DOI 10.1007/s10517-015-3145-9

VIROLOGY

Efficiency of Measurements of Hepatitis C Virus RNA

G. P. Pogosyan, A. A. Konovalova, V. V. Protas, and A. Sh. Dodonova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 160, No. 8, pp. 232-235, August, 2015
Original article submitted December 24, 2014

Hepatitis C virus loading was evaluated using modifications of PCR product detection: terminal point fluorescent detection, real time assay, and agarose gel electrophoresis. The advantages and flaws of the real time PCR and electrophoretic detection of amplification products for evaluation of the quantity of virus particle copies are evaluated.

Key Words: *PCR; hepatitis C RNA; reverse transcription; electrophoresis*

Viral infections remain a pressing problem of modern medicine, because of their overall prevalence and high incidence. One of diseases very difficult to cure and highly prevalent is viral hepatitis C [2,6]. The disease is caused by HCV, an RNA-containing hepatotropic virus of the *Flaviviridae* family [1].

We compare the efficiency of HCV measurements by PCR with electrophoretic and fluorescent detection.

MATERIALS AND METHODS

Screening studies were carried out by PCR – the most reliable method for measurements of HCV RNA. Evaluation of the infectious agent RNA copies is practically important for HCV detection [5], as PCR detects the infection during the earliest period of the disease: on week 1 after infection. The virus loading was evaluated by the real time quantitative PCR with agarose gel electrophoresis [3,4].

Plasma specimens from patients with the tentative diagnosis of HCV, made by clinicians, were studied. Measurements of HCV RNA were carried out with AmpliSense HCV Monitor-FI and AmpliSense HCV-Eph test systems with AmpliSense HCV-FL reagents.

E. A. Buketov Karaganda State University, Karaganda, Kazakhstan. ***Address for correspondence:*** gayane_63@mail.ru. G. P. Pogosyan

RESULTS

In order to evaluate HCV RNA, 324 specimens were screened by PCR with the terminal point hybridization fluorescent detection from November 2013 to February 2014.

Thirty-four positive specimens were selected, after which HCV RNA copies were analyzed by real-time PCR and electrophoresis.

Two test systems fit for analysis for evaluation of the quantity of hepatitis C viral particles in clinical material (blood): AmpliSense HCV-Monitor-FI and AmpliSense HCV-Eph. Specimens found positive in previous experiments were analyzed as follows: HCV RNA was isolated, the resultant molecules were used in two tests simultaneously: real time PCR and reverse transcription with subsequent amplification of cDNA fragments.

When using real-time PCR, the results of amplification of HCV RNA site and internal control specimen (ICS) were analyzed each time. Accumulation of ICS amplification product was evaluated by FAM fluorophore detection channel, of specific product by HEX channel. The calibrator concentration was preset before each analysis. The reactions of KB1 and KB2 calibrators were repeated twice for calibration curve plotting. The following calibrator values were preset for FAM and HEX channels: 250,000 for KB1 and 50 for KB2. A total of 11-12 specimens were analyzed in

TABLE 1. Evaluation of Viral Loading in Clinical Specimens (Data Obtained in AmpliSenseSoftMonitorFRT)

No.	Sample	Threshold cycles		Concentrations		Result	
		FAM Green IC	HEX Yellow spec	FAM Green IC	HEX Yellow spec		
		threshold concentration	threshold concentration	copies	copies	copies/ml	copies/ml
1	1	25.9	19.1	1489	14,296	1,295,810	Ok
2	2	26.2	29	1229	18	2000	Ok
3	3	26.6	17.7	951	36,692	5,207,101	Ok
4	4	26	24	1397	528	50,998	Ok
5	5	26.7	19.2	892	13,365	2,022,063	Ok
6	6	24.4	17.5	3892	41,981	1,456,190	Ok
7	7	24.1	17	4716	58,784	1,682,624	Ok
8	8	26.9	21.5	785	2841	488,516	Ok
9	9						ICS failure
10	10	26	17.2	1397	51,378	4,965,117	Ok
11	11	28.4	22	300	2029	911,698	Ok
12	12	27.1	17.9	691	32,070	6,268,639	Ok
13	NCS	24.4		3892			NCS Ok
14	PCS1	24.7	24.7	3212	329	13,846	PCS1 Ok
15	PCS2	24	18.2	5028	26,204	703,533	PCS2 Ok
16	IC1	16.6	15				Calibrator
17	IC1	19.2	14.7				Calibrator
18	IC2	31.5	27.9				Calibrator
19	IC2	30.9	27.1				Calibrator

Note. IC: internal control; spec: specific; NCS: negative control specimen; PCS: positive control specimen.

each experiment. Viral particle copies were counted after real time PCR (Table 1).

In order to compare the results with the results of electrophoretic detection, the data were converted from U/ml to copies/ml. The following distribution of the numbers of copies/ml was obtained: 10^3 in 2 cases, 10^4 in 3, 10^5 in 5, 10^6 in 14, and 10^7 in 8 cases. The concentration "below 3" was stated in 2 samples.

In order to carry out PCR with electrophoretic detection, serial 10-fold dilutions of HCV cDNA in TE (Tris-EDTA) buffer were prepared. Electrophoregrams of cDNA were analyzed with consideration for the test system sensitivity (10^3). The concentration of viral particles was not found in 3 of all specimens, because the electrophoregram showed no fluorescence at the level of 240 n. p., corresponding to HCV specific fragment. The following distribution of virus loading was detected: 10^3 in 1 specimen, 10^4 in 4, 10^5 in 13, 10^6 in 3, 10^7 in 8, and 10^8 copies/ml in 2 specimens.

The results obtained by two PCR methods are presented in Table 2. The numbers of viral particles evaluated by PCR with electrophoretic detection coincided with the results of the real time PCR in 9 specimns. In the rest specimens analysis by the real time PCR proved to be more sensitive (10-100-fold).

The data obtained by the above methods indicate their comparable sensitivities.

The real time PCR exhibited certain advantages. The procedure of analysis is simple, requirements to PCR laboratory are less stringent, quantitative analysis is carried out with completely automated estimation of the number of copies, and registration is electronic. However, the price of a single analysis by this method is very high, which impedes its introduction into everyday practice.

PCR with electrophoretic detection exhibited just two advantages: visualization of amplified PCR products and rather cheap reagents.

Hence, the advantages and flaws of the methods evaluated in our study can be useful when choosing the method for evaluation of HCV RNA in clinical and research laboratories.

TABLE 2. Results of Quantitative Analysis Using Two Methods for Amplification Product Detection

No.	Fluorescence	RT-PCR		Electrophoresis
		U/ml	copies/ml (U/ml×4)	copies
1	+	1.3×10^6 (1,295,810)	5×10^6 (5,183,240)	10^7
2	+	2×10^3 (2000)	8×10^3 (8000)	10^3
3	+	5×10^6 (5,207,101)	2×10^7 (20,828,404)	10^7
4	+	5×10^4 (50,998)	2×10^5 (203,992)	10^4
5	+	2×10^6 (2,022,063)	8×10^6 (8,088,252)	10^7
6	+	1.5×10^6 (1,456,190)	5.8×10^6 (5,824,760)	10^7
7	+	1.7×10^6 (1,682,624)	6.7×10^6 (6,730,496)	10^7
8	+	4.9×10^6 (4,965,117)	1.9×10^7 (19,860,468)	10^8
9	+	9×10^5 (911,698)	3.6×10^6 (3,646,792)	10^7
10	+	6×10^6 (6,268,639)	2.5×10^7 (25,074,556)	10^8
11	+	2.7×10^6 (2,737,349)	1×10^7 (10,949,396)	10^5
12	+	3.2×10^6 (3,218,951)	1×10^7 (12,875,804)	10^6
13	+	6.8×10^6 (6,755,003)	2.7×10^7 (27,020,012)	10^5
14	+	1.5×10^5 (154,843)	6×10^5 (619,372)	10^5
15	+	1.7×10^7 (16,688,699)	6.7×10^7 (66,754,796)	10^7
16	+	<3		
17	+	8×10^4 (8748)	3×10^4 (34,992)	10^5
18	+	1×10^5 (107,959)	4×10^5 (431,836)	10^5
19	+	7×10^3 (7548)	3×10^4 (30,192)	10^6
20	+	0.6×10^2 (63)	0.2×10^3 (252)	10^4
21	+	1.4×10^6 (1,416,733)	5.6×10^6 (5,666,932)	10^7
22	+	4.8×10^5 (477,219)	1.9×10^6 (1,908,876)	10^5
23	+	1.5×10^4 (15,347)	6×10^4 (61,388)	10^4
24	+	5×10^5 (543,052)	2×10^6 (2,172,208)	10^5
25	+	2×10^6 (2,005,436)	8×10^6 (8,021,744)	10^6
26	+	2×10^6 (2,005,436)	8×10^6 (8,021,744)	10^6
27	+	4×10^4 (43,293)	1.7×10^5 (173,172)	10^5
28	+	6.7×10^5 (668,224)	2.6×10^6 (2,672,896)	10^5
29	+	6.5×10^4 (65,285)	2.6×10^5 (261,140)	10^5
30	+	6.4×10^6 (6,350,980)	2.5×10^7 (25,403,920)	10^5
31	+	2×10^6 (2,269,730)	9×10^6 (9,078,920)	
32	+	<3		
33	+	4×10^5 (412,032)	1.7×10^6 (1,648,128)	10^5
34	+	5.8×10^5 (580,087)	2.3×10^6 (2,320,348)	10^5

REFERENCES

1. *Hepatitis C. World Health Organization. Information Bulletin* (2013).
2. K. P. Meyer, *Hepatitis and Hepatitis Aftereffects* [in Russian], Moscow (1999).
3. M. N. Melnikova, A. V. Vinogradov, B. M. Mednikov, and L. A. Chekanovskaya, *Priroda*, No. 4, 51-54 (2001).
4. C. A. Heid. J. Stevens, K. J. Livak, and P. M. Williams, *Genome Res.*, **6**, No. 10, 986-994 (1996).
5. I. M. Mackay, K. E. Arden, and A. Nitsche, *Nucleic Acids Res.*, **30**, No. 6, 1293-1305 (2002).
6. H. R. Rosen, *N. Engl. J. Med.*, **364**, No. 25, 2429-2438 (2011).