PCR for Hepatitis C: User Friendly?

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Saeed Hamid (Department of Medicine, Aga Khan University Hospital, Stadium Road, Karachi.)

A revolution has taken place in the field of infectious disease due to the introduction of molecular biology techniques[1]. In particular, the Polymerase Chain Reaction (PCR) has led the field due to the fact that it directly detects the antigen of interest, is relatively easy to perform and is extremely sensitive and specific. However, it is some of these very properties which have shown that there is a downside as well, to this elegant technique[2]. The concept behind PCR is relatively simple and the process essentially consists of three reactions. First, the DNA of interest is broken down into the two single stranded components (denaturation), then small pieces of complementary DNA called primers are attached to specific sites on the single DNA strands (annealing) and finally, the whole complementary copy of the DNA synthesized (extension). This process, which mainly takes place in an automated machine, is repeated many times over to give a large amount of DNA. Thus, finally, the antigen of interest is obtained in a huge amount which can be analyzed in various ways. PCR is particularly suited for the study of Hepatitis C Virus (HCV) infection due to a number of reasons. The virus has not yet been grown in culture or seen on Electron Microscopy, there is as yet no serologic assay for antigen detection and the virermia is usually of a low level and transient. In addition, the available antibody tests are not ideal because they may remain negative in the early stages of acute HCV infection and in a small number of chronic infections. HCV is a common infection in patients with chronic liver disease in our country. Hence, our group's interest in developing PCR for HCV in our own laboratory. We now offer this as a routine test in our hospital laboratory and are seeing an ever-increasing number of requests for HCV PCR. It is therefore, timely, that the exact value of HCV PCR indifferent clinical situations be determined so that the end-users can gain maximum benefit out of this-powerful but expensive test.

PCR for diagnosis of HCV infection

PCR usually does not have a major role to play in the diagnosis of HCV infection, an important point which clinicians must understand. Serologic tests are usually reliable and much less expensive, particularly with the introduction of second generation ELISA tests[3]. These tests have 90-95% sensitivity and specificity, particularly in the diagnosis of patients who have chronic liver disease. They may, however, have a slightly higher false positivity or false negativity rate when used to screen healthy populations, for example, blood donors[4]. in such a situation PCR can be employed as a confirmatory diagnostic test, just as a Western Blot would be used to confirm HIV infection in doubtful cases. HCV PCR can however, have good diagnostic value in cases of suspected acute HCV infection as it can become positive within the first few days, whereas, the antibodies may take up to 6 weeks to develop[5]. In such situations, it will be more cost effective to order a PCR rather than the antibody test. PCR would also be the test of choice in immuno-compromised patients suspected of HCV infection, who will not develop antibodies.

PCR for monitoring treatment of HCV

This is the clinical area where HCVPCR has the greatest potential benefit and may justify it's cost. Treatment of chronic HCV infection is expensive and the best means of monitoring response to treatment is still undecided. For example, it is clear that responses in transaminase levels and virological responses frequently do not correlate[6]. Transaminase levels may have normalized and yet the virus persists as demonstrated by PCR. Such patients are likely to relapse very quickly when treatment is stopped. Therefore, if the end point of treatment is virological clearance, then PCR is extremely important in monitoring therapy. We recommend that a PCR be done to monitor treatment

for HCV in the following manner. PCR should be done before start of treatment to confirm the presence of viremia and to establish a baseline test. During therapy, progress can be monitored with transaminase levels alone. However, before it is decided to stop therapy, the absence of viremia should be demonstrated, preferably by a series (2 to 3) of negative PCR tests. Serial testing is advisable because viremia may be transiently negative even in the natural course of HCV infection[7].

Specimen handling for PCR

A negative PCR test for HCV may be disturbing for the clinician when he expects a positive test in a given clinical situation. This may be a genuine false negative result due to a technical error in the tests. However, there are other much likelier explanations of which the physician should be aware. Firstly, as mentioned earlier, the viremia in HCV infection is a dynamic process and can become transiently negative even without treatment. A single PCR measurement is testing this dynamic process at just one instance in time and may not be reflective of the whole process. Secondly, efficient specimen handling has been shown to be very important for increasing the sensitivity of the PCR[8]. HCV is a fragile virus like most RNA viruses and viraemia levels will decay rapidly if blood samples are not handled quickly. For best results it is recommended that samples are processed and properly stored within an hour of collection. Therefore, logistics are an important consideration when a PCR test for HCV is ordered. In summary, PCR is an important addition in our range of tests already available for HCV. Applied properly, it will yield information that may be very important in patient management and is not currently available by any other means. Results have to be interpreted in the context of the whole clinical situation and serial testing may be required in particular situations.

References

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