JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3298–3300 Vol. 35, No. 12
0095-1137/97/$04.00+0

NOTES

GEMHEP Multicenter Quality Control Study of PCR Detection of GB Virus C/Hepatitis G Virus RNA in Serum

MARC BOGARD,[1]† CLAUDINE BUFFET-JANVRESSE,[2]† JEAN-FRANÇOIS CANTALOUBE,[3]† PHILIPPE BIAGINI,[3]† GILLES DUVERLIE,[4]† SANDRINE CASTELAIN,[4]† JACQUES IZOPET,[5]† MARTINE DUBOIS,[5]† CHRISTINE DEFER,[6]† ISABELLE LEPOT,[6]† JOLIETTE COSTE,[7]† PATRICK MARCELLIN,[8]† MICHÈLE MARTINOT-PEIGNOUX,[8]† PHILIPPE HALFON,[9]† VICTORIA GEROLAMI,[10]† LIONEL FRANGEUL,[11]† JEAN-MICHEL PAWLOTSKY,[12]† FRANÇOISE ROUDOT-THORAVAL,[12]† ELISABETH DUSSAIX,[13]† PASCALE LOISEAU,[14]† NADINE RAVERA,[14]† PATRICIA LEWIN,[15]† JERÔME LAMORIL,[16]† JOËLLE LERABLE,[17]† FRANÇOISE BOUCHARDEAU,[17]† SYRIA LAPERCHE,[17]† MARTINE MARIOTTI,[17]† JEAN-JACQUES LEFRÈRE,[17]†* THIERRY NICOT,[18] FRANÇOIS DENIS,[18] MICHÈLE GASSAIN,[19] PATRICK MÉREL,[20] CHRISTIAN TRÉPO,[21] VALÉRIE THIERS,[22] CHRISTIAN BRÉCHOT,[22] CLAUDE FÉREC,[23] BERNARD MERCIER,[23] FRANÇOISE STOLL-KELLER,[24] PIERRE PALMER,[25] AND PIERRE LEBON[25]

Centre Hospitalier, Meaux,[1] Centre Hospitalo-Universitaire (CHU), Rouen,[2] Etablissement de Transfusion Sanguine (ETS),[3] Laboratoire Alphabio,[9] and Hôpital de la Conception,[10] Marseille, CHU, Amiens,[4] CHU, Toulouse,[5] ETS, Lille,[6] ETS, Montpellier,[7] Hôpital Beaujon, Clichy,[8] CHU Pitié-Salpêtrière,[11] ETS de l'Assistance Publique-Hôpitaux de Paris,[14] Institut National de la Transfusion Sanguine,[17] CHU Necker,[22] and Hôpital Saint Vincent-de-Paul,[25] Paris, CHU Henri Mondor, Créteil,[12] Hôpital Paul Brousse, Villejuif,[13] Laboratoire Cerba, Cergy-Pontoise,[15] Hôpital Louis-Mourier, Colombes,[16] CHU, Limoges,[18] CHU, Nantes,[19] ETS, Bordeaux,[20] Hôtel Dieu, Lyon,[21] ETS de Bretagne Occidentale, Brest,[23] and CHU, Strasbourg,[24] France

Received 24 April 1997/Returned for modification 5 August 1997/Accepted 2 September 1997

PCR is, to date, the only available tool for the detection of GB virus C (GBV-C) and hepatitis G virus (HGV) RNAs. Twenty-two French laboratories participated in a quality control study to assess the sensitivity and specificity of their procedures. The panel included 13 positive controls and 7 negative controls. The laboratories used either in-house PCR techniques adapted from the literature or partly standardized commercial tests. Three laboratories performed faultlessly with the entire panel. Most laboratories had excellent specificity (100% in 20 of 22 laboratories). Sensitivity was acceptable (85 to 100%) in 15 centers and insufficient (38 to 77%) in 7. As with nonstandardized in-house PCR, the commercial assays gave discrepant performances in different laboratories. These results suggest that laboratories willing to use PCR for detection of GBV-C/HGV RNA for research or diagnostic purposes should participate in multicenter quality control trials.

GB virus C (GBV-C) and the so-called hepatitis G virus (HGV) are two recently identified variants of the same virus. GBV-C/HGV was shown to belong to the *Flaviviridae* family (8), but to a genus different from that of hepatitis C virus (HCV), and to induce acute and chronic infections in humans (10, 15). Whether GBV-C/HGV actually induces clinical manifestations remains disputed. Its role in chronic hepatitis seems to be limited (1, 3, 13), while it has recently been suggested to be associated with cases of fulminant hepatic failure (6, 16) or aplastic anemia (2, 7, 18). Whatever its actual pathogenetic role, GBV-C/HGV is highly prevalent in the general population, as shown by reported prevalences ranging from 1 to 4% in blood donors from industrialized countries (10, 11), raising the issue of screening blood donations in order to prevent transfusion-associated transmission. In the absence of any reliable serological assay, detection of GBV-C/HGV RNA by PCR remains the only available diagnostic tool and indicates ongoing infection (12). Because of its high sensitivity, PCR can generate false-positive results, usually due to laboratory contamination or DNA carryover, and technical problems can also be responsible for false-negative results. This was recently emphasized by multicenter quality control studies of PCR for the detection of HCV RNA, in which important discrepancies were observed among the participating laboratories (4, 5, 17). Strict guidelines for sample preparation, PCR conditions, and detection of amplified products are therefore required.

The Groupe Français d'Etudes Moléculaires des Hépatites (GEMHEP) initiated a multicenter quality control study of PCR for the detection of GBV-C/HGV RNA. Twenty-two specialized laboratories, including diagnostic, research, and blood bank laboratories, participated in the study, the aim of which was to assess the sensitivity and specificity of various in-house and commercial GBV-C/HGV PCR procedures in order to determine optimal PCR conditions for maximum diagnostic proficiency.

The quality control study used a test panel consisting of 20 serum samples, including 13 positive controls (4 in duplicate) and 7 negative controls. The samples were obtained from nine subjects who repeatedly tested positive for GBV-C/HGV RNA

* Corresponding author. Mailing address: Institut National de la Transfusion Sanguine, 53, boulevard Diderot, 75012 Paris, France. Phone: 01 43 44 46 83. Fax: 01 55 33 88 74. E-mail: lefrere@worldnet.fr.

† Member of the Groupe Français d'Etudes Moléculaires des Hépatites (GEMHEP).

and seven volunteers who tested negative for GBV-C/HGV RNA. The positive group included seven blood donors without risk factors for parenterally transmitted viruses, who had normal alanine aminotransferase activity and negative markers for infection with hepatitis B and C viruses, human immunodeficiency virus types 1 and 2, and human T-lymphotropic virus types 1 and 2; one patient with chronic hepatitis C associated with GBV-C/HGV replication who was receiving alpha interferon and had normal alanine aminotransferase activity at the time of sampling; and one GBV-C/HGV RNA-positive subject, likely infected through the transfusion of packed erythrocytes, who subsequently became GBV-C/HGV RNA negative (a self-resolving infection). The protocol was approved by the institutional ethics committee, and the subjects gave written informed consent to the study.

The panel was prepared by an investigator external to the participating laboratories. Aliquoted samples (0.5 ml) were frozen and stored at −80°C. In order to perform the study blindly, the panel was randomly coded (1 to 20) and frozen coded aliquots were supplied to the laboratories. The panel was tested by the routine procedures of each laboratory for RNA extraction, cDNA synthesis, PCR amplification, and the sequence of the oligonucleotide primers used (9, 14). Of the 22 laboratories, 10 used an in-house PCR technique adapted from the literature and 12 used a commercial, partly standardized GBV-C/HGV RNA PCR assay: either an HGV PCR enzyme-linked immunosorbent assay (Boehringer GmbH, Mannheim, Germany) (10 laboratories) or an HGV DEIA test (Sorin Biomedica, Saluggia, Italy) (2 laboratories). The results were sent to the same external investigator who coded the panel. Each center was identified by a letter chosen by random drawing and unconnected to the author list of this article.

Each laboratory was assigned a quality score corresponding to the percentage of correct results for the 20 samples tested. PCR sensitivity, specificity, and accuracy rates were calculated for the whole series of experiments and for each laboratory separately. The 95% confidence intervals (CI) were estimated according to the binomial distribution, or by approximation of the binomial distribution by the normal distribution when possible. Comparisons of quality scores were performed by the Mann-Whitney nonparametric test.

Table 1 shows the results of the study in terms of sensitivity (defined as the percentage of positive PCR results for positive controls) and specificity (defined as the percentage of negative PCR results for negative controls). Among the 440 tests performed by the 22 laboratories, 3 false-positive results and 62 false-negative results were observed. Thus, the overall sensitivity was 78% (95% CI, 73 to 83%) and the overall specificity was 98% (95% CI, 96 to 100%). Three laboratories (laboratories D, O, and V) had both 100% sensitivity and 100% specificity. One of these used the Boehringer test, one used an in-house PCR technique adapted from the method of Linnen et al. (10), and one used the Sorin test. A sensitivity of 100% was observed in four laboratories (laboratories D, H, O, and V), while a specificity of 100% was observed in all but two laboratories (laboratories H and S).

Deeper analysis showed that sensitivity could be considered satisfactory in 15 of the 22 laboratories, in which it ranged from 85 to 100%. Indeed, in all instances, 85% sensitivity was related to a failure to detect low-titer viremia in the duplicate samples from a patient receiving alpha interferon treatment. Among these 15 laboratories, 8 used an in-house PCR technique, 6 used the Boehringer assay, and one used the Sorin assay. The remaining seven laboratories clearly had insufficient sensitivity in detecting GBV-C/HGV RNA. Two of them used an in-house PCR technique, four used the Boehringer assay, and one used the Sorin assay. None of the steps of the procedure appeared critical to the results obtained by the laboratories, whether they chose a commercial or an in-house PCR method. The wide dispersion of the results given by the commercial assay indicated that the way in which the technique was handled was more critical than the technique itself, which was similar in all cases.

TABLE 1. Performance of 22 laboratories in PCR detection of GBV-C/HGV RNA on a panel of 20 serum samples

Laboratory	PCR technique	Sensitivity[a] (n = 13)	Specificity[b] (n = 7)	Quality score (%)
A	In-house	69	100	80
B	In-house	85	100	90
C	Boehringer	92	100	95
D	Boehringer	100	100	100
E	In-house	85	100	90
F	In-house	85	100	90
G	In-house	85	100	90
H	Boehringer	100	86	95
I	Boehringer	77	100	85
J	Boehringer	85	100	90
K	In-house	85	100	90
L	In-house	85	100	90
M	Boehringer	85	100	90
N	Boehringer	38	100	60
O	In-house	100	100	100
P	Boehringer	54	100	70
Q	Boehringer	31	100	55
R	In-house	77	100	85
S	Sorin	38	71	50
T	Boehringer	85	100	90
U	In-house	85	100	90
V	Sorin	100	100	100
Mean (95% CI)		78 (73–83)	98 (98–100)	85 (82–88)

[a] Defined as the percentage of positive PCR results for positive controls.
[b] Defined as the percentage of negative PCR results for negative controls.

The results of this quality control study indicated that the specificity of GBV-C/HGV RNA detection was satisfactory in the participating laboratories. This suggests that these laboratories benefited from the successive steps of a previous quality control study of HCV PCR aimed at improving the procedures used, in which both sensitivity and specificity were consistently improved (4, 5). However, several laboratories lacked sensitivity in detecting GBV-C/HGV RNA in the present study. It must be stressed, in addition, that, in the absence of a "gold standard" for the presence of GBV-C/HGV replication, the positive controls were recruited on the basis of positive RNA detection by a currently used PCR procedure. Therefore, the possibility that GBV-C/HGV variants could escape the detection procedures used in this study, and that sensitivity would thus be overestimated, cannot be ruled out.

A lack of sensitivity was observed with in-house procedures as well as with commercial assays. It is of interest that important discrepancies were observed among different laboratories using the same commercial assay. It must be stressed, however, that the commercial assays are not entirely standardized in their present form. Indeed, the first steps of the reaction (RNA extraction and PCR amplification) still require in-house procedures. The discrepant results observed among different laboratories, some of which lacked experience in RNA PCR, emphasize the critical role of the initial steps of the procedure for the success of PCR for the detection of GBV-C/HGV RNA, as well as the need for assays in which all steps of the procedure are standardized.

To date, the actual pathogenicity of GBV-C/HGV appears unclear. The role of this virus in fulminant hepatic failure is debated, and the long-term consequences of GBV-C/HGV infection are unknown (2, 6, 7, 11, 13, 16, 18). For these reasons, whether GBV-C/HGV should be sought in patients with various medical conditions and whether blood donations should be screened for GBV-C/HGV are questions that are still debated. The results of the present quality control study show that, whatever the decisions made regarding these two important issues, technical problems in GBV-C/HGV PCR need to be resolved. Sensitivity and specificity issues should also be addressed before the performance and publishing of clinical and epidemiological studies of GBV-C/HGV infection based on the detection of viral RNA in serum by PCR. Overall, the results of this quality control study strongly suggest that full standardization of GBV-C/HGV RNA detection is necessary and that the laboratories performing GBV-C/HGV PCR should participate in repeated quality control studies, whatever technique they use.

REFERENCES

1. **Alter, H. J.** 1996. The cloning and clinical implications of HGV and HGBV-C. N. Engl. J. Med. **334:**1536–1537.
2. **Byrnes, J. J., A. T. Banks, M. Piatack, and J. P. Kim.** 1996. Hepatitis G-associated aplastic anaemia. Lancet **348:**472.
3. **Fiordalisi, G., A. Bettinardi, I. Zanella, G. Parninfo, R. Stellini, and D. Primi.** 1996. Prevalence of the GBV-C virus in a group of Italian patients with acute and chronic hepatitis of unknown etiology, abstr. C225. *In* Abstracts of the IXth Triennial International Symposium on Viral Hepatitis and Liver Disease.
4. **French Study Group for the Standardization of Hepatitis C Virus PCR.** 1994. Improvement of hepatitis C virus RNA polymerase chain reaction through a multicentre quality control study. J. Virol Methods **49:**79–88.
5. **Groupe Français d'Etudes Moléculaires des Hépatites.** 1994. Multicentre quality control of hepatitis C virus RNA polymerase chain reaction, p. 49–56. *In* Groupe Français d'Etudes moléculaires des Hépatites (ed.), Hepatitis C virus: new diagnostic tools. John Libbey Eurotext, Paris, France.
6. **Heringlake, S., S. Osterkamp, C. Trautwein, H. L. Tillmann, K. Böker, S. Muerhoff, I. K. Mushahwar, G. Hunsmann, and M. P. Manns.** 1996. Association between fulminant hepatic failure and a strain of GBV virus C. Lancet **348:**1626–1629.
7. **Kao, J. H., J. Chen, M. Y. Lai, and D. S. Chen.** 1996. GBV-C/HGV infection and aplastic anaemia. Lancet **348:**1032–1033.
8. **Leary, T. P., A. S. Muerhoff, J. N. Simons, T. J. Pilot-Matias, J. C. Erker, M. L. Chalmers, G. G. Schlauder, G. J. Dawson, S. M. Desai, and I. K. Mushahwar.** 1996. Sequence and genomic organization of GBV-C: a novel member of the *Flaviviridae* associated with human non-A-E hepatitis. J. Med. Virol. **48:**60–67.
9. **Leary, T. P., A. S. Muerhoff, J. N. Simons, T. J. Pilot-Matias, J. C. Erker, M. L. Chalmers, G. G. Schlauder, G. J. Dawson, S. M. Desai, and I. K. Mushahwar.** 1996. Consensus oligonucleotide primers for the detection of GB virus C in human cryptogenic hepatitis. J. Virol. Methods **56:**119–121.
10. **Linnen, J., J. Wages, Z. Y. Zhang-Keck, K. E. Fry, K. Z. Krawczynski, H. Alter, E. Koonin, M. Gallagher, M. Alter, S. Hadziyannis, P. Karayiannis, K. Fung, Y. Nakatsuji, J. W. Shih, L. Young, M. Piatak, C. Hoover, J. Fernandez, S. Chen, J. C. Zou, T. Morris, K. C. Hyams, S. Ismay, J. D. Lifson, G. Hess, S. K. H. Foung, H. Thomas, D. Bradley, H. Margolis, and J. P. Kim.** 1996. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science **271:**505–508.
11. **Loiseau, P., M. Mariotti, C. Corbi, N. Ravera, R. Girot, M. Thauvin, E. Portelette, X. Mariette, F. Roudot-Thoraval, M. Benbunan, and J. J. Lefrère.** 1997. Prevalence of hepatitis G virus (HGV) RNA in French blood donors and recipients. Transfusion **37:**645–650.
12. **Pawlotsky, J. M., J. J. Lefrère, D. Dhumeaux, and F. Denis.** 1996. Nouveaux virus d'hépatites reliés aux Flaviviridae: le virus de l'hépatite G et les virus GB, p. 77–94. *In* J. J. Lefrère (ed.), Les virus transmissibles par le sang. John Libbey Eurotext, Montrouge, France.
13. **Pawlotsky, J. M., F. Roudot-Thoraval, M. Pellerin, and D. Dhumeaux.** 1996. GBV-C infection in HCV-infected patients: epidemiological characteristics, influence on HCV infection and response to interferon alfa therapy. Hepatology **24:**226A.
14. **Schlueter, V., S. Schmolke, K. Stark, G. Hess, B. Ofenloch-Haehnle, and A. M. Engel.** 1996. Reverse transcription-PCR detection of hepatitis G virus. J. Clin. Microbiol. **34:**2660–2664.
15. **Simons, J. N., T. P. Leary, G. J. Dawson, T. J. Pilot-Matias, A. S. Muerhoff, G. G. Schlauder, S. M. Desai, and I. K. Mushahwar.** 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat. Med. **1:**564–569.
16. **Yoshiba, M., H. Okamoto, and S. Mishiro.** 1995. Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown aetiology. Lancet **346:**1131–1132.
17. **Zaaijer, H. L., H. T. M. Cuypers, H. W. Reesink, I. N. Winkel, G. Gerken, and P. N. Lelie.** 1993. Reliability of polymerase chain reaction for detection of hepatitis C virus. Lancet **341:**722–724.
18. **Zaidi, Y., C. S. Chapman, and S. Myint.** 1996. Aplastic anaemia after HGV infection. Lancet **348:**471–472.