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Importance of the cutoff ratio for detecting antibodies against hepatitis A virus in oral fluids by enzyme immunoassay☆

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Multiple studies have examined the use of oral fluids in modified serum-based assays aiming to replace serum in antibody detection for hepatitis A. However, the reliable detection of HAV immunity in oral fluid requires an extremely sensitive assay; most immunoassays designed for serum antibody determination lack sufficient sensitivity for this purpose. Consequently, an "in-house" competitive enzyme immunoassay (EIA) designed specifically for use with oral samples collected using a ChemBio® device was developed to detect total anti-HAV antibodies (IgG and IgM). This system was compared to an in-house competitive EIA and a commercial EIA considered to be the "gold standard" using corresponding serum samples ($n = 225$) to determine the accuracy of the assay and to evaluate the importance of the cutoff ratio for the detection of anti-HAV antibodies in oral fluids. When the median serum cutoff and the optimal oral fluid cutoff (ROC analysis) obtained from the in-house competitive EIA were compared, the oral fluid cutoff was found to be 28.8% higher than the serum cutoff. When different oral fluid cutoff values were compared, a reduction of about 17% was shown to be essential to increase test accuracy. At an oral fluid cutoff value of 0.351, sensitivity and specificity were higher, reaching 91.7% and 86.2% ($p < 0.001$, AUROC = 0.915), respectively. The convenience, accuracy and non-invasive nature of the developed method make it a useful alternative to serum-based assays for discriminating between HAV-immune and non-immune individuals.

1. Introduction

Hepatitis A virus (HAV) belongs to the *Hepatovirus* genus, family *Picornaviridae*. Acute infection is diagnosed by detecting anti-HAV immunoglobulin M (IgM), while anti-HAV Immunoglobulin G (IgG) is the major class of antibody generated during the convalescent phase (Fiore, 2004; Iino, 2004). Conventionally, hepatitis A diagnosis is performed using serum; but the collection of blood by venipuncture is invasive, expensive and potentially painful (De Cock et al., 2004). Consequently, there is a need for alternative fluids for testing that do not involve invasive procedures and are easier to collect, such as oral fluid.

Oral fluid contains saliva from the salivary glands and gingival crevicular fluid, which is a transudative plasma derived from the capillary bed beneath the tooth–gum margin (Roitt and Lehner, 1983). Gingival crevicular fluid contributes most of the diagnostically important plasma-derived IgG and IgM anti-HAV antibodies (Parry, 1993). The advantage of using oral fluid to evaluate humoral immunity induced by HAV infection is the safety and painlessness of collection, making this a satisfactory and convenient alternative to testing blood (Parry et al., 1989), especially in children and other groups from whom it is difficult to collect blood specimens (Ochnio et al., 1997).

Several investigations have evaluated the use of oral samples for hepatitis A diagnosis and epidemiological studies (Amado et al., 2006; Hurni et al., 1993; Laufer et al., 1995; Ochnio et al., 1997; Parry et al., 1987). However, the kinetics of HAV infection dictate a critical time for the detection antibodies in oral fluids, because immunity induced by HAV infection in the convalescent phase is weaker than that found in persons infected recently (Zaaijer et al., 1993); this phenomenon is more pronounced in oral fluids, in which antibody concentrations are approximately 800- to 1000-

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fold lower than those found in serum and plasma (Challacombe et al., 1978; Parry, 1993; Roitt and Lehner, 1983). Therefore, oral fluid assays should be optimised to improve the sensitivity and specificity of their results.

Although commercial total anti-HAV (detecting both IgM and IgG) tests are highly sensitive and specific for detecting acute HAV infection in oral fluids, they are less able to detect previous HAV infection because these tests are designed specifically for serum samples presenting a limit of antibody detection greater than those found in oral fluid samples from patients in the convalescent phase. This fact indicates the need for more sensitive tests. Oral fluid samples could offer an efficient alternative to conventional serum assays because they allow the sensitivity to be adjusted for the detection of low-titre antibodies.

The aims of this study were to develop and optimise an in-house competitive EIA for the detection of anti-HAV antibodies in oral fluid and to demonstrate the importance of establishing a cut-off ratio for the detection of antibodies against HAV in oral fluid specimens.

2. Materials and methods

2.1. Study population

The population studied was made up of 225 volunteers, 140 patients and 85 healthy professionals, who participated in World Hepatitis Day (May 19th, 2009) at a hospital located in Rio de Janeiro (Brazil) after obtaining informed consent from each individual. Ethical permission for collecting and testing samples for assay development was obtained from the Hospital authorities and the Fiocruz Ethics Committee (536/09).

2.2. Sample collection

Paired serum and oral samples were collected from each individual. Five millilitres of peripheral blood was drawn by venipuncture using hypodermic needles and multi-type Vacutainer sterile tubes. Subsequently, the samples were centrifuged at $1800 \times g$ at 25 °C for 5 min, and the sera were stored at −20 °C. An oral fluid sample was obtained from each individual using a commercial device, ChemBio® (ChemBio® Diagnostic Systems, Inc., NY, USA), which consisted of a sponge swab attached to a handle. Participants were instructed to rub the swab along the tooth/gum line for approximately 1 min, after which the swab was returned to the plastic tube containing 500 μL of preservative solution and transported to the laboratory at 4 °C. The oral fluid was concentrated at the bottom of a plastic tube by centrifugation at $1300 \times g$ at 25 °C for 10 min and stored at 4–8 °C.

2.3. Detection of anti-HAV antibodies in serum

Sera were subjected to an in-house competitive EIA as described previously by Vitral et al. (1991) and to a commercially available EIA (Bioelisa HAV, Biokit S.A., Barcelona, Spain) in accordance with the manufacturer's instructions for detection of total anti-HAV antibodies. Considering that the total anti-HAV Bioelisa test is recognised to have more than 99% sensitivity and specificity, it was used as the gold standard kit for comparison with the in-house competitive EIA using oral fluid samples.

2.4. Development of an in-house EIA for anti-HAV antibody detection in oral fluids

For oral fluid samples, an in-house competitive EIA was designed for total anti-HAV detection, and its sensitivity and specificity were compared with those of matched serum samples.

2.4.1. Production of HAV antigen

Briefly, FRhK-4 (Foetal Rhesus Kidney-4) cells were grown to confluence in disposable 150 cm^2 Falcon flasks with growth medium (medium 199 with 10% foetal bovine serum). Monolayers were washed once with 0.01 M phosphate-buffered saline (PBS), pH 7.2, and inoculated with 3.0 ml of HAV strain HAF-203 (Gaspar et al., 1993). The virus was adsorbed for 1 h at 37 °C, and 40 ml of maintenance medium (medium 199 with 2% foetal bovine serum) was then added without removal of the inoculum. Cultures were kept at 37 °C for 10 days. The cell-associated virus was extracted by freeze–thawing the bottle contents, including the medium supernatant, four times. The harvested fluid was subjected to a short burst of sonication followed by a brief low-speed centrifugation, and the supernatant was aliquoted and stored at −70 °C. TaqMan real-time PCR was conducted to quantify viral particles as described previously by De Paula et al. (2007). To determine the efficiency of the HAV antigen in the competitive EIA, checkerboard titrations were performed between HAV antigen (ranging from 1:1 to 1:32) and undiluted positive and negative oral fluid controls.

2.4.2. Anti-HAV IgG capture and conjugated antibody

A pool of convalescent serum was obtained from patients with serologically confirmed hepatitis A exposure and an observed anti-HAV titre of ≥1:10,000 by commercial EIA (Bioelisa HAV, Biokit) approximately 100 days after the onset of the disease. Immunoglobulin G was purified from 10 ml of this serum by the protein A chromatographic method as per the manufacturer's instructions (Affi-Prep Protein A Matrix, Bio-Rad Laboratories, CA, USA). Purified anti-HAV IgG antibody (10.240 μg/μL) was used to cover the microplates as a capture antibody.

Human anti-HAV IgG (4 mg/ml) was conjugated to 8 mg/ml horseradish peroxidase (type VI; Sigma–Aldrich, USA) by the method of Nakane and Kawaoi (1974) and stored at −20 °C. The efficiency of the resulting capture antibody and conjugate was evaluated by checkerboard titration (dilutions ranging from 1:500 to 1:8000) in the in-house competitive EIA.

2.4.3. HAV competitive antibody EIA

The surface of the microplate was coated with 100 μL of specific purified anti-HAV IgG antibody (10.240 μg/μL) diluted 1:1000 and incubated overnight at 4 °C. Unbound waste was removed by rinsing the plate with PBS, pH 7.2. Then, 100 μL of undiluted oral fluid positive (oral fluid from convalescent volunteers in duplicate) and negative controls (oral fluid from non-infected individuals applied in triplicate) along with 50 μL of undiluted HAVAg (2×10^6 copies/ml) produced in FRhK-4 cells was applied to the plate. After 2 h at 37 °C, the unbound antigen–antibody complex was washed as described above. Anti-HAV human polyclonal antibody conjugated with peroxidase (586 μg/μL) was diluted 1:2000 in phosphate-buffered saline containing normal goat serum (1%) and normal human serum (1%). Then, 100 μL of conjugate was added onto the plate and incubated for 1 h at 37 °C. After washing, the reaction was visualised using 100 μL of 3,3′,5,5′-tetramethyl-benzidine substrate (Sigma–Aldrich, USA) and stopped after 15 min at room temperature with 100 μL of 1 N H_2SO_4. The plate was read at 450 nm using a reference filter of 620 nm. The background was determined by the optical density of the blank, and it was subtracted from all samples tested in the in-house competitive EIA. Serum and matched oral fluid samples were tested by in-house competitive EIA to compare serum and oral fluid median cut-offs.

2.5. Analysis of the HAV in-house competitive EIA

The cut-off point for HAV antibody detection was evaluated based on two models. In the first approach (model 1), the cut-off was calculated according to a commercial EIA manufacturer's

instructions {O.5 (CNX + CPX)} (Bioelisa HAV, Biokit S.A., Barcelona, Spain), where CNX and CPX are the averages of the negative and positive controls, respectively. Samples were classified following optical density/cutoff (OD/cutoff) criteria; positive samples presented OD/cutoff values equal to or lower than 1.0, negative samples presented OD/cutoff values higher than 1.1 and equivocal samples showed OD/cutoff values between 1.0 and 1.1. Fisher's Exact test (GraphPad Software, CA, USA) was applied to calculate sensitivity, specificity and predicted values of the in-house competitive EIA using the cutoff value calculated by this first model.

The second approach (model 2) compared results from matched serum and oral fluid samples using receiver operating characteristic curve (ROC curve) analysis (Medcalc, Mariakerke, Belgium). The ability of the model to differentiate between positive and negative individuals for total anti-HAV (discrimination) was quantified using the area under the curve (AUROC) test (Hanley and Mcneil, 1982; Steyerberg et al., 2001). AUROC values can range from 0.5 (purely random discrimination) to 1.0 (perfect discrimination). ROC curve analysis was applied to calculate sensitivity, specificity and predicted values of the competitive EIA using the cutoff calculated by this second model. The false-positive matched samples were tested with a commercially available immunoblot assay (ImmunoComb II HAV Ab, Orgenics, Israel) to exclude the possibility of false-positive results from the in-house EIA after ROC analysis.

2.6. Performance of in-house HAV competitive EIA

The Kolmogorov–Smirnov test was used to test serum and oral fluid OD/cutoff ratios for normality. Spearman's rank correlation was applied when appropriate to assess the correlation between serum and oral fluid OD/cutoff values. The Kappa coefficient (κ) was used to assess the degree of agreement between oral fluid and serum antibody status following the interpretation: $\kappa < 20\%$ poor; $\kappa = 21$–40% fair; $\kappa = 41$–60% moderate; $\kappa = 61$–80% good and 81–100% excellent agreement (Altman, 1991). The coefficient of variation (CV%) was used to analyse the inter- and intra-assay reproducibility of the in-house competitive EIA. Univariate logistic regression was performed using serum samples as the independent variable. Two-tailed p-values < 0.05 were considered statistically significant. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS for Windows, 13.0; SPSS Inc., Chicago, IL) and GraphPad Prism (GraphPad Software, CA, USA).

3. Results

3.1. Evaluation of the inputs produced

The antigenicity of HAV as an EIA antigen was confirmed by checkerboard titration with undiluted positive and negative oral fluid controls. The positive controls reacted only with undiluted HAV antigen, and this reaction correlates with their ability to neutralise the virus. Real-time PCR was used to quantify HAV RNA accumulation in FRhK-4 cells. RNA extracted from supernatant 10 days post-infection showed an expected viral load of 2×10^6 copies/ml for HAV EIA. For these reasons, HAV antigen was used undiluted in the in-house competitive EIA.

Preliminary tests in a checkerboard titration were also used to determine the optimal dilutions of anti-HAV capture antibody and anti-HAV IgG conjugate. Optimal performance was obtained using anti-HAV IgG at a coating dilution of 1:1000 and anti-HAV IgG conjugate at 1:2000. The antibody dilution chosen was that which discriminated optimally between the positive and negative control ODs.

3.2. Serological data

Serum and oral fluid samples were obtained from 225 volunteers, of which 132 (58.7%) were female and 93 (41.3%) were male. The age of the studied population ranged from 8 to 87 years with a median age of 39 years. Total anti-HAV antibodies were detected in 145 sera by commercial EIA (Bioelisa HAV, Biokit), showing a hepatitis A prevalence of 64.4%. The results of all serum samples tested by the in-house competitive EIA were concordant with those obtained in the commercial assay.

3.3. Analysis of in-house competitive EIA

Oral fluid samples were examined using the in-house competitive EIA for total anti-HAV antibodies and these results were compared with matched serum samples tested by the in-house competitive EIA (Vitral et al., 1991) and by the commercial EIA. To establish an optimum cutoff for oral fluid in the competitive EIA, the oral fluid cutoffs calculated by the two approaches were compared to serum cutoffs in the in-house competitive EIA and to each other.

In the in-house competitive EIA, using the cutoff value calculated according to the manufacturer's instructions (model 1), the median cutoff for oral fluid controls was 0.423, ranging from 0.080 to 0.440 (95% CI 0.310–0.343, $p < 0.001$), whereas the median cutoff for serum controls was 0.250, ranging from 0.151 to 0.368 (95% CI 0.263–0.285, $p < 0.001$). The median optical density of the blank, which was discounted in all samples tested by the in-house competitive EIA, was 0.022. However, receiver operating characteristic curve analysis (model 2) showed that lowering the cutoff point from 0.423 (calculated according to the manufacturer's instructions) to 0.351 (calculated by ROC analysis) would give better discrimination for oral fluid samples by the competitive EIA. Median analysis demonstrated that the oral fluid cutoff value was significantly higher than that for serum, showing that this specimen has lower antibody titres than serum.

The frequency of true-positive results for total anti-HAV in oral fluid was 57.6% according to the manufacturer's cutoff, corresponding to 125 reactive competitive EIA tests out of 217 samples tested (8 indeterminate results). However, adopting the optimal cutoff of 0.351, this frequency increased to 59.1%, corresponding to 133 reactive competitive EIA tests out of 225 samples tested. Table 1 shows concordantly negative, concordantly positive and discordant results of matched serum and oral fluid samples tested in the competitive EIA according to the cutoff. The 11 false-positive matched samples were subjected to an immunoblot (ImmunoComb II HAV Ab, Orgenics, Israel), and the results confirmed the false-positivity of these oral fluid samples.

3.4. Evaluation of test performance

Spearman's rank coefficient analysis was applied to correlate the commercial and in-house competitive EIA results using the cutoff calculated according to the manufacturer's instructions. Although the OD/cutoff values varied between the assays (Fig. 1), a significant positive correlation was found among anti-HAV OD/cutoff ratios of the specimens (rho = 0.659, $p < 0.0001$) (Fig. 2).

Using ROC curve analysis for the in-house competitive EIA, a cutoff value of 0.351 yielded the highest combined sensitivity and specificity for oral fluid samples. The sensitivity for each cutoff value was plotted versus one minus the specificity as a ROC plot (Fig. 3). A cutoff value of 0.250 ($p < 0.0001$, AUROC = 1.0, 95% CI 0.858–1.000) was demonstrated by ROC analysis to be optimum for serum samples in the in-house competitive EIA (Fig. 4).

The competitive EIA model for oral fluid provided good discrimination capacity with an AUROC value above 0.910 ($p < 0.0001$,

Table 1
Results of the in-house competitive EIA according to the oral fluid cutoff value.

Manufacturer's cutoff (0.423; $n = 225$)				ROC analysis cutoff (0.351; $n = 225$)			
	Oral fluid +	Oral fluid −	Total		Oral fluid +	Oral fluid −	Total
Serum +	125 (57.6%)	12 (5.5%)	137 (63.1%)	Serum +	133 (59.1%)	12 (5.3%)	145 (64.4%)
Serum −	26 (12%)	54 (24.9%)	80 (36.9%)	Serum −	11 (4.9%)	69 (30.7%)	80 (35.6%)
Total	151 (69.6%)	66 (30.4%)	217 (100%)[a]	Total	144 (64%)	81 (36%)	225 (100%)

Manufacturer's cutoff = {0.5 (CNX + CPX)}, where CNX and CPX is the average of negative and positive controls, respectively. ROC analysis cutoff = calculated based on the effect of varying the threshold on the numerical outcome of the test.

[a] Indeterminate results = 8.

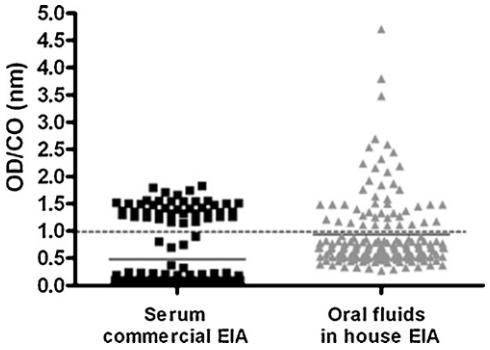

Fig. 1. OD/cutoff ratios of each serum and oral fluid sample plotted according to the respective EIA based on the manufacturer's cutoff. The *y*-axis represents the OD/cutoff ratio, the dashed line is OD/cutoff = 1. Samples with OD/cutoff ratios below 1.0 are considered positive for anti-HAV. The solid lines represent the average OD/cutoff ratios for the serum and oral fluid samples, which were 0.481/0.938, respectively.

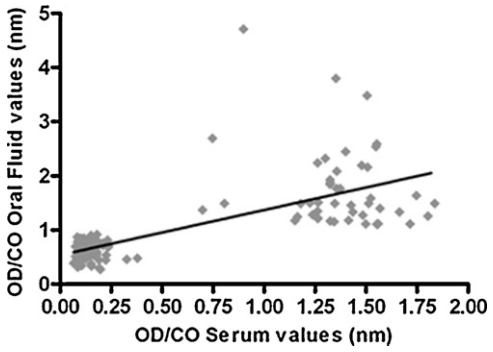

Fig. 2. Scatter plot of matched oral fluid and serum optical density/cutoff values (nm), showing positive correlation between the in-house competitive EIA and commercial EIA results. The OD/cutoff ratios (OD/CO) of the samples are plotted as a regression line (rho = 0.659, $p < 0.0001$).

AUROC = 0.915, 95% CI 0.836–0.964). Using this cutoff value (0.351), the AUROC value of the competitive EIA model is closer to the ideal of 1.0. However, the accuracy of the test would be compromised if the manufacturer-recommended cutoff, calculated for oral fluid (0.423), was adopted in the ROC analysis. The optimal cut-off calculated using ROC curve analysis improved test performance: sensitivity increased from 91.2% to 91.7% and specificity increased from 67.5% to 86.2% compared to the first model (Table 2).

The intra- and inter-assay reproducibility values from the oral fluid samples resulted in overall coefficients of variation value of 4.23% and 14.9%, respectively.

4. Discussion

Serological testing is the gold standard method of screening for HAV infection, but several studies have analysed oral fluid specimens by modified serum-based assays (Amado et al., 2006; Chohan

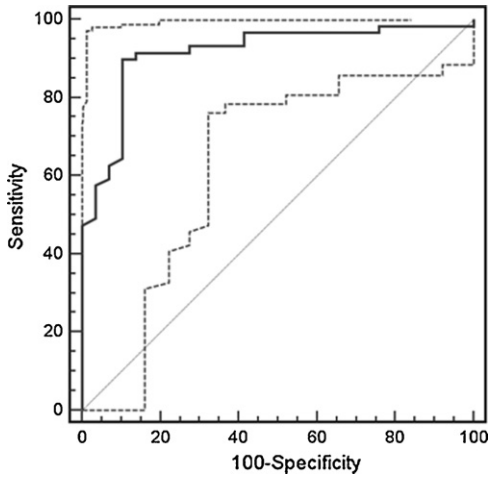

Fig. 3. Receiver operating characteristic curve analysis of anti-HAV antibody detection in the in-house competitive EIA using oral fluid samples. The area under the curve is 0.915 ($p < 0.0001$, 95% CI: 0.836–0.964). The solid line is the ROC curve and the dashed lines are the 95% CI limits.

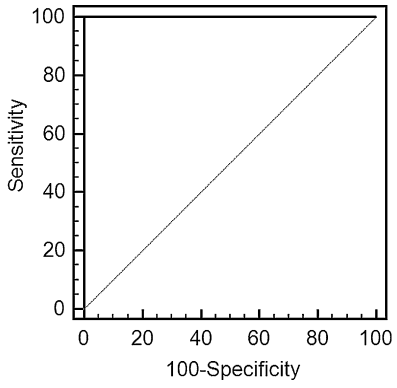

Fig. 4. Receiver operating characteristic curve analysis of anti-HAV antibody detection in the in-house competitive EIA using serum samples. The area under the curve is 1.000 ($p < 0.0001$, 95% CI 0.858–1.000). The solid line is the ROC curve and the dashed lines are the 95% CI limits that are overlapping on the figure.

Table 2
Validation parameters of the in-house competitive EIA among oral fluid samples.

	Manufacturer's cutoff (0.423; $n = 225$)[a]	ROC analysis cutoff (0.351; $n = 225$)
Sensitivity (%)	91.2	91.7
Specificity (%)	67.5	86.2
Positive predictive value (%)	91.4	92.4
Negative predictive value (%)	82.7	85.2
Concordance (%)	82.4	89.8
κ Coefficient (%)	61.6	77.8

[a] Indeterminate results = 8.

et al., 2001; Judd et al., 2003; McIntyre et al., 1996; Parry, 1993). However, reliable detection of immunity induced by HAV in oral fluids requires an extremely sensitive assay and most immunoassays designed for serum antibody determination lack sufficient sensitivity for this purpose (Ochnio et al., 1997). The oral fluid samples analysed in this study were tested first by a commercial EIA without modifications and demonstrated a low level of sensitivity (40%). Consequently, an in-house competitive EIA using oral fluid samples to detect anti-HAV antibodies was developed.

Serum and oral fluid samples tested by the in-house competitive EIA were used to compare median serum and oral fluid cutoffs. The median serum and oral fluid cutoffs by the in-house EIA were 0.250 (as demonstrated similarly by Vitral et al., 1991) and 0.351, respectively. Because the in-house EIA is a competitive assay, non-reactive samples presented a yellow colour as opposed to reactive ones. Thus, it was observed that the oral fluid cutoff value was 28.8% higher than serum cutoff value, confirming that the serum controls used in commercial assays are not appropriate for hepatitis A surveillance using oral fluids due to the difference in antibody titres.

One critical aspect of detecting antibodies in oral fluids is the collection device. Numerous acceptable and easy-to-use devices have been used to facilitate oral fluid collection and to increase the concentration of immunoglobulins; cotton swabs are among the most common (Kozaki et al., 2009). Some collection devices, such as Salivette® (Sarsdedt, Germany), Orasure® (Orasure Technologies Inc., Bethlehem, PA, USA) and Omni-SAL® (Saliva Diagnostic Systems, Singapore), have been used to collect oral fluids to detect anti-HAV antibodies using immunoassays (Amado et al., 2006; Oba et al., 2000; Ochnio et al., 1997; Parry, 1993). In these studies, different levels of sensitivity and specificity were observed, showing that oral collection devices may interfere with antibody detection, but appropriate oral fluid collection devices can offer suitable samples for diagnosis (Tamashiro and Constantine, 1994).

The results presented above, supported by the Kappa coefficient (77.8%), indicate a close agreement between serum and oral fluid. This fact suggests that oral fluid collected by a device that targets specifically crevicular fluid may provide the sensitivity needed. Other collection devices that collect crevicular fluid, such as OraSure® (Epitope Incorporated, Beaverton, OR, US), have had relative success in increasing sensitivity by drawing the secretion of plasma immunoglobulins into the mouth, as was also shown by Vyse et al. (2001). Therefore, it is important to adapt the collector device to the test used.

In this study, oral fluids were collected with a ChemBio® device (ChemBio® Diagnostic Systems, Inc., NY), and two approaches were used to determine the optimum assay cutoffs along with the highest sensitivity, specificity and accuracy. The selection of the cutoff point can change the accuracy of the test. By reducing the oral fluid cutoff by about 17%, from 0.423 (calculated according to manufacturer's instruction) to 0.351 (calculated by ROC analysis), sensitivity increased from 91.2% to 91.7% due to indeterminate samples that, after ROC curve analysis, were determined to be truly positive. Specificity increased from 67.5% to 86.2% due to a reduction in the false-positive rate. The in-house competitive EIA was developed to improve the sensitivity for detecting low antibody titres in oral fluid. To obtain adequate sensitivity for the test, false-positive results were obtained (manufacturer's cutoff), which were minimised by lowering the cutoff value (ROC analysis), giving appropriate sensitivity and specificity values.

The in-house competitive EIA showed better sensitivity (91.7%) compared to the commercial EIA using the OraSure® collection device (86%) (Amado et al., 2006). Previous studies using the Salivette® collection device (Sarsdedt, Germany) in an in-house EIA reported sensitivity and specificity of 98.7% and 99.6%, respectively, (Ochnio et al., 1997). The Omni-SAL collection device (Saliva Diagnostic Systems, Singapore) provided sensitivity and specificity values of 82.1% and 100%, respectively (Oba et al., 2000). The higher specificity found in the previous study might be related to the smaller anti-HAV-negative population ($n = 15$) compared to the size of the negative population in this study ($n = 80$).

In this study, few false-positive or -negative results were observed. Parry et al. (1988) also reported low rates of false positivity and negativity after immunoglobulin prophylaxis in salivary RIA. Even with the optimum cutoff determined by the upper limit of the ROC plot, discordant oral fluid results represented 10.2% (23/225) of the study population, unlike with the serum test. False-positive oral fluid test results might be due to non-specific interaction between HAV antigen and oral fluid elements, as demonstrated in previous studies (Ochnio et al., 1997). The false negative salivary test results observed might reflect nonspecific waste bound to the class-specific antibodies used to coat the microplate or could be linked to the low concentration of antibodies in some oral fluid samples (Parry et al., 1987).

The convenience, accuracy and completely non-invasive nature of the method developed here could provide a useful alternative to serum-based assays for discriminating between HAV-immune and non-immune individuals. The sensitivity and specificity obtained from ROC curve analysis showed that in-house competitive EIA can be used appropriately in epidemiological studies. ROC curve analysis has been used to characterise the accuracy of a diagnostic test and to compare results between tests. Based on the relative costs of false-positive and false-negative errors and the pretest probability of disease, the optimal decision threshold for each sample can be chosen. The further away a ROC curve is from the chance diagonal, the better discriminating power and diagnostic accuracy the test shows (Obuchowski, 2005). In characterising the accuracy of a diagnostic (or screening) test, the ROC curve provides much more information about how the test performs than just a single estimate of the test's sensitivity and specificity (Weinstein et al., 2005; Zhou et al., 2002).

5. Conclusions

The data presented above indicate that the in-house HAV competitive EIA can identify susceptible and immune individuals with cutoff points specifically adapted for oral fluids. These results reinforce the idea that oral fluids can be used as an alternative to serum for HAV screening and epidemiological studies (Amado et al., 2006; Ochnio et al., 1997; Parry et al., 1987; Vyse et al., 2001).

In conclusion, an in-house competitive EIA designed specifically for oral fluid samples is a promising technique for epidemiological application.

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