Peptide based enzyme immunoassays for detecting hepatitis C antibodies in sera of people at high risk

F G Gabriel, C G Teo

Abstract

***Aim*—To evaluate the performance of three newly introduced enzyme immunoassays (EIAs) for hepatitis C virus (HCV) antibodies, based on synthetic oligopeptides as antigens.**

***Methods*—Referred serum samples (n = 173) from people representing groups at high risk of HCV infection were studied. An EIA based on second generation recombinant polypeptide antigens was used for comparison. EIA reactivities were validated by testing repeatedly reactive samples in two recombinant antigen based immunoblot assays.**

***Results*—In samples from patients with liver dysfunction and those with bleeding disorders sensitivity of the three peptide based EIAs, manufactured by Innogenetics NV, Biokit SA, and United Biomedical Inc., were all 93%; specificity and efficiency were all greater than 95%. In samples from blood donors (previously tested as positive by the Ortho and Abbott Second Generation EIA) specificity, sensitivity, and efficiency were 95% or greater in all three peptide assays. Sensitivity, specificity, and efficiency of the recombinant antigen based *Ortho* Second Generation EIA were 100%, 89%, and 93%, respectively, in sera of patients with liver disease and those with bleeding disorders; and 100%, 43%, and 83%, respectively, in prescreened samples from blood donors.**

***Conclusion*—The peptide EIAs are more specific but less sensitive than the Ortho EIA. Peptide based EIAs should be useful in validating the specificity of Ortho EIA reactivities.**

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The first assays for detecting hepatitis C virus (HCV) antibodies used a recombinant HCV polypeptide as antigen.[1] This protein (c100–3) is derived from a region of the genome that putatively codes for the non-structural (NS) proteins NS3 and NS4. Radioimmunoassays and enzyme immunoassays (EIAs) based on c100–3 have been successfully used for screening blood and organ donors, seroprevalence studies, and the determination of HCV disease associations. However, such assays occasionally fail to detect HCV positive samples[2–4] or yield false reactivities.[5 6]

Newer assays have recently been introduced that should improve the performance of first generation assays. They use as antigens recombinant fusion proteins or synthetic oligopeptides derived from regions of the genome other than those encoding c100–3. We have compared the prototype recombinant protein based second generation EIA with the prototype EIA that used peptide antigens and found divergent specificity and sensitivity results.[7] In this study, using sera from those at risk of infection with HCV, we evaluate other, more recent peptide EIAs against EIAs and immunoblot assays based on second generation recombinant polypeptides.

Methods

Samples (n = 173) sent to this laboratory for HCV antibody screening and confirmation were studied. These were consecutive samples drawn from people in three risk groups for HCV infection: patients with liver dysfunction (L) (n = 58); patients with bleeding disorders (B) (n = 45); and blood donors whose samples had previously been tested as HCV antibody positive in a blood transfusion centre using the Abbott HCV EIA Second Generation (Abbott Laboratories, Abbott Park, Illinois) (D) (n = 70). All samples except those from D had not been pretested by the referring laboratories.

All samples were tested for antigens to HCV antibodies using the following EIAs: Ortho HCV Antibody ELISA Test System (Ortho Diagnostic Systems Inc., Raritan, New Jersey); Innotest HCV Antibody (Innogenetics NV, Antwerp, Belgium); Bioelisa HCV (Biokit SA, Barcelona, Spain); and UBI HCV EIA (United Biomedical Inc., Lake Success, New York). The Ortho EIA uses two recombinant antigens expressed by yeast): c22–3, which is derived from the genomic region of the prototype HCV strain that encodes the core (capsid) polypeptide; and c200, translated from regions encoding NS3 and NS4.[8] EIAs from Innogenetics, Biokit, and UBI use synthetic peptide antigens. Sequences of the peptides are not specified; however, it is known that peptides in the UBI assay are derived from core and NS regions.[9] All four assays are indirect EIAs. Results were expressed as optical density (OD) values related to a cutoff value calculated according to the manufacturers' instructions. OD values above cutoff indicated reactivity.

Samples reactive in any one assay were tested once more in that assay. Repeatedly

Hepatitis and Retrovirus Laboratory Virus Reference Division, Central Public Health Laboratory, Public Health Laboratory Service, 61 Colindale Avenue London NW9 5HT
F G Gabriel
C G Teo
Correspondence to:
Dr C G Teo

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reactive samples were further tested with the Second Generation Chiron RIBA HCV Test System (Ortho Diagnostic Systems Inc.), a recombinant immunoblot assay (RIBA) that uses recombinant fusion proteins 5–1–1, c100–3, c33c and c22–3 immobilised as individual bands on nitrocellulose strips. The first three antigens are shorter polypeptides of c200, and c100–3 encompasses 5–1–1. Reactivities to each antigen were classified as +/−, 1+, 2+, 3+ and 4+, and overall reactivities were expressed as reactive (R^+), indeterminate (R^i), and non-reactive, as recommended by the manufacturer. This assay is referred to as RIBA 2 in this study.

Samples reactive in an EIA but not in the RIBA 2 were further tested by another immunoblot assay, the third Generation Chiron RIBA HCV Test System (Chiron Corporation, Emeryville, California) (referred to as RIBA 3). The test format is similar to RIBA 2. Each test strip is successively coated with the following antigens: c100 (p), c33c, c22(p) and NS5. c33c and NS5 are recombinant fusion proteins encoded from the NS3 and NS5 genomic regions, respectively; c100(p) and c22(p) comprise synthetic peptides derived from the c100 and core coding regions, respectively. In this study samples that tested negative in this assay were considered to be RIBA negative (R^-) and those that were negative in RIBA 2 but indeterminate in this assay were considered R^i.

Results

One hundred and seven (63%) of the total number of samples studied were repeatedly reactive in at least one EIA. Of these, 82 (70%) were reactive in all four EIAs; five (6%) were reactive in various combinations of two and three EIAs; and 20 (24%) were reactive in one EIA only. In this last group most (n = 19) were reactive in the Ortho EIA.

EIA reactivities were validated using two RIBA systems. Seventy nine (74%) reactive samples could be categorised as R^+, 13 (12%) as R^i, and 15 (14%) as R^-. R^i reactivities were of two groups: reactivity with either c22–3 (n = 7) or c22 (p) (n = 1); and reactivity with either c100–3 (n = 4) or c100 (p) (n = 1). In this study R^i samples that were c22–3– or c22 (p) reactive are referred to as R^i (c22 reactive); similiarly, R^i samples that were c100–3 or c100 (p) reactive are referred to as R^i (c100 reactive). While the staining intensity found in R^i (c22 reactive) samples varied (with intensities of 2+ to 4+), that in R^i (c100 reactive) samples was invariably weak (all with 1+ intensity). Of the eight R^i (c22 reactive) samples, seven were reactive in all EIAs and one was reactive in three EIAs. All five R^i (c100 reactive) samples were reactive in the Ortho EIA only; four of these were blood donor samples.

In this study EIA reactive samples are regarded as truly reactive when R^+ or R^i (c22 reactive), falsely reactive when R^-, and truly negative when EIA non-reactive. In view of the low intensity bands in all R^i (c100 reactive) samples these were also regarded as falsely reactive. Using these criteria, the sensitivity, specificity, and efficiency of the EIAs were calculated.[10] Efficiency is regarded as the degree to which results of an assay agree with the true antibody response of the samples tested, and is defined as the percentage of the sum of true positive and true negative results divided by the total number of samples tested. Because samples from the D group were preselected, data from this group were considered separately from the L and B groups. The results are shown in the table. The peptide based EIAs performed similarly. None was as sensitive as the Ortho EIA but all were more specific.

Comparative performance of four HCV EIAs

	Ortho		*Innogenetics*		*Biokit*		*UBI*	
	L+B	*D*	*L+B*	*D*	*L+B*	*D*	*L+B*	*D*
Sensitivity (%)	100	100	93	96	93	96	93	96
Specificity (%)	89	43	98	95	98	100	100	95
Efficiency (%)	93	83	96	96	96	97	97	96

L = patients with liver dysfunction; B = patients with bleeding diatheses; D = blood donors.

Discussion

This study shows that peptide based HCV EIAs display similar performance characteristics; they are all specific and sensitive. However, their sensitivity does not match that of the second generation Ortho EIA, which uses the recombinant antigens c200 and c22–3. There are several possible reasons for this. First, HCV epitopes are more fully represented in the recombinant polypeptides, because they are expressed from larger regions of the viral genome. Second, some HCV epitopes may not be linear but conformational. This explanation is, however, conjectural because the sequences of peptides used as antigens have not been disclosed. Third, the proportion of the various antigenic components coated on to the solid phase varies among assays, and some antigens might be found in suboptimal quantities while combined with other antigens in the same assay.

The relative poor specificity of the second generation Ortho EIA has reduced the overall efficiency of the assay in spite of its very high sensitivity. This low specificity is reminiscent of that obtained from EIAs based on c100–3. The c200 component in the new Ortho EIA probably contributes, because the genomic region encoding it also encodes a fragment of c100–3. The particularly low specificity in the blood donor group very likely results from prescreening by the Abbott assay which, being based on recombinant antigens similar to the Ortho assay, may yield equivalent false positivity rates.

This consideration also raises doubts about the positivity of R^i (c100 reactive) samples, particularly as they all yielded very weak reactivities in the c100–3 or c100 (p) bands. Persistent, weak, solitary c100–3 reactivities have not been demonstrated in recipients of

hepatitis C infected blood products and HCV inoculated chimpanzees who have been closely followed up for long periods after the acute stage of infection.[11–14] As this study shows, samples manifesting such reactivities may be derived from blood donors, and it is important that these people are not falsely implicated as being infected with HCV. Notably none of the peptide based EIAs found that samples with solitary c100–3/c100 (p) reactivities were positive.

There are two limitations to this study. Calculation of sensitivity, specificity, and efficiency as performed here is not the only one approach to the comparison of EIA sensitivities. A more comprehensive evaluation of sensitivities might include, in addition, the testing of EIAs against a seroconversion panel[14] and the determination of end point titres of samples known to contain HCV antibody. The second caveat arises from our consideration that EIA reactive but RIBA unreactive samples are HCV antibody negative, and it is based on the assumption that the sensitivity of RIBA equals or exceeds that of EIAs. In the absence of gold standards for HCV antibody testing, it is currently difficult to ascertain the true negative status of such samples. However, we have attempted in this study to determine true negativity further by subjecting EIA reactive samples that were RIBA 2 unreactive to another, newer RIBA, and classified such samples as negative only if they were also unreactive in the latter assay.

We have already advocated a low cost strategy of HCV antibody confirmation in which EIAs of different antigenic configurations are used to cross validate EIA reactivities.[7] We suggested that the most sensitive EIA should be used as the screening assay and that the most specific EIA which uses another antigenic configuration should be used as the validation assay; only samples that are discordantly reactive after testing with the validation EIA need to be tested using the more costly assays—for example immunoblot. This study shows that the peptide based assays will be useful as validation assays.

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