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The polymerase chain reaction—a time of transition from research to routine

The identification of hepatitis B virus (HBV) as the cause of "serum hepatitis" followed the discovery of a new protein (Australia antigen) in blood samples and its later recognition as a viral surface protein (HBsAg). Detection of HBsAg remains the front line diagnostic assay for hepatitis B, both in the routine virology laboratory and for screening donated blood, and most infected individuals also are positive for antibodies to the nucleocapsid protein (anti-HBc). However, not all HBsAg carriers have a significant viraemia: HBsAg may be produced from HBV DNA and integrated into the genomes of the hepatocytes without virus replication. The situation is confounded further by the fact that some individuals who are believed to have recovered from infection, with seroconversion to anti-HBs, may have very low levels of virus—detectable by the polymerase chain reaction (PCR)[1]—which may reactivate in the face of immunosuppression.

Detection of hepatitis B viraemia is facilitated by another protein, HBeAg, which is secreted by the infected hepatocytes. HBeAg in serum invariably indicates ongoing virus replication in the liver. The converse, however, does not necessarily apply, and HBeAg may be absent from—and its antibody (anti-HBe) present in—viraemic individuals, especially those infected with precore, core promoter, and some other HBV mutants. Hepatitis B viraemia can only be established unequivocally by the detection of the genome. HBV DNA was first detected indirectly by assaying the endogenous polymerase activity and, following its cloning and sequencing, directly by a variety of hybridisation assays and the PCR.

In the February issue, van Duersen *et al* reported their evaluation of the PCR to resolve diagnostic uncertainties following serological testing of five individuals for markers of HBV infection.[2] The detection of HBsAg in a blood donor five days after hepatitis B immunisation is reassuring, as this testifies to the exquisite sensitivity of current assays. The fear for this front line assay is that HBsAg with amino acid sequence variation in the major antigenic determinant may escape detection, especially by assays which rely on monoclonal antibodies for antigen capture or detection. Such variants have been described most often in immunised children ("vaccine failures") and in liver transplant recipients treated with hepatitis B immune globulin in an attempt to prevent infection of the graft, but may be detected as minority species in persistently infected individuals and selected during natural seroconversion to anti-HBs.

Of the four remaining patients tested,[2] one was HBsAg negative but gave a false positive result when tested for HBeAg. This patient was from a high risk group for HBV infection and a negative PCR could itself cause a diagnostic dilemma: if sequences encoding HBsAg are variant, the primer binding sites may be too. As van Duersen and his colleagues point out, PCR protocols and their performance in detecting HBV DNA are not yet standardised for general use. Three patients were positive using the PCR; one was also HBsAg positive and of interest for the absence of anti-HBc, which was not pursued. A patient with a single change in the immunodominant region of HBsAg illustrates the paradox that polyclonal antibody may be more likely than monoclonal antibody based assays to fail to detect variant HBsAg with single amino acid substitutions,[3] especially where the antigen is present in low titre. The final case, with multiple substitutions in HBsAg, illustrates the real threat to surface antigen testing. The concern is that failure to detect HBsAg will lead, not to diagnostic dilemmas, but to the use of a donated unit of blood which proves to be HBV positive.

Despite a long standing reluctance to introduce assays which detect nucleic acid into routine diagnostic laboratories, it seems inevitable that assays based on the PCR will be used more and more in such laboratories. The recently discovered GB virus C[4] (GBV-C, also known as hepatitis G virus) is a case in point. While antibodies to the viral surface protein (anti-E2) seem to be a marker of recovery, attempts to devise assays for antigens or antibodies present during infection have not been successful, and diagnosis of viraemia depends on detecting the genomic RNA. But for the lack of evidence of pathogenicity, it seems inevitable that routine PCR testing of donated blood for GBV-C would already have been introduced. However, experience with another recently discovered virus, HHV-8 or KSHV, illustrates all too well that even established research laboratories may fall foul of PCR contamination.[5]

To comply with an EC requirement from 1 January 1999, PCR testing of plasma pools used for blood products must begin for hepatitis C virus in the near future. The aim is to detect those rare donations in the window period between infection and seroconversion to anti-HCV. Testing of "minipools" of around 500 donations seems the most likely scenario, and there will be an obligation to analyse positive pools to identify the source. One suspects that pressure will follow to test for HIV and other viruses (for example, HBV and parvovirus, perhaps in a multiplex format) and, later, to test blood used for individual transfusions.

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