Evaluation of the Automated COBAS AMPLICOR Hepatitis C Virus PCR System

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To evaluate the reliability and feasibility of the automated Roche COBAS AMPLICOR PCR system for routine detection of hepatitis C virus (HCV) RNA, a total of 405 serum samples previously tested by an in-house nested PCR and manual Roche AMPLICOR microwell plate HCV test were examined. Complete concordance was found between the results with the HCV COBAS AMPLICOR system and the previously determined HCV RNA status. The automated HCV COBAS AMPLICOR system provides the clinical microbiology laboratory with a specific and sensitive PCR method for rapid and reliable detection of HCV RNA.

The determination of viral RNA in serum and tissue samples by amplification techniques is indispensable in the diagnosis of hepatitis C virus (HCV) infection and in monitoring of response to treatment (1). Of the available methods, PCR commands itself by virtue of its high sensitivity and its consequent ability to detect the very low levels of HCV RNA that are present in many clinical samples. However, although PCR is in principle a simple test in daily laboratory practice, many problems have been encountered with this method, the most important being frequent generation of false-positive results due to the amplification of contaminating nucleic acids, mainly the PCR products from previous amplifications (2). In addition, problems have also been encountered with false-negative PCR results, caused mainly by amplification inhibitors present in clinical specimens or by insufficient isolation of nucleic acids. Therefore, standardized procedures are needed to ensure reliable PCR results (2). In 1993 the first-generation AMPLICOR HCV test (Roche Molecular Systems, Branchburg, N.J.) was developed to increase the availability and improve the standardization of HCV RNA PCR testing. This commercial test was designed for amplification in the Perkin-Elmer (Norwalk, Conn.) 9600 thermal cycler, with enzyme immunoassay-like colorimetric detection taking place in standard microtiter wells coated with an oligonucleotide designed to capture the biotinylated PCR product. To avoid contamination by previously produced amplicons, the uracil-N-glycosylase procedure was incorporated into the assay. This first-generation test compared favorably with HCV in-house PCR protocols in several preclinical and clinical evaluations in Europe, Japan, and the United States (6–9, 11, 12).

Recently, a second-generation automated HCV COBAS AMPLICOR system has been developed by Roche Molecular Systems (3). This system, which automatically amplifies and detects PCR products, contains a single thermal cycler with two independently regulated heating and cooling blocks, an incubator, a magnetic particle washer, a pipettor, and a photometer. The biotinylated amplified products are captured on oligonucleotide-coated paramagnetic microparticles and detected with an avidin-horseradish peroxidase conjugate and tetramethylbenzidine as the substrate. Additionally, an internal control which monitors possible inhibition of the PCR is incorporated into the assay. The internal control is ampliﬁed with target nucleic acid in the specimen and detected after capture by paramagnetic microparticles coated with an internal control-speciﬁc probe. In specimens that are negative for target nucleic acid, a positive signal for the internal control provides assurance that the specimen was successfully ampliﬁed and detected (3).

In the present study we evaluated the reliability and feasibility of HCV COBAS AMPLICOR on a total of 405 previously well-characterized serum samples obtained from 262 subjects, all from Slovenia. The test was performed according to the manufacturer’s instructions, as previously described (4). Serum samples were removed from each erythrocyte clot 1 to 4 h after venipuncture and aliquotted into five tubes, which were stored at −70°C until being thawed for testing. Samples included in the study were purposely preselected in order to increase the possibility of the appearance of false-negative and false-positive results. In all samples, anti-HCV status was determined with the Ortho HCV 3.0 ELISA (Ortho Diagnostic Systems, Neckargemünd, Germany). Reactive sera were supplementaly tested by Chiron RIBA HCV 3.0 SIA (Chiron Corporation, Emeryville, Calif.). The presence of HCV RNA was determined by both in-house nested PCR and the first-generation AMPLICOR HCV microwell plate assay, as previously described (8, 9). All known precautions were rigorously taken to avoid sample-to-sample contamination and PCR product carryover. Briefly, these included (i) strict physical separation of areas for clinical specimen preparation, amplification, and detection, with separate supplies of aerosol-resistant tips (ART; Molecular Bio-Products, San Diego, Calif.) and dedicated pipettors for each area; (ii) meticulous pipetting techniques; (iii) frequent changes of gloves; (iv) the use of multiple negative controls; and (v) daily cleaning of all working areas, test tube holders, and sample racks with sodium hypochlorite. The anti-HCV and HCV RNA status of all subjects included in the study was evaluated in collaboration with responsible physicians. Clinical assessment included risk factors for HCV infection, liver function test results, liver histology findings (if available), clinical and laboratory follow-up observations, and results of amplification and serological tests obtained with additional specimens from the same subjects. Hence, 191 HCV RNA-positive (all anti-HCV positive) and 214 HCV RNA-negative samples (160 anti-HCV negative and 54 anti-HCV positive) were included in the study. Among the 191 HCV RNA-positive samples obtained from 132 patients, 14 samples had below 2,000 copies of HCV RNA/ml, 18 had between 2,000 and 15,000 copies, 15 had between 15,000 and 50,000 copies, 28 had between 50,000 and 100,000 copies, 36 had between...
100,000 and 300,000 copies, and 80 had over 300,000 copies of HCV RNA/ml, as determined by the AMPLICOR HCV Monitor assay (Roche Molecular Systems) (5). According to the manufacturer’s recommendations, all samples which tested positive by the AMPLICOR HCV microwell plate assay and negative by AMPLICOR HCV Monitor were defined as having below 2,000 copies of HCV RNA/ml. The HCV genotype distribution was as follows: 62 patients had HCV subtype 1b, 32 had genotype 3, 21 had subtype 1a, 11 had subtype 2c, 5 had subtype 2b, and 1 had genotype 4, as determined by the Inno LiPa HCV II assay (Innogenetics, Ghent, Belgium) (10).

Complete concordance between the results with the HCV COBAS AMPLICOR system and the previously determined HCV RNA status of 405 serum samples was found in the present study. Interestingly, despite the numbers of low-level viremia samples, samples with an HCV genotype other than 1a, and anti-HCV-positive and HCV RNA-negative samples included, no false-negative results were obtained by HCV COBAS AMPLICOR. Analysis of the internal control results indicated that there was no PCR inhibition in any of the anti-HCV-positive and HCV RNA-negative samples, proving the usefulness of the internal control. The uracil-N-glycosylase procedure incorporated into the system seems to effectively control PCR carryover.

To the best of our knowledge, our study is the second published evaluation of HCV COBAS AMPLICOR. Results comparable to ours were obtained by Jungkind et al. (4), who established a complete correlation between the results of the novel automated HCV PCR system and those of the first-generation AMPLICOR HCV test in a study performed on 214 serum samples (60 HCV RNA positive and 154 HCV RNA negative). However, those authors made no attempt to confirm the obtained results by additional testing and examination of clinical findings, since, as they stated, a detailed comparison of the PCR results with other test results and clinical history was not a primary goal of their study.

In conclusion, our study confirmed that the HCV COBAS AMPLICOR system provides the clinical microbiology laboratory with a specific and sensitive PCR method for rapid and reliable routine detection of HCV RNA.

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REFERENCES