that the correctional facilities, which at present have inadequate infection control practices and isolation facilities to prevent transmission of *M. tuberculosis*, fully comply with the current guidelines to reduce the risk of tuberculosis spread [14,15].

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Gingivocrevicular transudate for HIV screening

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The use of saliva as an alternative biological fluid to serum in diagnosis or screening of infectious diseases by antibody detection has been the main subject of several articles in the last few years [1–6]. Saliva samples can be obtained simply, without specialized personnel, and the process is non-traumatic for the patient and economic and poses no contamination risks for health workers. For these reasons, saliva samples may be of great utility in underdeveloped nations, where there is a severe shortage of personnel and specialized equipment. Saliva is a mixture of the secretions produced by the salivary glands and gingival crevicular transudate (GCT). The use of GCT, which has a greater concentration of immunoglobulins (Ig) of the IgG type than does complete saliva [7], seems to improve detection of such Ig in the samples [8].

An oral device without preservatives (Salivette, Starstedt, Leicester, UK) was used to obtain the GCT samples. The device is a cylinder of cotton wool contained in a polystyrene double tube with a snap top (price USA $0.20). The cotton wool was placed in the lateral gingival fold until the individual could perceive that it had become soft because of its absorption of GCT. The samples thus obtained were centrifuged at 4000 *g* for 15 min, producing a supernatant which was

aliquoted and frozen at −80°C until processing. A serum sample was simultaneously obtained by venepuncture from each of the individuals included in the study. These samples were then aliquoted and stored at −80°C until processed.

A total of 183 paired samples of GCT and serum were analyzed (50 CDC stage II, 10 CDC stage III, 33 CDC stage IV, 26 risk factor individuals and 64 blood donors). In both types of sample, the presence of IgG against HIV-1 and HIV-2 was detected. With GCT samples, we used a new dot blot (Multispot HIV-1/HIV-2, Sanofi Diagnostics Pasteur, France) capable of discriminating between both IgG HIV-1 and IgG HIV-2. For the screening of serum samples, an indirect enzyme immunoassay (EIA) (VIDAS HIV 1+2, bioMérieux, France) was used. All reactive samples were confirmed by Western blot (WB) (Bioblot HIV-1 plus, Genelabs Diagnostics, Switzerland). The WB used combines specific proteins for HIV-1 and a synthetic peptide (gp36) for HIV-2 in the same strip; WHO criteria [9] were used for confirmation of screening reactivity.

The results presented in Table 1 show that all GCT samples non-reactive by dot blot belonged to non-infected individuals (EIA negative), except for one sample from a patient of the CDC stage IV group, which was negative by dot blot but positive by GCT WB (reactivity against gp160, gp120 and gp41). The GCT samples reactive by dot blot correspond to infected individuals, with the exception of one sample from a non-infected individual also negative by GCT WB. The characteristics of this test in comparison with serum WB results have been as follows: 99% sensitivity and 99% specificity (positive predictive value (PPV) and negative predictive value (NPV) were 0.9).

Several reasons may be suggested in the case of the non-reactive sample from the individual belonging to CDC stage IV. The first is the immunologic deterioration presented by individuals in the final stages of the infection, yielding a lower IgG serum concentration and even loss of concrete antibodies (i.e. p24 antibodies); second, GCT may therefore not present reactivity when analyzed by insufficiently sensitive techniques. The use of a more sensitive test (WB) leads in this case to the detection of IgG against envelope proteins in the GCT. The confirmed presence of HIV-1 envelope antibody in GCT is highly suggestive of HIV-1 infection and also correlates with the reactivities obtained in the serum WB of this individual.

The salivary device used presents two main advantages. The first and most important is that it can obtain an adequate volume (≃1 ml) for sample analysis by different techniques and storage. Second, in contrast to the case with other devices, IgG sample concentration does not vary so much, because the sample is not diluted. There is no experimental evidence that the concentration of Ig in GCT declines during storage, but any such changes would be minimal, because the samples were not diluted in any preservative buffer and they were frozen after recovery, thus minimizing the action of saliva enzymes. GCT samples (USA $0.20) are cheaper to obtain than serum samples (USA $0.60 for syringe+needle+tube). Furthermore, the GCT process permits a single individual to collect samples from several patients at once, instead of the individualized sample collection required with serum.

Our results confirm previous work by others [10,11] but using a simple device for sampling and testing. The use of GCT instead of saliva has been proposed in several articles [8,10,11] to facilitate the detection of HIV IgG due to the increased amount of this type of Ig.

In conclusion, the use of dot blot together with the oral GCT device could offer an alternative for screening of infection produced by HIV in wide population groups, because of its low cost and easy application. Further studies should be performed in patients with advanced stages of the disease to determine if the loss in sensitivity could be due to the immunologic condition in this particular stage of the disease.

Table 1 Detection of HIV-1 antibodies in the serum and GCT of different population groups

Group	No.	Dot_{GCT}	EIA_{serum}	WBp[a]
Stage II	50	50	50	50
Stage III	10	10	10	10
Stage IV	33	32	33	33
Subjects at risk for HIV infection	26	0	0	0
Blood donors	64	1[b]	0	0
Total	183	93	93	93

[a]Samples with positive Western blot in serum and GCT.
[b]Sample with negative Western blot in GCT.

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More on HBe Ag-negative mutants in chronic HBV infection

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Chronic infection with hepatitis B virus (HBV) leads to a wide spectrum of liver diseases, including asymptomatic carrier state, chronic active hepatitis, cirrhosis and hepatocellular carcinoma. Patients presenting with chronic active hepatitis typically have evidence of HBV replication marked by high levels of HBV DNA, HBV surface antigen (HBs Ag) and the e antigen (HBe Ag) in their serum [1]. In later stages of the disease, seroconversion from HBe Ag to anti-HBe antibody classically indicates a transition to a 'non-replicative' phase, in which necro-inflammatory activity ceases. In some cases, however, anti-HBe seroconversion is not followed by such a favorable outcome, and markers for HBV replication remain detectable [2]. Carman et al [3] have studied such patients and found that they carried HBV mutants which cannot synthesize the precore precursor protein and therefore HBe Ag.

The HBe Ag precursor is encoded by the 637-bp precore/core open reading frame starting at nucleotide 1814 of the HBV minus strand genome (nomenclature according to Galibert et al [4]). The most frequently described mutation is a G to A substitution at the 3′ end of the pre-C gene (nucleotide 1896), which converts codon 28 for tryptophan (TGG) to a termination codon (TAG) [5], resulting in the failure of HBe Ag production and the cessation of its secretion into the bloodstream. It is often associated with a G to A silent mutation at nucleotide (nt) position 1899. The frequency of the nt 1896 mutation varies in different studies, ranging from 46% [6] to 80% [3]. This variability led us to investigate the frequency of the nt 1896 mutation and the presence of other putative mutations in a cohort of French patients.

We analyzed 32 serum samples from chronically infected patients showing discordant serologic profiles, i.e. positive for HBs Ag and HBV DNA, negative for HBe Ag and positive for anti-HBe antibody. Serum samples were submitted to heat treatment (100 °C) for 30 min and then clarified by centrifugation (12 000 *g*) for 30 min. This rapid technique for HBV DNA preparation had been validated in preliminary studies as compared either to classical proteinase K–sodium dodecylsulfate (SDS) lysis followed by phenol extraction or to NaOH treatment of the serum. We applied an amplification-created restriction site method derived from that described by Lindh et al [7] for detecting the nt 1896 G to A mutation. According to this procedure, the nt 1865–2058 region of HBV DNA was amplified using P1 primer with a mismatch at position 1893. When the nt 1896 G to A mutation was present, the 194-bp PCR product exhibited a BSU 361 restriction site which could be shown by enzyme digestion and agarose gel electrophoresis. Prior to digestion, a plasmid containing a unique BSU 361 restriction site was added as a control for the completion of digestion. The specificity of the PCR reaction was checked by hybridization with a digoxigenin-labeled probe specific for the HBV precore region. In parallel, the nt 1728–2160 region of HBV DNA was amplified for the determination of nucleotide sequence using a T7 polymerase sequencing kit (Pharmacia).

After digestion with BSU 361, we observed three patterns of migration in an agarose gel among the 32 samples tested. Five cases (16%) showed a digested product of 165 bp characteristic of the G to A mutation. Twenty cases (62%) showed an undigested PCR product of 194 bp. In seven (22%) cases, two fragments comigrated in the agarose gel, one of 194 bp, and one of 165 bp, corresponding to a mixed viral population (wild type and mutant). In these cases, the possibility of partial digestion with BSU 361 was ruled out by