS, Sattar SA. Activity of selected oxidizing microbicides against the spores of *Clostridium difficile*: relevance to environmental control. *Am J Infect Control* 2005; **33** (6): 320–5.

4 Ungurs M, Wand M, Vassey M *et al*. The effectiveness of sodium dichloroisocyanurate treatments against *Clostridium difficile* spores contaminating stainless steel. *Am J Infect Control* 2011; **39** (3): 199–205.

5 Speight S, Moy A, Macken S *et al*. Evaluation of the sporicidal activity of different chemical disinfectants used in hospitals against *Clostridium difficile*. *J Hosp Infect* 2011; **79** (1): 18–22.

Detecting HIV antibodies in oral fluid: validation of a commercial antigen-antibody assay

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The use of saliva and oral fluids for detecting antibodies to human immunodeficiency virus (HIV) has long been suggested as an alternative to the use of blood.[1–3] Oral fluid is a safe, simple and convenient sample to collect for this purpose for a number of reasons. First, the occupational risks associated with needlestick accidents and injuries from phlebotomy are eliminated. Second, although oral fluid from HIV-1-infected individuals contains antibodies to HIV-1, the presence of infectious virus is rare. Third, oral fluid samples are easy to collect and the procedure is non-invasive, increasing patient comfort, acceptability of the method, and compliance with repeated testing.[4,5]

Oral fluid is a mixture consisting of the secretions of the salivary glands together with oral cavity microorganisms, cells and a gingival-crevicular transudate (GCT). The GCT is a fluid that contains immunoglobulin (IgA and IgG) and other blood components which have passed through the mucosa into the oral cavity.[6,7] It has been shown that the GCT of HIV-infected individuals contains high concentrations of HIV-specific IgG antibodies.[8] This antibody concentration, although lower than that found in serum, is quite sufficient to render GCT an adequate sample for anti-HIV antibody detection in epidemiological studies.[9,10] Studies have shown that a modified serum HIV assay can be used with acceptable sensitivity and specificity to test for HIV antibodies in GCT, regardless of the rate of prevalence of HIV-1 infection in the population under study.[11–14]

The introduction of specialised collection devices designed to improve the suitability of samples for HIV testing has seen an improvement in the sensitivity compared to tests performed on whole saliva. This is attributed to the presence of preservative fluid in the collection device, which contains antibacterial and antiproteolytic substances that protect the IgG from proteolytic degradation.[15,16]

The HIV assay used by Quest Diagnostics for three years to test saliva samples from non-hospital sites was due to be phased out by the manufacturer (Adaltis) and there was a

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Table 1. Intra-assay reproducibility and precision studies.

Control	Positive/ negative	Mean absorbance	SD	%CV
Negative	100%	0.118	0.02	20.4
Antibody-positive	100%	1.995	0.14	7.0
Antigen-positive	100%	3.246	0.07	2.2

Table 2. Inter-assay reproducibility and precision studies.

Control	Positive/ negative	Mean absorbance	SD	%CV
Negative	100%	0.093	0.02	20.2
Antibody-positive	100%	1.898	0.23	12.2
Antigen-positive	100%	2.369	0.18	7.6

need to validate a new method in order to maintain continuity of service. Originally, a number of rapid point-of-care (POC)-type assays had been considered as a replacement, but due to the large number of samples processed and tested on a daily basis, this did not seem a viable option, both in terms of time management and cost. The assay chosen to be validated was the BioRad Genscreen Ultra HIV Ag-Ab, as its predecessor (Genetic Systems HIV-1/HIV-2 Plus O EIA) had been used by other laboratories to good effect. This method offered high sensitivity and specificity (97.0% and 99.7%, respectively) when used with oral fluids.[17]

It is important however, that any modification of a standard assay is validated carefully in-house and correlated against the incumbent method to ensure any changes are effective without compromising the assay.

Oral fluid samples were collected by trained personnel in the genitourinary medicine clinic at West Middlesex University Hospital (WMUH) using the OmniSal oral fluid collector (Oral fluid Diagnostics Systems, Massachusetts) and transported to the laboratory.

The Omni-Sal collector employs 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 (approximately 1 mL). The collection pad is then inserted in a stoppered transport tube containing 1.1 mL phosphate-buffered saline (pH 7), protease inhibitors, surfactants, antimicrobial agents and 0.2% sodium azide as a preservative.

Intra-assay and inter-assay assessments were carried out using both kit controls. For the intra-assay assessment the controls were tested 20 times in one run and compared to the precision stated by the manufacturer. For the inter-assay assessment the controls were run five times over five separate runs and compared to the manufacturer's data.

Two oral fluid samples were collected from known HIV-positive patients using the OmniSal collectors and sent to the laboratory. The oral fluid was extracted and serially diluted (neat to 1 in 1024) in OmniSal buffer, tested in duplicate over five days and stored at 2–8°C.

The BioRad Genscreen Ultra HIV Ag-Ab assay recommends using 75 µL serum as the sample; however, as this was an oral fluid sample, a checkerboard titration of the known positive samples was carried out to determine optimum volume. Serial dilutions (neat to 1 in 1024) of the samples were made in OmniSal buffer and were added at 75, 100, 125, 150, 175, 200, 225 and 250 µL volumes and tested in duplicate.

Twenty-five oral fluid samples (20 negative, five positives [confirmed by a reference laboratory GACPAT test]) previously tested with the Adaltis assay were retested using the new modified BioRad Genscreen Ultra HIV Ag-Ab assay. Forty patients (25 HIV seropositive patients, 15 seronegative) were asked to provide oral fluid samples, and these were tested using the new modified method.

Control samples were run with each batch of specimens. A mixed positive control of HIV-1 and HIV-2 (supplied by NIBSC) was run with the kit controls and a negative control (BioRad Viroclear) was run to ensure the assay had been successful. These controls were processed and assayed as specimens. The run was considered valid if the following criteria were met: i) absorbance of the kit positive controls was greater than 0.90; ii) absorbance of external positive and negative controls was greater than 0.90 and less that 0.15, respectively.

Reproducibility and precision studies showed 100%

Table 3. Bayesian plot of patient results: **a)** positive/negative agreement between assays; **b)** positive/negative agreement between saliva results and serum results.

A

			Primary method	
			Adaltis assay	
			Positive	Negative
Secondary method	BioRad HIV-1/ HIV-2 Ag/Ab assay	Positive	5	0
		Negative	0	20

B

			Primary method	
			Abbott AxSym (serum result)	
			Positive	Negative
Secondary method	BioRad HIV-1/ HIV-2 Ag/Ab assay	Positive	25	0
		Negative	0	15

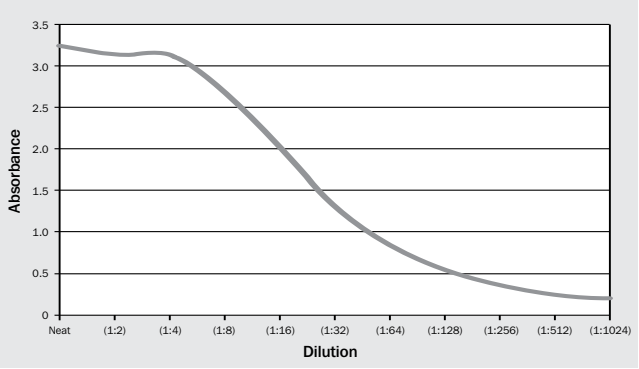

Fig. 1. Limit of detection using a 200 µL sample volume in a modified assay.

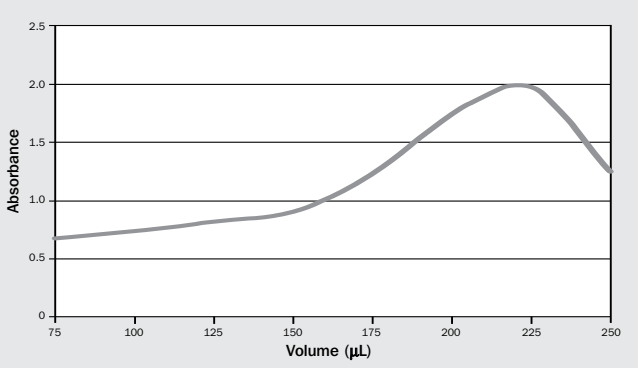

Fig. 2. Hook effect seen at higher volumes of saliva.

agreement with the manufacturer's quoted data ranges for the kit controls (Tables 1 and 2). The limit of detection showed that the modified assay would detect HIV antibodies down to a dilution of 1 in 256 (Fig. 1) when using the larger sample amounts, but could still detect HIV antibodies even if a smaller volume was used. However, at a dilution greater than 1 in 256 the coefficient of variation (CV) between the absorbance values was greater than 20%. The checkerboard titration showed the optimal volume of oral fluid to be 200 µL, although larger volumes (225 and 250 µL) were also tested. There was a definite decrease in absorbance when using larger volumes, as shown when the average of all the absorbance values was calculated and plotted (Fig 2). All the samples tested showed 100% agreement, both with the old assay and the new modified assay (Table 3a and b).

Using oral fluid instead of serum or plasma to test for antibodies to HIV has many advantages. The samples are easy to obtain, the procedure is less invasive and can be collected in a non-clinic setting or by the patient. This would be useful for needle-phobic patients and those difficult to bleed, including children, the obese and the elderly. They are also more likely to be willing to give multiple samples for sequential studies. The use of a collection device has also improved the type of sample obtained by providing stability for the antibodies that may be present. Certainly, the UK Health Protection Agency (now Public Health England) has shown in pilot projects that the use of salivary testing increases patient acceptability and can be implemented quite readily, but the risk of false positives can be increased particularly in areas of low prevalence.[18]

Owing to the lower concentration of antibody in oral fluid, a range of larger-than-recommended sample volumes was tested to investigate the effect on the sensitivity of the assay. As expected, an increase in absorbance with volume was noted; however. at volumes greater than 200 µL there was what appeared to be a hook effect, and absorbance values began to decrease. This could have been due to the relative proportions of antigen and the antibody being incorrect, as an excess of either will impair adequate immune complex formation. This has been recognised in two-step sandwich and one-step immunoassays.[19,20] Also, when using the higher volumes (225 µL and 250 µL) the microtitre wells were very full, and this could have led to carryover or splashing during the automated wash cycle. Therefore, a 200 µL volume became the standard for this assay.

These results show that a simple modification to the BioRad Genscreen Ultra HIV Ag-Ab assay, simply by increasing the sample volume for oral fluid, would allow this kit to be used as an alternative to the current saliva HIV test when used in conjunction with the OmniSal collection device. The results obtained when testing seropositive patients were concordant with their serum results, and the laboratory was able to show that these antibodies could still be detected at low concentration (1 in 256) in their oral fluid samples. Interestingly, the assay did detect HIV antibodies when the 75-µL sample volume was used at a 1 in 16 dilution. Some studies suggest that the cut-off be decreased by up to 30% to allow the lower concentration of oral fluid antibody to be more readily detected;[17] however, the results obtained during this validation suggest that this change is unnecessary and could increase the number of false-positive results that would need confirmation or repeat by an alternative method.

This assay would not be suitable for detection of early infection in the 'window period' as this is dependent on a short time period after HIV acquisition that an individual is tested. Also, studies have shown that oral fluid can have an inhibitory effect on the HIV antigen which could make detection more difficult.[21] For antibody detection, however, the safety of using oral fluid, the ease of collection, and the sensitivity and specificity of the modified HIV kit made the assay an ideal alternative for general HIV testing for surveillance studies outside the hospital and in a variety of other settings. Certainly, in a non-clinic setting, the ability to self-take a sample without supervision would be beneficial. However, positive saliva samples taken during screening would need to be confirmed by subsequent blood tests taken by a clinician or phlebotomist. □

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References

1 Tamashiro H, Constantine NT. Serological diagnosis of HIV infection using oral fluid samples. *Bull World Health Organ* 1994; **72** (1): 135–43.

2 Pink R, Simek J, Vondrakova J *et al*. Saliva as a diagnostic medium. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2009; **153** (2): 103–10.

3 Malamud D. Saliva as a diagnostic fluid. *Dent Clin North Am* 2011; **55** (1): 159–78.
4 Pant Pai N. Oral fluid-based rapid HIV testing: issues, challenges and research directions. *Expert Rev Mol Diagn* 2007; **7** (4): 325–8.
5 Debattista J, Bryson G, Roudenko N *et al*. Pilot of non-invasive (oral fluid) testing for HIV within a clinical setting. *Sex Health* 2007; **4** (2): 105–9.
6 Holm-Hansen C, Tong G, Davis C, Abrams WR, Malamud D. Comparison of oral fluid collectors for use in a rapid point-of-care diagnostic device. *Clin Diag Lab Immunol* 2004; **11** (5): 909–12.
7 Segal A, Wong DT. Salivary diagnostics: enhancing disease detection and making medicine better. *Eur J Dent Educ* 2008; **12** (Suppl 1): 22–9.
8 Granade TC, Phillips SK, Parekh B, Pau CP, George JR. Oral fluid as a specimen for detection and confirmation of antibodies to HIV type 1. *Clin Diagn Lab Immunol* 1995; **2** (4): 395–9.
9 Soto-Ramírez LE, Hernández-Gómez L, Sifuentes-Osornio J *et al*. Detection of specific antibodies in gingival crevicular transudate by ELISA for diagnosis of HIV type 1 infection. *J Clin Microbiol* 1992; **30** (11): 2780–3.
10 Martínez PM, Torres AR, Ortiz de Lejarazu R, Montoya A, Martin JF, Eiros JM. Human HIV antobody testing by enzyme linked fluoresecent and Western blot assays using serum, gingival-crevicular transudate and urine samples. *J Clin Microbiol* 1999; **37** (4): 1100–6.
11 Hunt AJ, Connell J, Christofinis G *et al*. The testing of saliva samples for HIV-1 antibodies: reliability in a non-clinic setting. *Genitourin Med* 1993; **69** (1): 29–30.
12 Pasquier C, Bello PY, Gourney P, Puel J, Izopet J. A new generation of serum anti-HIV antibody immunocapture assay for saliva testing. *Clin Diagn Virol* 1997; **8** (3): 195–7.
13 Granade TC, Phillips SK, Parekh B *et al*. Detection of antibodies to HIV type 1 in oral fluids: a large-scale evaluation of immunoassay performance. *Clin Diagn Lab Immunol* 1998; **5** (2): 171–5.
14 Wesolowski LG, Sanchz T, MacKellar DA *et al*. Evaluation of oral fluid enzyme immuinoassay for confirmation of a positive rapid HIV test result. *Clin Vaccine Immunol* 2009; **16** (7): 1091–2.
15 Chamnanpunt J, Phanuphak P. Value of saliva collection device, Omnisal, in preserving the anti-HIV activities of stored saliva. *Int Conf AIDS* 1993; **9**: 539 (www.aegis.org/search/Default.aspx?key=Omnisal).
16 Chohan BH, Lavryes L, Kishorchandra N *et al*. Validation of a modified commercial enzyme-linked immunoassay for detection of human immunodeficiency virus type 1 immunoglobulin G antibodies from oral fluid. *Clin Diagn Lab Immunol* 2001; **8** (2): 346–8.
17 Louie B, Lei J, Liska S, Dowling T, Pandori MW. Assessment of sensitivity and specificity of first, second and third generation EIA for the detection of antibodies to HIV-1 in oral fluid. *J Virol Methods* 2009; **159** (1): 119–21.
18 Health Protection Agency. Evidence and resources to commission expanded HIV testing in priority medical services in high prevalence areas. London: HPA, 2012
19 Spencer DV, Nolte FS, Zhu Y. Heterophilic antibody interference causing false-positive rapid human immunodeficiency virus antibody testing. *Clin Chim Acta* 2009; **399** (1–2): 121–2.
20 Fernando SA, Wilson GS. Multiple epitope interactions in the two-step sandwich immunoassay. *J Immunol Methods* 1992; **151** (1–2): 67–86.
21 Malamud D, Abrams WR, Barber CA, Weissman D, Rehtanz M, Golub E. Antiviral activities in human saliva. *Adv Dent Res* 2011; **23** (1): 34–7.

Identification of *Clostridium difficile*: evaluation of genotypic, phenotypic and proteomic methods

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Clostridium difficile infection (CDI) is an important cause of nosocomial diarrhoea. Stool culture, a sensitive method for the detection of *C. difficile*, is necessary for epidemiological investigation, for monitoring antibiotic resistance, and for providing a reference standard against which to validate assays. It is, however, expensive, time-consuming, requires toxin testing and also technical expertise.[1]

The clinical significance of culture remains unclear as carriage may occur.[2] Although the UK national standard operating procedure (SOP)[3] provides guidance for culture and identification of *C. difficile*, wide variations in methods of identification were reported in a survey of eight European countries.[4] The aim of this study is to compare phenotypic identification using the UK national SOP[3] with identification by 16S ribosomal DNA (rDNA) sequencing and matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS).

The local research ethics committee confirmed that, as the study represented a service evaluation of recognised diagnostic methodologies performed on anonymised excess diagnostic materials, further formal ethical approval was not required. Consecutive anonymised faeces samples from patients aged ≥18 years submitted to pathology laboratories at St George's Hospital, London, for faecal occult blood (FOB) or *Helicobacter pylori* antigen detection between 4 January 2010 and 9 February 2010 were analysed. Additionally, cytotoxin enzyme immunoassay (EIA)-positive faeces samples from patients suspected of CDI, and stored *C. difficile*-positive samples (collected between August and December 2009) were also included. Repeat samples received ≤ 28 days after the first sample were excluded. Samples (stored at 4°C) were analysed within five days of collection.

The samples were cultured for *C. difficile* after alcohol shock[3] for spore selection. The alcohol-faeces suspension (50 µL) was streaked on cycloserine cefoxitin fructose agar (CCFA; Oxoid, Basingstoke, UK) and Brazier's cycloserine cefoxitin egg yolk plate (CCEY; E&O Laboratories, Bonnybridge, Scotland). Plates were incubated anaerobically at 37°C for 48 h and examined by two readers (blinded). Positive controls for both selective media were used daily.

The colonies were identified by typical morphology (CCFA: ~2–4 mm in diameter, non-haemolytic, grey/white with rhizoid edge; CCEY: ~1.5–3 mm in size, grey, flat growth, no opacity around colonies). Suspected colonies were identified further after anaerobic subculture for up to

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