Synthetic Viral Particles Promise To Be Valuable in the Standardization of Molecular Diagnostic Assays for Hepatitis C Virus

Hepatitis C virus (HCV) is a primarily parenterally transmitted pathogen first discovered in the late 1980s. Infection with HCV is common throughout the world, and disease attributable to this virus is a major public health issue in both developed and developing countries. In the United States alone, ~4 million individuals are infected with HCV, with an estimated 30,000 new infections occurring annually (1). Of those infected by this virus, between 70% and 90% develop chronic HCV infection (compared with only 10–20% of those infected with hepatitis B virus), and 20–30% of chronically infected patients progress to liver disease (liver failure, cirrhosis, and/or hepatocellular carcinoma) within 20–30 years (2). Given these statistics and the lack of an effective vaccine, it is not surprising that the morbidity and mortality attributable to HCV are considerable and on the increase. HCV is responsible for 8000–10,000 deaths per year in the United States, a figure that some experts postulate will triple in the next 10–20 years, and chronic HCV infection is the single most common etiology of hepatic injury in patients undergoing liver transplantation (1).

The discovery of HCV in 1989 represents one of the most important accomplishments of molecular biotechnology. An infectious agent that had never been seen or cultured was discovered by cloning fragments of its genome from clinical material; the entire genetic and biochemical composition of the virus was then reconstructed in vitro (3). HCV is a member of the Flaviviridae family of viruses and possesses a single-stranded RNA genome of some 9.5 kb. The RNA sequence contains a single open reading frame and encodes a viral precursor protein of ~3000 amino acids that undergoes posttranslational processing to form six proteins. The HCV genome also contains two untranslated regions, one of 351 nucleotides at the 5’ end and a smaller region of just 35 nucleotides at the 3’ end. Enzyme immunoassay and immunoblot techniques for detecting anti-HCV antibodies were developed soon after the virus was discovered, and current iterations of these assays enable past exposure to HCV to be determined with a high degree of accuracy. In the United States, universal use of antibody assays to screen blood donors for HCV has been responsible for an 85–90% decrease in the incidence of transfusion-associated hepatitis during the past decade (1). It is perhaps fitting, given the manner in which HCV was discovered, that differentiating active HCV infection from past, resolved infection currently can be achieved only by using molecular assays to detect the presence of viral RNA in peripheral blood. More conventional microbiological approaches for detecting infection, such as culture or antigen detection, have been difficult to develop for HCV and currently play no role in diagnosis. Several molecular techniques, most prominently PCR and branched-chain DNA hybridization, have been used to enable either qualitative or quantitative detection of HCV RNA in serum or plasma (4). In addition, tests have been developed to determine the particular genotype of the HCV population infecting an individual patient. Because therapeutic response rate is influenced by HCV genotype (5), determination of HCV genotype may have prognostic and therapeutic value. Although debate continues among those responsible for the care of HCV-infected individuals regarding optimal use of molecular tests, molecular diagnosis and monitoring of HCV infection are clearly destined to become the standard of care in the relatively near future.

Unfortunately, given the increasing clinical importance and laboratory utilization of nucleic-acid testing for HCV, there are as yet no Food and Drug Administration-approved assays for diagnosis of active infection, monitoring of viral response to therapy, or viral genotyping. Comparative evaluations of commercially available research or investigational use products and so-called “home brew” assays have produced widely divergent results in terms of sensitivity, specificity, and reproducibility (6), and it is clear that assay standardization remains a major problem. One of the key elements in developing standardized assays is the preparation and distribution of appropriate control materials for validation and verification studies, quality-control testing, and proficiency testing. Both the NCCLS (7) and the College of American Pathologists [CAP; see Ref. (8)] recommend that, at a minimum, such control materials should be able to verify that sample preparation ensures release of nucleic acid from the agent being sought and eliminates specimen-specific amplification inhibitors, and that amplification and postamplification procedures are adequate for sensitive, specific, and (if necessary) quantitative detection of the desired agent.

Given the evidence that genotype-specific variability in amplification or hybridization efficiency can be a potential source of error in qualitative or quantitative HCV RNA detection (9), it would be highly advantageous to have such control reagents available for each of the principal HCV genotypes. Genotype-specific control reagents are also an obvious prerequisite for adequate quality control and quality assurance of any assay that purports to be able to determine the genotype of HCV from infected plasma or serum. The technical and logistic difficulties in preparing such reagents for an infectious agent such as HCV that has a ribonucleic acid genome (the ubiquity of ribonucleases in biological fluids precludes simply adding synthetically generated HCV RNA to clinical matrices) and cannot be propagated in vitro are considerable. Indeed, at the present time, the only reagent
that adequately satisfies NCCLS and CAP recommendations for a positive control is HCV-infected human plasma or serum, preferably pooled from a single donor to ensure a reasonably homogeneous viral population. Obtaining large volumes of infected plasma or serum and then characterizing them with respect to the quantity and genotype of HCV present is both an expensive and laborious proposition. In addition, because HCV-infected samples are considered biohazardous, complying with hazardous material shipping regulations further adds to the cost of acquiring this material. As a direct consequence of the expense and difficulty of producing and distributing such controls, manufacturers of commercially available HCV assays currently provide only synthetically generated HCV RNA transcripts or complimentary DNA as positive-control reagents in their kits. These reagents control for the performance of the amplification and (or) hybridization components of the assay but cannot validate the performance of sample preparation reagents and protocols. For laboratories to test the performance of the entire assay, they must purchase HCV-infected plasma from an independent vendor and perform testing of such material in addition to manufacturer-recommended quality control. Many laboratories are reluctant to add this additional financial burden to the already considerable cost of performing molecular diagnostic testing for HCV. Although formal data regarding the number of laboratories using controls other than those provided by the manufacturer are not available for HCV molecular testing, an educated guess can be made by extrapolating from data collected from laboratories performing HIV-1 RNA testing. In a survey conducted recently by the CDC (10), only 103 (47%) of 219 respondents performing nucleic-acid testing for HIV were using controls other than those provided by the manufacturer, and of those laboratories using such controls, only 69% were including them in each analytical run. Thus, only 32% of the laboratories performing molecular testing for HIV were following the recommendations of the NCCLS and CAP with respect to quality-control procedures.

It seems reasonable to surmise that, given the substantial overlap in laboratories performing HIV and HCV molecular testing, the use of extramanufacturer controls is as infrequent a component of routine quality control in HCV assays as it apparently is in those tests designed to detect and quantify HIV. In large part because of the absence of a Food and Drug Administration-approved test, however, the number of laboratories performing home-brew assays for detecting HCV RNA by PCR is considerably higher than for HIV, making the lack of stringent quality control substantially more worrisome.

Set against this rather depressing background, the description in this issue of Armored RNA as a technological solution to the standardization conundrum (11) makes for mood-elevating reading. These investigators have now successfully prepared synthetic viral particles containing components of either the HCV (11) or HIV (12) genome, and have demonstrated that such particles are stable after prolonged incubation in a clinical matrix. There are two clear and immediate indications for using these or similar reagents. First and foremost, this type of control should replace the positive-control reagents currently included in commercial HCV molecular assays. The inclusion of such a control would allow the routine assessment of the adequacy of RNA recovery and of the elimination of sample inhibition during sample processing. In view of the complex nature of HCV RNA testing and the clinical significance of the results, stringent testing of all aspects of assay performance should be mandatory. Because Armored RNA controls are noninfectious, stable, and presumably substantially less expensive to manufacture than HCV-infected plasma is to obtain, the incorporation of such controls should not lead to a substantial increase in kit cost and would eliminate the need to acquire costly extramanufacturer controls. The second obvious use of Armored RNA controls is in the production of proficiency testing materials. Over the past several years, the CAP has made available to laboratories several HCV-RNA-containing samples for proficiency testing purposes. The inconsistent results of these surveys have served to illustrate both the aforementioned lack of laboratory standardization and, unfortunately, the difficulty of using clinical materials for proficiency testing (13). HCV RNA degradation led to the invalidation of one survey, for example, and only limited proficiency testing for genotypes other than 1a (the most common HCV genotype in the United States) has been possible. The use of Armored RNA to produce proficiency testing samples could alleviate these problems and provide an assessment of the performance of laboratories without the need for caveats regarding the “quality” of the material supplied for testing.

It seems entirely apropos that the same technology that both enabled the discovery of HCV and provided the tools to develop assays for laboratory diagnosis and monitoring of infection with this virus should be used to generate the control reagents so necessary for assuring the performance of clinical laboratory assays. Publication of the current article describing the Armored RNA system (11) will, hopefully, be followed in the not too distant future by the commercial availability of control reagents formulated using this technique. Such a development will constitute a major milestone in the process of integrating high-quality molecular diagnostic testing into the routine clinical laboratory.

References


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