J Clin Pathol 1996;49:994–997

Assessment of commercial enzyme immunoassay for hepatitis C virus serotyping

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Abstract

***Aims*—To assess a commercial enzyme immunoassay (EIA) for the serotyping of hepatitis C virus (HCV) for routine use in a diagnostic laboratory setting, as well as for noting the serotype prevalence of selected specimens.**

***Methods*—Seventy six serum specimens, submitted to the laboratory for routine hepatitis studies between May 1992 and February 1996 and stored at −20°C, were evaluated. These specimens were categorised into specific hepatic, renal, and paediatric clinical conditions. The specimens all tested positive for HCV antibodies on a screening EIA, with confirmation on a recombinant immunoblot assay (RIBA). Certain specimens were also HCV RNA positive by the reverse transcription polymerase chain reaction (RT-PCR). All the specimens were serotyped using the newly developed serotyping EIA.**

***Results*—Twenty seven (35.5%) specimens were typable. Type 5 predominated (56%), followed by type 1 (33%), types 1 and 6 (7%) and type 3 (4%). The serotype 5 specimens showed 85% and 90% reactivity with recombinant antigens c100-3 and c22-3c, respectively; serotype 1 specimens showed 75% and 100% reactivity with these antigens. All serotype 5 specimens reacted with the c33-c antigen, but only 60% of serotype 1 specimens reacted with this antigen. The differences in the reactivity of the serotype 5 and serotype 1 specimens for c33-c antigen in the RIBA were significant, but no significant differences in reactivity for antigens c-1-1, c100-3, and c22-3 were noted. Serotype 3 specimens showed equal reactivity with all four antigens used in the RIBA.**

***Conclusion*—The serotyping EIA was easy to use, rapid, and cost effective compared with molecular assays. This assay seems to be ideal for the routine diagnostic laboratory setting, but could not be used for certain clinical specimens. The demonstration of serotypes 5, 1, and 3 was not unexpected in this cohort. The occurrence of serotype 6, although concurrent and more likely to be a false cross reaction with serotype 1 peptides, requires confirmation by molecular genotyping before it can be claimed that this type is present in South Africa.**

(*J Clin Pathol* 1996;49:994–997)

Keywords: Hepatitis C virus, enzyme immunoassay, serotypes, recombinant immunoblot assay.

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Accepted for publication
4 September 1996

Hepatitis C virus (HCV) has a seroprevalence of 0.5% to 8.0% among blood donors in many regions of the world, and is also a major aetiological agent of community acquired non-A, non-B hepatitis.[1 2] Hepatitis C disease is usually benign, often only mildly symptomatic, and may be slowly progressive.[1 2] HCV infection is progressive in about 80% of cases and may give rise to liver cirrhosis and hepatocellular carcinoma.[1 3] About 20% of chronic carriers may develop liver cirrhosis after about 20 years and a further 10% hepatocellular carcinoma after about 30 years.[1 3] Thus the most serious consequences of HCV infection will not be manifest for many years, suggesting that early identification of those patients at the greatest risk for progression to liver disease is advisable.[3 4]

The considerable genomic heterogeneity displayed by the different HCV isolates prompted the current classification of HCV into six major genotypes, of which types 1 to 4 are further divided into subtypes.[2 4 5] The clinicopathological importance of each genotype is currently the subject of intense investigation, with many studies reporting differing disease outcomes and responses to treatment with interferon-α.[3 4] The different genotypes differ in their nucleotide sequence, which is reflected in the amino acid composition of the epitopes on the HCV proteins.[6] Although molecular genotyping has paved the way in determining the viral types, recent serological techniques have been introduced and are considered to be as effective.[7] Several investigators have suggested that certain HCV serotypes (serological genotypes) vary in their propensity to produce clinically relevant liver disease, but other clinical studies have recorded severe and progressive disease with all the genotypes.[3 4 6] These serotyping methods could facilitate larger population based studies as well as contribute to the prognostic profile of eventual long term patient management.[6 7] Additionally, the particular geographical distribution of each serotype can be determined.[8]

We report on an assessment of a newly developed commercial enzyme immunoassay (EIA) for the serotyping of HCV, as well as noting the HCV serotype prevalence associated with particular clinical entities in the Pretoria area of South Africa.

Methods

Seventy six serum specimens, submitted to the Department of Medical Virology laboratory, Pretoria, between May 1992 and February 1996, and found to be positive for HCV antibodies during routine hepatitis testing,

Table 1 Patient demographics and laboratory data for serotyped specimens

		Patient data			Laboratory data					
					Recombinant immunoblot assay				Serotyping	
Specimen number	*Clinical category*	*Age*	*Sex*	*Screening EIA*	*c-1-1*	*c100-3*	*c33-c*	*c-22-3*	*(EIA)*	*RT-PCR*
2b	CAH	61	Female	+	+	+++	+++	+	5	NT
3b	CAH	61	Female	+	+++	+++	+++	+++	1	NT
4b	CAH	60	Female	+	−	−	++	+	5	NT
5a and 5b	CAH	52	Female	+	+	+	++	+	5	−
10	CAH	54	Male	+	++	++	++	++	3	+
11	CAH	?	Female	+	+	+	+	+	1	NT
14	CAH	40	Male	+	+	+	++	+++	1	+
22	CAH	?	Male	+	+	+	++	+	1	NT
23	CAH	60	Female	+	−	+	+	+++	5	NT
24	CAH	35	Female	+	−	+	++	−	1 and 6	NT
38	Liver cirrhosis	26	Female	+	+	++	++	++	5	+
39	Liver cirrhosis	5	Male	+	++	+	++	+++	1 and 6	NT

NT = not tested; CAH = chronic active hepatitis.

were included in the study. After initial screening all specimens were stored at −20°C with limited freeze-thawing. All specimens had tested positive for antibodies to HCV on a screening EIA (Murex anti-HCV, Murex Diagnostics Ltd, Dartford, Kent, UK) and the seropositivity was confirmed on a recombinant immunoblot assay (RIBA) (Chiron RIBA HCV 2.0 SIA; Chiron Corporation, Emeryville, California, USA). Seven of the 76 specimens were tested and found to be positive for HCV RNA by a reverse transcription polymerase chain reaction (RT-PCR) (HCV Probe/Primer Set, Digene Diagnostics Inc, Beltsville, Maryland, USA). The specimens were from patients in the following clinical categories: hepatoma (n = 8), chronic active hepatitis (CAH) (n = 26), acute hepatitis (n = 5), liver cirrhosis (n = 8), renal transplantation follow up (n = 9), pre-renal transplantation assessment (n = 1), chronic haemodialysis (n = 4), nephrotic syndrome (n = 1), a staff member working in a haemodialysis unit (n = 1), and paediatric conditions (n = 2). Eleven of the 76 serum specimens were serial follow up specimens.

All specimens were serotyped using a newly developed commercial serotyping EIA (Murex HCV serotyping 1–6 assay). The assay was performed and interpreted strictly according to the manufacturer's instructions. This serotyping EIA differentiates between the serotypes by detection of antibodies to the type specific antigenic regions of NS4. Synthetic peptides representing the variable antigenic regions from the non-structural protein NS4 of HCV types 1–6 are coated on the solid phase. Diluted serum is added together with competing heterologous peptides. The partial cross reaction between antibodies to one type of HCV and peptides from heterologous types results in type specific binding of the antibody to the solid phase.

Table 2 Hepatitis C virus serotypes in relation to patient data, clinical category, and laboratory data

	No of positive patients, hepatitis C virus serotype			
	5	*1*	*1 and 6*	*3*
Sex:				
Male	7	2	1	1
Female	7	6	1	0
Patient age (years):				
Mean age (range)	41 (26–71)	47 (35–61)	20 (5–35)	54
Clinical category:				
Acute hepatitis	2	0	0	0
CAH	4	4	1	1
Liver cirrhosis	1	0	1	0
Hepatoma	3	2	0	0
Haemodialysis	1	0	0	0
Before transplantation	0	1	0	0
After transplantation	2	1	0	0
Nephrotic syndrome	1	0	0	0
Laboratory assays:				
RIBA: positive	14	6	2	1
Indeterminate	0	2	0	0
RT-PCR: positive	1	3	0	1
Negative	2	0	0	0

CAH = chronic active hepatitis; RIBA = recombinant immunoblot assay.

Results

Patient demographics, clinical data, and laboratory results of the specimens from which HCV could be serotyped are summarised in tables 1 and 2. The HCV from 27 of the 76 (35.5%) specimens tested was typable. Of those which were untypable, 73% were stored for more than two years and the remainder for 24 months or less. Table 3 shows the number of specimens in each storage time category and the proportion which were typable. Specimens were divided into two categories: those stored for longer than 24 months and those stored for 24 months or less. Statistical analysis showed that the proportion of typable specimens stored for ≤ 24 months (18/20) was significantly larger ($P<0.0001$, $\chi^2 = 35.16$) than the specimens stored for periods > 24 months (9/56). Serotype 5 was the predominant identifiable serotype (56%), with type 1 (33%), types 1 and 6 (7%), and type 3 (4%) also evident. No serotypic predominance was noted in any of the clinical categories. The percentage reactivity of the serotype 5 and serotype 1 positive specimens to the four recombinant antigens—c-1-1, c100-3, c33-c and c-22-3—was compared. Serotype 5 specimens showed 85% and 100% reactivity with antigens c100-3 and c33-c, respectively, whereas serotype 1 specimens showed 75% and 60% reactivity with these antigens. Serotype 5 specimens showed 90% reactivity with c-22-3 and serotype 1 100% reactivity. Serotype 5 had 55% and serotype 1 50% reactivity, respectively, with c-1-1. There was no significant difference between the reactivity of serotypes 5 and 1 for antigens c-1-1, c100-3, and c22-3 (Fisher's exact test, $p<1.0000$; $p = 0.6024$, $p<1.0000$, respectively); but a significant difference between

Table 3 Proportion of serotypable hepatitis C specimens in relation to specimen storage time

Storage time (months)	No of specimens assessed	No of specimens serotypable
> 24 months:		
44	14	0
42	2	1
40	7	2
38	4	0
36	9	3
35	3	0
32	4	2
30	3	0
28	6	0
26	4	1
Total	56	9 (16%)
≤ 24 months:		
24	2	2
22	2	2
20	8	7
18	3	3
12	4	3
9	1	1
Total	20	18 (90%)

Mantel-Haenszel χ^2: $p < 0.0001$.

serotypes 5 and 1 was noted for antigen c33-c (Fisher's exact test, $p = 0.0364$). Type 6 occurred concurrently with type 1 in two of the specimens. One of the specimens was from a 35 year old patient with CAH and the other from a five year old child with liver cirrhosis. Sera from the former patient showed reactivity with c100-3 and c33-c, while sera from latter patient showed high reactivity with antigens c-22-3, c33-c, and c-1-1. HCV type 3 was noted in only one patient, a 54 year old man with CAH, and reactivity to all four RIBA antigens was noted.

Discussion

HCV genotypes have a role in the distinct pathogenesis associated with this virus, with certain types influencing eventual clinical progression.[5 9] Factors that can help to predict the response of HCV infection to interferon treatment include circulating viral RNA and the HCV genotype.[10–14] These parameters can influence the HCV antibody titres detectable on a third generation EIA.[10 12] HCV genotyping using molecular typing methods, such as restriction fragment length polymorphism (RFLP), however, require expertise and are labour intensive and expensive. An easy to use serological assay would therefore be a viable alternative for laboratories where no molecular technology was available.[10] However, comparative data of genotyping and serotyping techniques suggest that the sensitivities of the serotyping methods are significantly lower than those of the molecular based assays.[7 10 12]

In this assessment of a newly available commercial serological method for HCV serotyping, 27 of the selected 76 specimens were serotypable. As genotype 5 has already been shown to predominate in the South African community,[2] the high prevalence of this serotype (56%) was not unexpected. Serotype 5 was evident among the patients with hepatoma, CAH, acute hepatitis and liver cirrhosis, and although the patient numbers are small, this might indicate a clinicopathological role. Serotype 5 specimens had 90% and 100% reactivity with the core region (c-22-3) and the NS3 region (c33-c), respectively, of the genome, which is known to be highly conserved among all serotypes,[15] yet had a lower percentage reactivity with the other non-structural regions. Only the serotype 5 reactivity with antigen c33-c was significantly greater than the serotype 1 reactivity with antigen c33-c. One of the serotype 5 specimens had a low response to the c33-c and c100-3 antigens. Serotype 1 also had a relatively high prevalence (33%) and was seen in most of the selected clinical categories. Most of the serotype 1 positive patients had antibodies to the NS4 encoded antigens which correlates with findings from earlier studies.[8 15] Serotype 3 was detected in only one (4%) of the specimens and displayed uniform reactivity to the recombinant antigens. The low prevalence of this type in the South African community, albeit from a different geographical region, has been noted before.[2]

Evidence of mixed infection with more than one serotype—that is, serotypes 1 and 6—was seen in a 35 year old woman with CAH and a five year old who presented with liver cirrhosis. This phenomenon has been documented in blood donors from Hong Kong.[8] Only retrospective clinical details of the former patient were available; consequently contact with sources other than South African cannot be excluded. The five year old patient, however, was of African ethnic origin, but no HCV relevant risk factors were reported in the clinical history. However, the serotype 6 reactivity in the above two cases is more likely to have been caused by a false cross reaction with the serotype 1 peptides. Therefore, the presence of serotype 6 infection needs to be confirmed by molecular genotyping before it can be concluded that this serotype occurs in the South African population.

The high percentage of unserotypable specimens could possibly be ascribed to low antibody titres either due to prolonged storage at −20°C or to a possible genotypic influence on serological reactivity.[12] Many of the clinical groups were also potentially difficult to serotype because of innate or iatrogenic antibody depletion. The sera stored for long periods had discordant reactivity on the RIBA on repeat testing, varying from indeterminate to negative. Most of the more recent specimens were serotypable. Analysis shows that a higher percentage (90%) of specimens stored for ≤24 months were typable, and this was significant. This supports the use of serotyping for routine assays, yet caution should be exercised with retrospective studies. Ongoing research and improved molecular techniques, however, continuously reveal evidence of new hepatitis C virus variants.[16–20] This could well account for some of the untypable specimens and would thus necessitate continual upgrading of current serotyping assays.

Serotyping, although one of the important predictors for HCV infection response to treatment, should not be considered in isolation, but in conjunction with the clinical and other laboratory variables determined for each individual patient. The serotyping assay used in this investigation was easy to use in a routine

diagnostic laboratory setting. The assay was cost effective, being half the cost of molecular methods. The turnround time of the molecular methods involved three working days; the serotyping assay could be performed within three and a half hours. Although there are limitations to the assay—namely, the inability to differentiate subtypes and reduced sensitivity on stored specimens, it provides a viable alternative to molecular based assays.

We thank Ms Louise Scholtz for technical assistance; Drs ABW van Niekerk and I Pike for helpful comments; and Dr PJ Becker for the statistical analyses.

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