An inter-laboratory study of anti-HCV antibody detection in saliva samples

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SUMMARY
Aim — The purpose of this study was to evaluate the efficacy of anti-hepatitis C virus (HCV) antibody detection in the saliva samples of 108 drug users in an inter-laboratory study.
Methods — Between January and June 2001, 108 subjects in Lille, Metz and Lens received a test to detect anti-HCV antibodies in their saliva. Two consecutive saliva samples were taken in each subject (Salivette system, Sarstedt). An HCV serology (Axsym HCV 3.0, Abbott) was also performed and serum HCV RNA detection by Ampli
corc HCV 2.0 (Roche) was performed when HCV serology was positive. Sixty three patients had a negative HCV serology, 45 had a positive HCV serology, and 31 of these had positive HCV RNA as well. Tests for the detection of the anti HCV antibody in saliva samples were performed as a blind study in both the Lille and the Thionville laboratories.
Results — The sensitivity of saliva anti-HCV antibody tests was respectively 71% (32/45) and 78% (35/45) in Lille and Thionville. In the event of positive HCV viremia, the sensitivity was respectively 90% (28/31) and 93% (29/31). The specificity was respectively 97% (61/63) and 98.5% (62/63). Results from the two laboratories agreed for 101 saliva tests while discrepancies were found in 7 (Kappa Concordance Coefficient: 0.85).
Conclusions — This study confirms, in a large, unselected population sample, that anti-HCV antibody detection tests in saliva allow the detection of 90% of viremic HCV-antibody-positive patients with excellent specificity. The simplicity and reproducibility of this technique makes it a precious tool for epidemiological studies.

RÉSUMÉ
Étude inter-laboratoires de la détection des anticorps anti-VHC sur prélèvements salivaires

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But — Évaluer les performances de la détection d’anticorps anti-virus de l’hépatite C (VHC) dans les prélèvements salivaires de 108 sujets toxicomanes grâce à une étude inter-laboratoires.
 Méthodes — Entre janvier et juin 2001, 108 sujets de Lille, Metz et Lens ont bénéficié d’une recherche d’anticorps anti-VHC dans la salive. Deux prélèvements salivaires consécutifs étaient effectués chez chaque sujet (système Salivette, Sarstedt). En parallèle étaient réalisées une sérologie VHC (Axsym HCV 3.0 Abbott) et la recherche d’ARN-VHC par la technique Cobas Ampli
corc HCV 2.0, Roche diagnostic si la sérologie VHC était positive. Soixante trois malades présentaient une sérologie VHC négative et 45 une sérologie positive avec ARN VHC positif dans 31 cas. La recherche d’anticorps anti-VHC dans les prélèvements salivaires était réalisée en aveugle dans les laboratoires de Lille et de Thionville.
 Résultats — Les sensibilités du dépistage salivaire des anticorps anti-VHC étaient respectivement de 71 % (32/45) et 78 % (35/45) à Lille et Thionville. En cas de virémie VHC positive, les sensibilités étaient respectivement de 90 % (28/31) et 93 % (29/31). Les spécificités étaient respectivement de 97 % (61/63) et 98,5 % (62/63). Cent un résultats salivaire étaient concordants et 7 discordants entre les deux laboratoires (coefficient de concordance kappa : 0,85).
 Conclusions — Cette étude confirme sur un large échantillon non sélectionné que la recherche des anticorps anti-VHC dans la salive permet de dépister 90 % des sujets anticorps anti-VHC positifs virémiques avec une excellente spécificité. La simplicité et la reproductibilité de la technique en font un outil précieux dans les études épidémiologiques de terrain.
monitor therapeutic effects [1]. Saliva has also been proposed for the detection of certain viral diseases: such as human immunodeficiency virus (HIV) [2], hepatitis B virus (HBV), and hepatitis C virus (HCV) [2-9].

Most of the studies on detection of anti-HCV antibodies in saliva have been conducted in intravenous drug users because this population does not readily attend regular healthcare centers and blood drawing may be problematic. In recent studies, the sensitivity and specificity of detecting HCV infection on saliva samples has varied from 80-85% to 98-100% [8, 9]. This method enables detection of most subjects with a positive viremia [8-10].

To our knowledge, the reproducibility of this method has not been evaluated. The purpose of this study was to determine the inter-laboratory sensitivity, specificity and reproducibility of the Monolisa anti-HCV plus V.2 kit (Biorad) adapted to saliva samples in a large population of intravenous drug users with known or unknown HCV serology.

Methods

Subjects

HCV screening tests were performed in 108 intravenous drug users in three French cities (Lille, Metz, and Lens) between January and June 2001. Blood samples were obtained for serum ASAT, serum ALAT, anti-HIV antibodies, AgHBs, and anti-HCV antibodies. All HCV serologies were performed in a single laboratory using the Axsym HCV 3.0 kit (Abbott, Rungis, France). Search for HCV RNA was performed with the Cobas Amplicor HCV 2.0 kit (Roche diagnostics) when HCV serology was positive. Sixty-three subjects were HCV negative and 45 were HCV-positive, including 31 HCV RNA positive subjects.

Saliva tests

Duplicate saliva samples were obtained with the Salivette® system (Sarstedt). Samples were rapidly centrifuged and stored at −20 °C until tested with the Monolisa anti-HCV kit (Biorad, Marnes La Coquette, France). Search for anti-HCV antibodies on saliva samples was conducted in two laboratories (laboratory A = Biochemistry laboratory, Saint-Philibert Hospital, Lomme, France; laboratory B = Microbiology laboratory, Thionville Hospital, Thionville, France) using a double blind protocol. The laboratory technique for detection of HCV antibodies in serum was adapted to saliva samples in accordance with methods reported in the literature [8]. Saliva samples (80 µl) were diluted in 20 µl diluant then incubated 20 h at room temperature. The conjugate was then incubated 1 h at 40 °C with constant stirring before incubation with the substrate (ortho-phenylene diamine) in the dark for 30 min at room temperature. Optical density was measured at 450/620 nm. The positivity threshold was set at 0.200. Results were expressed as an index of the threshold value. Tests were performed manually in laboratory A and with a microplate automate in laboratory B.

Statistical analysis

The sensitivity and specificity of the ELISA tests on saliva samples were calculated using serum HCV antibody and HCV RNA results as the gold standard. The kappa coefficient of concordance between the saliva results from the two laboratories was calculated.

Results

Detection sensitivity in saliva samples was 71% (32/45) and 78% (35/45) in laboratory A and laboratory B respectively and specificities were 97% (61/63) and 99% (62/63), respectively. Specificities were 90.3% (28/31) and 93.5% (29/31) in laboratory A and laboratory B respectively in saliva samples from subjects with positive HCV RNA. Optical densities of the HCV-positive serologies were significantly lower for HCV RNA-negative subjects compared to HCV RNA-positive subjects (figure 1). Results from laboratory A and laboratory B were concordant for 101 saliva samples and discordant for 7 saliva samples (kappa coefficient = 0.85) (table I). Discordant results were found for 4/45 (8.9%) subjects with positive anti-HCV serum tests and in 3/63 (4.8%) of the subjects with negative anti-HCV serum tests (p = NS). The discordance for the 4 subjects with positive anti-HCV serum tests resulted from false negative saliva tests (3 in laboratory A and 1 in laboratory B). The discordance for the 3 subjects with anti-HCV serum tests resulted from false positive tests (two in laboratory A and one in laboratory B).

Discussion

The purpose of this work was to confirm the sensitivity and specificity of the Biorad test and determine its reproducibility for detecting HCV antibodies in saliva. The Biorad test was chosen because it can be adapted to automated analysis and provides good sensitivity and specificity [8]. The Salivette® system was used because saliva samples can be easily collected and stored in proper conditions of hygiene.

Using serology results as the standard, sensitivity was rather moderate (71% and 78% for laboratories A and B, respectively) while specificity was very good (98%). Since the presence of HCV antibodies in saliva results from passive diffusion, saliva tests can

<table>
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<th>Laboratory B</th>
<th>Laboratory A</th>
<th>Negative</th>
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<td>Total</td>
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Table I. – Comparison of salivary test results between the 2 laboratories.

Kappa: 0.85
be negative when serum titers are very low. We were unable however to determine the serum threshold under which saliva tests are negative (figure 1).

Using viremia as the gold standard, sensitivity was to the order of 90% among anti-HCV-positive subjects. The much lower serum antibody titer (and thus the very low antibody titer in saliva) observed in viremia-negative subjects (figure 1) could explain the false negatives.

The inter-laboratory reproducibility was rather good with a kappa coefficient > 0.8. The difference observed may be related to the technique used (manual in laboratory A and automatic in laboratory B). These good results demonstrate that this method can be performed with a microplate automate, similar to the technique used for serum samples.

The methodology used for this study bears three strong points: 1) saliva results were obtained in a double blind manner; neither the serum results nor the saliva results from the other laboratory were known to the testing laboratory. The positive threshold for HCV antibodies in saliva was predefined in accordance with the literature [8] and was not modified during the study according to the results obtained. 2) The sensitivities were calculated using both serum antibody level (determined with the same diagnostic test, Abbott HCV EIA, 3.0 for all samples) and detection of HCV RNA in serum from antibody-positive subjects as the gold standard. For routine practice, it is important to have good sensitivity for viremic subjects since all subjects requiring treatment are found among the viremia-positive population. 3) Recruitment bias was avoided since saliva samples were obtained from a large French population of intravenous drug users selected for viremic status and not from HCV antibody-positive subjects regularly attending a hospital clinic who are generally viremic. The rate of seropositivity (40%) and the rate of viremia amongst seropositive subjects (70%) were in agreement with earlier data reported in the literature (Table II). Thus, while detection of HCV antibodies in saliva is a simple, reliable and reproducible screening test, its usefulness in routine practice remains to be determined as serum tests remain the gold standard. One of the principle advantages of saliva testing is that it enables screening larger populations to obtain epidemiological data [7, 12, 13]. In this framework, saliva sampling avoids the negative selection bias which eliminate subjects with a high risk of HCV seropositivity from whom a blood sample cannot be obtained. A preliminary report concerning five subjects showed that seroconversion in saliva occurred at about the same time as seroconversion in serum [14].

Saliva sampling is not the only approach that can be used to simplify screening strategies in the intravenous drug user population. Fingertip capillary blood sampling using the dried blood spot method is under development as another way of screening for HCV antibodies [15, 16]. The first results appear to be superior to those obtained with saliva screening [16], but there is a risk of exposure to contaminated blood and a risk of superinfection due to insufficient aseptic. Furthermore, the acceptability of this approach remains to be evaluated.

In summary, this study conducted in a large unslected population confirmed that saliva screening tests can detect 90% of viremic HCV-antibody-positive patients with excellent specificity. Although the sensitivity is insufficient for individual screening, the simplicity and reproducibility of this technique makes it a precious tool for epidemiological studies.

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REFERENCES

Table II. – Performances of saliva tests: review of the literature.

<table>
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<th>Authors</th>
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<td>76.5%</td>
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se: sensitivity; sp: specificity


