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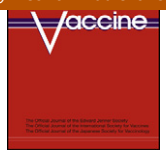

Could oral fluid be used to evaluate anti-hepatitis A virus status in individuals living in difficult-to-access areas?

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ABSTRACT

A strategy adopted by different countries to reduce the number of new cases of hepatitis A is the vaccination. However, the mosaic of the epidemiological profile in developing countries has hampered the establishment of a unified nationwide vaccination program. To determinate national vaccination policies, the results of epidemiological studies need to be carefully considered. For this monitoring, the use of oral fluid is very important due to the painless and non invasive collection characteristics. There are few studies investigating which oral fluid collection device is optimal to detect low antibody levels and its use in selecting individuals for vaccination. So, the present study aimed to evaluate different oral fluid collection devices to detect humoral immune response against hepatitis A virus and its application in epidemiological studies. Therefore, 90 matched serum and oral fluid samples were collected from volunteers with different immune status, under ideal conditions of collection (optimization panel); and 224 matched samples in difficult-to-access areas (epidemiological study). Serum was collected by venipuncture and the oral fluid was obtained using three commercial devices: Salivette®, OraSure® and ChemBio®. Serum and oral fluid were submitted to a commercial immunoblot to detect total anti-HAV antibodies. The optimization panel demonstrated that ChemBio® device had the best performance (100% agreement), followed by OraSure® (95.4%) and Salivette® (90.8%). The optimal collection device (ChemBio®), tested in a difficult-to-access area and evaluated under precarious conditions of collection, showed similar prevalence of total anti-HAV between serum and oral fluid, 80.8% and 79%, respectively. A follow-up was performed to evaluate the stability of oral fluid and it was observed that 210 days after the collection it was possible to detect anti-HAV antibodies. Oral fluid can be used to detect low levels of specific-antibody, being important to select age groups to be vaccinated. Therewith, the choice of proper collection device is essential to evaluate HAV antibodies in the epidemiological scenario.

1. Introduction

Hepatitis A is an endemic illness in Brazil and mainly affects individuals during early childhood. However, because of improvements in sanitary conditions, the epidemiologic pattern of the disease has changed, and there has been an increase in the number of clinically evident cases in adolescents and adults [1].

In countries with low or intermediate rates of the disease (USA and Argentina), a routine pediatric vaccination program is thought to be the best strategy to control hepatitis A virus (HAV) infection because children play a critical role in disease transmission [2,3]. The epidemiological pattern and economic factors of HAV should be considered when selecting individuals and/or age groups for vaccination to prevent hepatitis A outbreaks.

One strategy for understanding the epidemiology of hepatitis A is investigating immunity status by detecting anti-HAV antibodies in age-specific groups [4]. Although these studies, which are based on anti-HAV prevalence, are conventionally performed using serum samples, blood collection by venipuncture is invasive and potentially painful [5]. Furthermore, the subsequent transport (to avoid hemolysis), storage (temperature control), and processing (centrifugation) of serum samples require specific conditions that are mostly unavailable in surveillance settings. Thus, alternatives to blood analysis are needed that are non-invasive and easy to collect. Oral fluid could be a satisfactory and convenient alternative to blood analysis [6], particularly when considering children or other

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individuals from whom it is difficult to collect blood specimens as well as communities in difficult-to-access areas [7].

Although several studies have demonstrated the suitability of oral fluid as an alternative to serum for detecting HAV-specific antibodies [7–10], inadequate sensitivity and/or specificity of the available tests makes these assays inappropriate for clinical use. These features are intrinsically related to the pathogenesis of HAV infection and are critical for evaluating the antibody response that is induced by vaccination. The immune protection induced by the HAV vaccine is at least 10 times lower than that resulting from natural infection [11], and it is more pronounced when using oral fluids, in which HAV-specific antibody concentrations are approximately 800–1000 fold lower than those observed in serum samples [12–14].

Currently, there are several available methods for collecting oral fluid. However, few community-based studies have investigated which method is optimal for anti-HAV detection; important factors such as low cost, ease of collection, the validity of the results when the samples are stored under sub-optimal storage conditions, and use in a low-tech setting should be considered [15]. The aim of this study was to evaluate different oral fluid collection devices to determine which is more suitable for distinguishing between HAV-susceptible and -protected individuals in community survey studies.

2. Materials and methods

2.1. Optimization panel of oral fluid samples

The optimization panel was composed of matched serum and oral fluid samples collected from 90 health care workers without epidemiological or clinical factors associated with acute or chronic hepatitis. The health care workers were from the Oswaldo Cruz Institute and were stratified according to the total anti-HAV status of their serum. A total of 55 individuals had documented immunity to HAV (post vaccination, $n = 25$; previous infection, $n = 30$), and 35 individuals were non-reactive for anti-HAV antibodies.

The optimization panel was designed to determine the optimal salivary collection device and the most favorable parameters (dilution, incubation time and temperature) for the detection of low titers of anti-HAV antibodies in a commercial immunoassay (ImmunoComb® II HAV Ab, Orgenics, Israel) using serum samples as a reference (referred to as the "gold standard").

2.2. Sample collection and processing

Matched serum and oral fluid samples were collected from each participant. Five milliliters (mL) of peripheral blood was drawn by venipuncture using hypodermic needles and multiple sterile vacuum blood collection tubes (Vacutainer system, Franklin Lakes, NJ, USA). Subsequently, the samples were centrifuged at $1800 \times g$ at 25 °C for 5 min, and the sera were stored at −20 °C. Oral fluid samples were obtained with three different commercial devices: ChemBio® (ChemBio Diagnostic Systems Inc., NY, USA), OraSure® (originally provided as an HIV-1 Oral Specimen Collection Device) (Epitope Inc., Beaverton, USA), and Salivette® (Sarstedt, Germany). Oral fluid sample collection and processing procedures are shown in detail in Table 1.

2.3. Sample screening

Total anti-HAV antibodies were detected with a commercially available, solid-phase enzyme immunoassay (EIA) based on the principle of immunocapture (ImmunoComb® II HAV Ab, Orgenics, Israel). The solid phase is a comb composed of 12 projections. Each projection is sensitized at two positions: an upper spot with a monoclonal anti-HAV antibody (internal control) and a lower spot with rabbit anti-human IgG and IgM antibodies.

The test was performed according to the manufacturer's instructions and adapted for oral fluid samples; 25 μL of oral fluid was added without sample diluents. The results of the test are visible as gray-blue spots on the surface of the projections, and the visual results are determined semi-quantitatively by comparing the intensity of the color of the lower spot on each projection with the color scale provided by the manufacturer. The results of the samples were classified according to the cut-off point (10 IU/L) of the test. A spot with an intensity greater to or equal than the cut-off point indicated the presence of protecting anti-HAV levels. A spot with an intensity slightly less than that of the cut-off was considered an equivocal result, and the sample was retested. A spot with a lower intensity than that of the cut-off was considered negative. The ImmunoComb® II HAV Ab assay has a limit of detection of 10 IU anti-HAV antibodies/L, which is regarded as the minimum concentration of anti-HAV antibodies that indicates immunization has occurred. All of the samples were assayed three times, and identical visual readings for HAV were consistently observed by multiple investigators (three) for all samples.

2.4. Applicability of the optimal salivary collection device in surveillance settings

After determining the optimal salivary collection device, its applicability in a surveillance setting was determined. This study was performed in four isolated communities in South Pantanal, Brazil, in difficult-to-access areas that are 661 km from the city of Campo Grande. This region is sparsely populated and is characterized by wetlands that hinder access to the coastal communities; access is only available by boat. For these reasons, fishing is the primary source of income and livelihood for the majority of the population. The survey was conducted between April and June 2010, and the ChemBio® device was used to collect 224 matched serum and oral fluid samples using a non-probability sampling method from all consenting occupants of households. The entire population consisted of 691 individuals. The samples were placed in a cool box and returned to the laboratory after 15 days of collection for a total anti-HAV screening test. The sociodemographic characteristics of each member of the study were obtained with questionnaires.

2.5. Effects of time exposure and temperature on the detection of anti-HAV antibodies in oral fluids

The influence of temperature and time exposure on the detection of anti-HAV antibodies in oral fluid samples was investigated. The parameters were based on the manufacturer's storage instructions. Five concordant, matched samples (3 anti-HAV positive and 2 negative) that were collected in difficult-to-access areas of South Pantanal were selected for follow-up to evaluate anti-HAV antibody stability. Due to the unavailability of cooling in the surveillance setting, the oral fluid samples remained at unstable temperature conditions for 15 days. At the end of this exposure, the samples were sent to a laboratory in Rio de Janeiro and were centrifuged and refrigerated at 2–8 °C until the first analysis (15 days after collection). The samples were stored for 210 days after collection and were retested every 30 days.

2.6. Ethical aspects

Ethical permission for collecting and testing samples was provided by the FIOCRUZ Ethical Committee (536/09), and written informed consent was obtained from each participant before entering into the study. The specimens and questionnaires were

Table 1
Description, collection, and extraction characteristics of the three commercially available oral fluid devices used in the present study.

ChemBio®	
Description	A sponge swab attached to a handle with a plastic tube containing 500 μL of preservative solution.
Collection	The swab is rubbed along the teeth/gum line for approximately 1 min, after which it is returned to the plastic tube.
Extraction	Oral fluid is concentrated at the bottom of the plastic tube by centrifugation at 1300 × *g* at 25 °C for 10 min and stored at 2–8 °C.
OraSure®	
Description	A flat absorbent cotton pad pretreated with 800 μL of preservatives and stabilizing reagents supported by a plastic stem.
Collection	The pad is placed against lower gum on one side and keeps stationary for 2 min.
Extraction	As previously described and stored at −20 °C.
Salivette®	
Description	A polypropylene tube with a perforated inlay containing an absorbent cotton wad.
Collection	As previously described for OraSure®. Then it is applied to the wad 1 mL of phosphate-buffered saline (PBS).
Extraction	As previously described and stored at −20 °C.

anonymous, and feedback was given to all participants of the study, including their results. All unprotected participants were advised to be vaccinated against hepatitis A.

2.7. Statistical analysis

Data are presented as medians and frequencies. The performance of the laboratory tests with the collected oral fluid samples was determined by comparing the sensitivity, specificity, and positive and negative predictive values and their respective 95% confidence intervals (95% CI) with the serum results, which were used as a gold standard control. The linear and weighted kappa (*k*) statistic was used to evaluate the rate of agreement between the oral fluid and serum anti-HAV antibody status for each device used. According to the strength of the agreement, the *k* value was interpreted as follows [16]: <20%: poor; 21–40%: fair; 41–60%: moderate; 61–80%: good; and 81–100%: very good. To compare proportions, the Chi-square (χ^2) test for independence with Yate's continuity correction, χ^2 for trend, and Fisher's exact test (when appropriate) were used. The Spearman's coefficient of rank correlation (*rs*) was used to evaluate the degree of the relationship between the values of color intensity on the colorimetric scale obtained after using the oral fluid collection devices. A two-tailed $p < 0.05$ was considered statistically significant. All analyses were performed with MedCalc for Windows, version 8.1.0.0 (MedCalc Software, Mariakerke, Belgium), and GraphPad InStat version 3.05 (GraphPad Software, CA, USA) software.

3. Results

3.1. Determination of the optimal oral fluid dilution for detecting anti-HAV antibodies

The optimal oral fluid dilution for detecting anti-HAV antibodies in the ImmunoComb® II HAVAb was determined using matched samples from the optimization panel. Among the 30 individuals with natural immunity to HAV, oral fluid samples collected by OraSure® and Salivette® devices presented concordant results with those from serum samples until a 1:25 dilution. However, false-negative results were observed after the 1:5 dilution when the ChemBio® device was used. For the 25 HAV-vaccinated individuals, all of the diluted samples presented false-negative results, irrespective of the oral fluid collection device used. False-positive results were not observed in the group of 35 individuals who were non-reactive for anti-HAV antibodies. Based on these findings, the detection of anti-HAV antibodies by all of the devices was optimal when undiluted oral fluids were used; the evaluation of other parameters (temperature, incubation time, etc.) was not required to optimize these samples.

3.2. Performance of oral fluid collection devices for detecting anti-HAV antibodies

The rate of agreement between the oral fluid and serum anti-HAV antibody status for each device was evaluated for each group of individuals. Oral fluid samples collected by OraSure®, Salivette®, and ChemBio® yielded concordant results ($k = 100\%$) with the corresponding serum samples in individuals with a natural immunity to HAV and in individuals that were orally non-reactive for anti-HAV antibodies. For the 25 HAV-vaccinated individuals, all of the samples that were collected with ChemBio® device were reagent. Two and four samples yielded false-negative results after collection by OraSure® and Salivette®, respectively. However, half of these false-negative results (1/2 – OraSure®) were observed in individuals that were not fully vaccinated (1 dose administered of a 2-dose schedule) against HAV, while the other half (2/4 – Salivette®) were observed in individuals that were fully HAV-vaccinated (2-dose schedule completed).

When analyzing the results from individuals with natural immunity to HAV and those from HAV-vaccinated individuals, a variation in the color scale values was observed in the oral fluid and serum samples. HAV-vaccinated individuals presented median color scale values that were significantly lower than those for individuals with natural immunity to HAV ($p < 0.05$). Moreover, there was a significant trend of values with a more intense color in the samples from individuals with natural immunity to HAV relative to those from HAV-vaccinated individuals ($p < 0.05$) (Table 2). Among the oral fluid devices used, ChemBio® yielded median values of color intensity that were more similar to those of serum from the group of HAV-vaccinated individuals ($n = 25$; $p = 0.1250$) than from the total group of individuals with immunity to HAV ($n = 55$; $p = 0.0020$).

ChemBio® was the most sensitive and specific of the tested oral devices, with positive and negative predictive values equal to 100%. A correlation analysis was used to evaluate how the values of the visual readings of the color scale for the serum and oral fluid correspondingly changed for each oral fluid device; a significant positive correlation existed between these two variables ($p < 0.0001$).

The weighted kappa value revealed a perfect rate of agreement ($k = 100\%$) between the serum and oral fluid samples collected with the ChemBio® device. Moreover, the highest positive correlation was found with the ChemBio® device. The parameters evaluating the performance of the EIA used in the experiments are presented in Table 3.

3.3. Applicability of oral fluid specimens for epidemiological study

After determining that the ChemBio® oral fluid collection device yielded the best results for the anti-HAV antibody detection test, an epidemiological study was conducted to assess the applicability of this device in surveillance settings.

Table 2
Level of anti-HAV antibodies in individuals with immunity to HAV and evaluated by visual reading of the color scale[a] (CombScale™) according to oral fluid collection devices.

Group	Sample			
	Serum	Oral fluid collection device		
		ChemBio®	OraSure®	Salivette®
Vaccinated[a] ($n=25$)				
Median	2.0	2.0	1.0	1.0
Range	1.0–5.0	1.0–5.0	0.0–5.0	0.0–4.0
Color scale	(n/%)	(n/%)	(n/%)	(n/%)
0	0 (0.0)	0 (0.0)	2 (8.0)	4 (16.0)
1	4 (16.0)	6 (24.0)	12 (48.0)	12 (48.0)
2	12 (48.0)	11 (44.0)	6 (24.0)	6 (24.0)
3	4 (16.0)	4 (16.0)	2 (8.0)	0 (0.0)
4	4 (16.0)	3 (12.0)	2 (8.0)	3 (12.0)
5	1 (4.0)	1 (4.0)	1 (4.0)	0 (0.0)
Natural immunity ($n=30$)				
Median	4.0	3.0	3.0	3.0
Range	1.0–5.0	1.0–5.0	1.0–5.0	1.0–5.0
Color scale	(n/%)	(n/%)	(n/%)	(n/%)
0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1	1 (3.3)	3 (10.0)	4 (13.4)	5 (16.7)
2	5 (16.7)	4 (13.4)	7 (23.3)	7 (23.3)
3	8 (26.7)	10 (33.3)	8 (26.7)	6 (20.0)
4	15 (50.0)	12 (40.0)	10 (33.3)	11 (36.7)
5	1 (3.3)	1 (3.3)	1 (3.3)	1 (3.3)
p-Value[†]	**0.0022**	**0.0054**	**0.0008**	**0.0003**

[a] Presence of protecting anti-HAV antibodies levels is indicated by a spot with an intensity greater than or equal to that of the cut-off point (10 IU/L).
[†] $p<0.05$ (in bold) are statistically significant. Vaccinated versus natural immunity groups (Chi-square for trend).

Table 4
Total anti-HAV results of serum and oral fluid matched samples from a population-based prevalence study conducted in difficult-to-access areas of South Pantanal ($n=224$).

	Serum+	Serum−	Total
Oral fluid+	176 (78.6%)	1 (0.4%)	177 (79.0%)
Oral fluid−	5 (2.2%)	42 (18.8%)	47 (21%)
Total	181 (80.8%)	43 (19.2%)	224 (100%)

Table 5
Effect of time exposure and temperature for detecting anti-HAV antibodies in oral fluid.

Collection device	Storage temperature	Stability
ChemBio®	Room temperature	2 years[a]
	2–8 °C	Up to 210 days[d]
	20 °C	Not recommended[a]
OraSure®	Room temperature	21 days[b]
	2–8 °C	21 days[a]
	−20 °C	45 days[a]
Salivette®	Room temperature	5 days[b]
	2–8 °C	20 days[c]
	−20 °C	1 year[a]

[a] According to manufacturer's instruction for HIV test.
[b] According to Ref. [14].
[c] According to Ref. [17].
[d] Current study.

In a population-based prevalence study conducted in difficult-to-access areas of South Pantanal, 224 matched serum and oral fluid (ChemBio®) samples were obtained from volunteers; 100 (43.9%) of the volunteers were female, and 124 (56.1%) were male. The age of the study population ranged from 3 to 86 years with a mean age of 26.91 ± 17.35 years.

Total anti-HAV antibodies were detected in 181 sera samples using the commercial immunoassay ImmunoComb® II HAVAb (Orgenics, Israel); the HAV seroprevalence was 80.80%. The prevalence of total anti-HAV yielded by the oral fluid samples collected with the ChemBio® device was 79.01%, corresponding to 177 reactive samples. Table 4 shows the concordant and discordant results of the serum and oral fluid matched samples. These data showed that the ChemBio® device had a sensitivity of 97.24% (95% CI: 0.936–0.991), a specificity of 97.67% (95% CI: 0.877–0.999), a positive predictive value of 99.44% (95% CI: 0.968–0.999), a negative predictive value of 89.36% (95% CI: 0.768–0.964) and a kappa coefficient of 91.7% (95% CI: 0.851–0.982).

The range of the colorimetric scale of the reagent samples was similar between the serum and oral fluid (ChemBio®) samples, resulting in a median of 3.0 for both specimens. There was no variation among the non-reactive samples.

3.4. Stability of anti-HAV antibodies in oral samples collected in difficult-to-access areas

The stability of the anti-HAV antibodies was determined by monitoring the five serum and oral fluid (ChemBio®) matched samples at different time exposures and temperatures.

When samples were collected and stored at unstable storage conditions for 15 days (temperature variation, 2–25 °C), anti-HAV antibodies could be detected from the oral samples. When samples were stored at 2–8 °C, there was no change in the anti-HAV antibodies within the 180 first days after collection. However, on day 210 after collection, a one-level decrease in the colorimetric scale was observed for the reactive samples. Antibodies against hepatitis A remained detectable in the oral fluid samples for more than 210 days. A comparison of Salivette®, OraSure® and ChemBio® sample stability based on both the literature and the results obtained in this study is summarized in Table 5. The ChemBio® device exhibited the best performance at both room temperature and 2–8 °C relative to the Salivette® and OraSure® devices, as has been observed in other studies [14,17].

Table 3
Parameters of performance evaluation of the ImmunoComb® II by using different oral collection devices.

Parameters	Oral fluid collection device		
	ChemBio®	OraSure®	Salivette®
Sensitivity (95% CI)[a]	100.0 (0.9351–1.000)	96.36 (0.8748–0.9956)	92.73 (0.8242–0.9798
Specificity (95% CI)[a]	100.0 (0.9001–1.000)	100.0 (0.9001–1.000)	100.0 (0.9001–1.000
Positive predictive value (95% CI)[a]	100.0 (0.9351–1.000)	100.0 (0.9328–1.000)	100.0 (0.9302–1.000
Negative predictive value (95% CI)[a]	100.0 (0.9001–1.000)	94.5 (0.8179–0.9934)	89.74 (0.7577–0.9714
Spearman's correlation coefficient (p-value)[†]	0.987 (<**0.0001**)	0.969 (<**0.0001**)	0.948 (<**0.0001**)
Kappa statistic	100.0	95.4	90.8

[a] 95% confidence interval.
[†] $p<0.05$ (in bold) are statistically significant.

Table 6
Summary of reports on performance data of oral fluid collection devices.

Author	Year	Studied population	Objective	Antibodies detected	Collection device	Sensitivity (%)	Specificity (%)
Ochinio et al.	1997 [7]	General population	Standardization	IgG	Salivette®	98.70	99.60
Oba et al.	2000 [8]	General population	Diagnostics	IgM, IgG and IgA	Omni-SAL®	82.10	100
Amado et al.	2006 [10]	Outbreak	Diagnostics	IgM and IgG	OraSure	86.67	100
Quolin et al.	2007 [24]	General population	Prevalence	IgG	Oracol®	84.70	100
Current Study	2011	General population	Prevalence	IgM and IgG	ChemBio®	100	100

4. Discussion

To date, HAV vaccination strategies have been implemented on the basis of cost-effectiveness and epidemiological studies. Routine large-scale infant vaccination programs are not recommended for individuals living in areas of high endemicity [18]. In 2006, the U.S. Advisory Committee on Immunization Practices (ACIP) [18] recommended routine HAV vaccination of all children aged 12–23 months, irrespective of risk category or location, resulting in a significant decrease in hepatitis A incidence in the next year. A more recent assessment of hepatitis A vaccine coverage among USA children between the ages of 12 and 23 months from 2006 through 2009 revealed improved coverage that had reached a plateau, leading to a push for hepatitis A vaccination of all children beginning at age 12 months by immunization programs and vaccine providers [19].

In developed countries, the implementation of a nationwide routine vaccination program against hepatitis A is still an important issue, mainly because of the changing HAV epidemiological pattern in some regions. Although a vaccine against hepatitis A has been licensed since 1995, some countries do not recommend routine hepatitis A vaccination due to the cost of the vaccine and differences in HAV epidemiology across the nation (for example, whether the vaccine is indicated for the groups known to be at high risk for the infection). In the epidemiological context, the utilization of oral fluid to determine HAV protection has been demonstrated to be appropriate because of its advantages and high accuracy for surveillance studies in different rate groups [7,8,10,14,20–22]. The advantages of oral specimen collection and testing and the performance of several oral fluid collection devices and modified EIAs have led to increased interest in the utilization of oral fluid as a surrogate for serum samples.

To be useful for HAV epidemiological studies and the screening of groups with a high seroprevalence rate of anti-HAV antibodies, the EIAs originally designed for use on serum samples were modified to detect the antibodies in oral fluid; the levels of anti-HAV antibodies are lower in oral fluid than in serum. As a result, an improvement in the sensitivity and specificity of the assays using matched oral fluid and serum samples has been demonstrated in several studies [7,8,10]. However, some studies have reported results of HAV testing in oral fluid collected from patients during hepatitis A outbreaks, during which oral fluid is known to have higher titers of anti-HAV antibodies [6,10]. Thus, the optimization of EIAs for detecting anti-HAV antibodies in oral fluid collected during outbreaks does not appear to be appropriate to validate these assays for use in evaluating oral fluid anti-HAV levels associated with vaccine-induced immunity. Moreover, the optimal oral fluid collection device for the determination of anti-HAV status must be identified because the commercial product used for specimen collection can affect the recovery of antibodies and thus yield a lower accuracy result [7,8,23,24]. In the present study and in accordance with a previous study, the use of oral fluid for anti-HAV antibody detection was optimized; the use of an oral fluid sample without dilution is ideal for the detection of anti-HAV antibodies by a modified EIA [10]. The three commercial oral fluid collection devices yielded different values of sensitivity and specificity for the detection of anti-HAV antibodies. The efficiency of oral fluid collection devices in extracting antibodies can be affected by the commercially available product used for their collection [24].

The levels of IgG anti-HAV-specific antibodies vary widely according to how immunity is acquired and the biological fluid assayed. Higher levels are detected in serum samples from patients recently infected with HAV than in oral fluid from vaccinated individuals [11]. The differences in the sensitivity rates found here could be partially explained by false-negative results from the OraSure® (2/25) and Salivette® (4/25) devices in the group of vaccinated individuals. Nevertheless, the specificity rates did not appear to be affected by the use of different oral fluid devices. Among the devices used for oral fluid collection, Salivette® had the lowest sensitivity rate (92.73%), with four oral fluid samples from vaccinated individuals testing negative for anti-HAV antibodies. These results are in line with previous studies reporting negative results when using this oral fluid device [14,21,25]. The damaging effect of plain cotton on the analytical performance of this device is conceivably attributed to substances derived from the cotton, which affect the results by interfering with the detection of antibodies [26].

The efficiency of antibody elution from the device's sorbent material may vary among the oral fluid collection devices and may reflect different procedures of collection. The ChemBio® device is designed to specifically target the gums, which is the region of the oral cavity most likely to be rich in crevicular fluid; additionally, the ChemBio® device is used more vigorously inside the mouth than the other two devices. This characteristic of the product may explain why oral fluid samples collected by devices that specifically target crevicular fluid may contain anti-HAV antibodies in quantities that more reliably reflect the levels in serum samples [27]. The other devices, OraSure® and Salivette®, are placed inside the oral cavity adjacent to the gums and thus have a similar collection procedure, as reported by a study comparing three different oral-fluid collection devices including OraSure® [15]. Nevertheless, OraSure® performed better than Salivette®, a finding that may be related to substances that are present in the OraSure® device that stimulate the transudation of immunoglobulins from the vascular space to the oral cavity [14].

A comparative analysis of the median color scale values revealed higher values in samples from individuals with a natural immunity to HAV than in those from HAV-vaccinated individuals. Of the three oral collection devices tested, the results provided by the ChemBio® device were the most similar to the results from the reference serum samples. Additionally, the ChemBio® device exhibited the best combination of evaluation performance parameters, which were higher than those reported in previous studies (Table 6).

To determine the effectiveness of the ChemBio® device and its applicability in a surveillance setting as a substitute for serum samples, we performed an investigation of HAV infection in difficult-to-access areas of South Pantanal. Using samples collected from individuals belonging to different communities, we observed similar values of prevalence of anti-HAV antibodies (79.01%) and anti-HAV seroprevalence (80.8%) in oral fluid collected with ChemBio®.

The suitability of oral fluid in an epidemiological scenario is closely related to the stability of the sample. Because the stability of the OraSure® and Salivette® collection devices has been previously reported in the literature, this study evaluated the effect of these parameters only in oral fluid samples collected with the ChemBio® device. The temperature variation during in-field sample storage and delayed processing did not significantly interfere with the detection of anti-HAV antibodies among oral samples when compared to the serum results. Sample storage at temperatures of 2–8 °C caused no significant changes during the first 180 days after collection. However, at day 210, a decrease of one level on the colorimetric scale for reactive samples was observed, but the qualitative results remained the same. This stability should be considered in an epidemiological scenario in which there is no refrigeration, in developing countries that can have large and difficult to accommodate variations in temperature [28], or when samples are sent to the laboratory by mail service [23].

The collection methodology and sample preservation by the use of stabilizers in the ChemBio® device were considered an important strategy to avoid the problems of rapid antibody degradation during storage as reported by Gröschl and colleagues [26] for other collection devices. In this study, we observed that this preservation was sufficient to increase the stability of the sample. Thus, these results showed that the ChemBio® device is suitable for vaccination and epidemiological surveillance in difficult-to-access areas because freezing is not required for sample storage.

5. Conclusion

Oral fluid samples collected with the ChemBio®, OraSure® and Salivette® devices provided qualitative results that were sufficient for detecting anti-HAV antibodies under optimal conditions. However, the ChemBio® device had the best performance in the optimization panel, and the stability of samples collected with this device demonstrated that this device was most appropriate for a surveillance scenario.

Moreover, oral fluid can be used to detect low-level, specific antibody levels in vaccinated individuals, although the choice of the appropriate collection device is essential to evaluate HAV antibodies in difficult-to-access areas. Oral fluid was used to demonstrate that it is possible to collect this clinical specimen when ideal storage conditions are not available, which is indispensable to determining the epidemiological profile of the disease and selecting age groups for vaccination.

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