Appendix

Oral fluid testing procedures

Testing for antibodies to HIV was done with the Murex 1+2 GACELISA (VK61, Abbott Diagnostics, Maidenhead),21 with positive results confirmed by using a modified Clonosystems enzyme immunoassay (ELISA; Bistat Diagnostics, Stockport). Anti-HBc testing used Murex ICE (Abbott Diagnostics, Maidenhead), with positive results confirmed by using an in-house radioimmunoassay.21 Testing for antibodies to hepatitis C virus used a modified protocol for the Ortho HCV 3.0 SAVe enzyme linked immunosorbent assay (ELISA; product No 940982, Ortho Diagnostics, Amersham); borderline results were further investigated with a modified Chiron recombinant immunoassay test (RIBA HCV 3.0; product No 930780, Ortho Diagnostics, Amersham).

Estimation of sensitivity and specificity of oral fluid tests

The Ortho HCV 3.0 eSAVe ELISA was selected for the development of an assay for antibodies to hepatitis C virus in oral fluid on the basis of its superior sensitivity on serum testing. Dilutions were prepared of well characterised serum specimens positive and negative for antibodies to hepatitis C virus in which the IgG content was similar to that found in oral fluid. These dilutions were used to optimise conditions for the assay such that the discrimination between positive and negative specimens was maximised. Specimen volume and the duration, temperature, and effect of agitation on incubation of the specimen, conjugate, and substrate were studied. By using the optimum conditions identified, oral fluid specimens collected by Orasure from 291 blood donors who were negative for antibodies to hepatitis C virus were tested to establish the cut off for the assay. Tests on Orasure specimens from 318 people serologically negative for antibodies to hepatitis C virus were all negative (specificity 100%; 95% confidence interval 98.8% to 100%). Of 216 Orasure specimens from seropositive subjects, 188 (sensitivity 87.0%; 82.6% to 91.5%) were positive. Of the 216 oral fluid specimens from seropositive patients, however, 126 had been collected from patients with liver disease, and 116 (92%; 85.9% to 96.1%) of these were positive. The remaining 90 seropositive specimens came from a randomly sampled population of injecting drug users from London. Of these, 72 (80.0%; 70.2% to 87.7%) yielded positive results. As this latter group probably better represents the population of prisoners at risk of hepatitis C infection, this observation was used as a guide to the sensitivity of oral fluid antibodies to hepatitis C virus testing in the population of prisoners described in this paper.


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