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Application of the Serodia-HCV Particle Agglutination for the detection of antibodies to Hepatitis C Virus

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Abstract

The Serodia-HCV Particle Agglutination (HCV-PA) for the detection of HCV antibodies was compared with the Enzyme Immunoassay Test (UBI HCV EIA) for possible in-house use. A total of 150 specimens were analysed using UBI HCV EIA and Serodia-HCV PA. Of these, 80 (53.3%) were both PA and EIA positive and 59 (39.3%) were negative by both techniques. Eleven sera (7.4%) were found to be EIA-positive but PA-negative. These 11 discordant sera were further tested by the LiaTek-HCV III Irnmunoassay (Organon Teknika). Ten were found to be line immunoassay negative and one was line immunoassay positive. Failure of the PA to detect the HCV positive serum meant that a small proportion of HCV antibody positives may be missed by the PA test. We conclude that (i) EIA should continue to be the first line screening test in our laboratory, (ii) PA with its 100% specificity could be a useful supplementary screen for all EIA-positive sera and finally (iii) line immunoassay could be used on sera to resolve discordant results in the EIA and PA assays.

Key words: Particle agglutination, enzyme immunoassay, Hepatitis C virus

INTRODUCTION

The cloning of the Hepatitis C Virus (HCV) genome and development of tests to detect anti-HCV in the serum[1,2] has made it possible to diagnose most of non-A non-B hepatitis cases, the aetiology of which was previously unknown. HCV, an RNA virus, has been associated with post transfusion hepatitis[3] and chronic liver diseases such as cirrhosis and hepatocellular carcinoma.[4] The HCV genome is about 9.4 kb long and consists of structural and non-structural proteins (Fig. 1). Three regions viz. C, E1 and E2/NS1, putatively code for structural proteins which include a nucleocapsid and two envelope glycoproteins respectively. Four non-structural proteins viz. NS2, NS3, NS4 and NS5 include a membrane-bindingprotein, a protease, a helicase and an RNA polymerase protein. Antibodies to HCV can only be detected about fifteen weeks after acute infection and in some cases appears after one year of infection.[3] The initial first generation commercial ELISA kits for the detection of anti-HCV consisted only of non-structural proteins viz. the c-100-3 antigens.

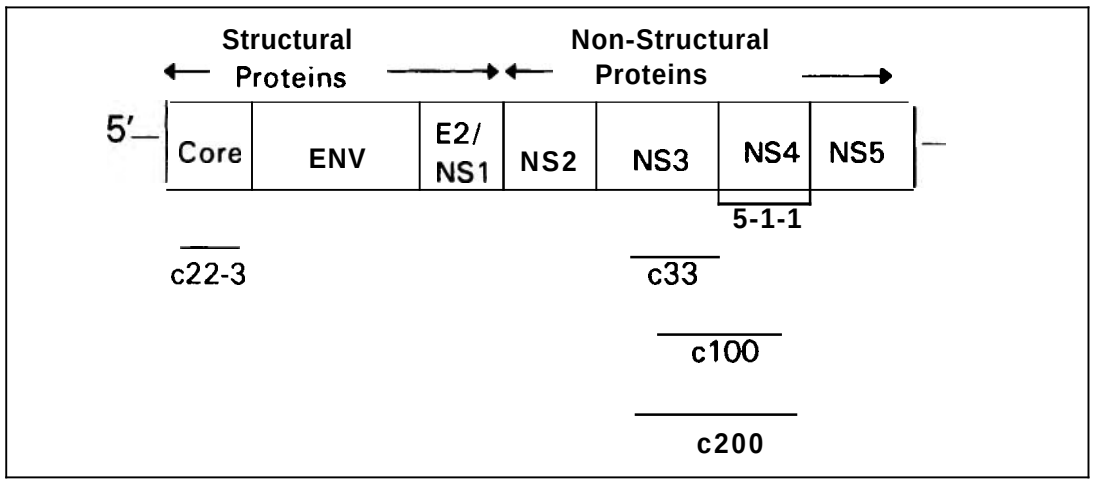

FIG. 1: Organisation of the HCV genome.

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Subsequently, an increase in sensitivity of the commercial ELISA tests was achieved with the incorporation of more non-structural protein (viz c33 antigen) and structural protein (viz c22-3 antigen). Later the development of immunoblot assay/line immunoassay helped to increase the specificity of anti-HCV screening and is now widely used as the "gold standard for serological tests. The Line immunoassay consists of several antigens representing HCV core, NS1, NS3, NS4 and NS5 proteins coated as discrete horizontal lines across a membrane strip.

The Particle Agglutination (PA) assay consists of gelatin particle carriers coated with recombinant structural antigens viz. c22-3 and non-structural protein (c200). It works on the principle that HCV antigen coated particles are agglutinated by HCV antibodies present in serum samples.

Our objectives were to compare the Serodia-HCV Particle Agglutination with the HCV-UBI EIA test and to study the possibility of using PA as a supplementary test in place of the commercially available but expensive, tedious immunoblots and line immunoassays.

MATERIALS AND METHODS

Serum samples

A total of 150 sera samples received from patients with chronic hepatitis were screened for UBI HCV EIA (Organon Teknika) and Serodia-HCV PA (Fujirebio Inc). All sera positive by both test kits and sera showing discordant results between PA and EIA were tested using the LiaTek HCV 111 Line immunoassay (Organon Teknika).

UBI HCV ELISA

The manufacturer's recommendation was adhered to during the testing procedure. 200 ul of specimen diluent was dispensed to a microtitre plate-well coated with HCV synthetic peptides, then 10 ul specimen or control was added. The plate was incubated at 37°C for 30 minutes and then washed 6 times with phosphate buffer. 100 ul of working conjugate was added and plate was incubated at 37°C for 15 minutes, followed by a wash procedure and the addition of 100 ul of OPD substrate solution. Incubation was then carried at 37°C for 15 minutes and the reaction was stopped with 1 mol/l sulphuric acid. The absorbance was read at 492 nm. Specimens which were reactive were then retested. Absorbance equal or greater than the cut-off value was considered as positive and those lower than the cut-off was negative. Cut-off value was calculated as 0.2 X Mean Strongly Reactive Control.

Serodia-HCV Particle Agglutination

The test procedure followed was that of the manufacturer. Using the serum diluent provided in the test kit, test sera and control sera were serially diluted in three wells viz, 1:4, 1:8 and 1:16.25 ul of reconstituted control particles was added into well 2 thereby giving a serum final dilution of 1:16; another 25 ul of sensitised particles was added into well 3 to give a final dilution of 1:32. The plate was then shaken and incubated at room temperature for 2 hours. A specimen was positive if agglutination occurred at dilution 1:32 with sensitised cells and negative at dilution 1:16 with control particles. When a test serum showed agglutination with both control and positive particles, the manufacturer's absorption procedure was carried out and the serum retested.

LiaTek HCV III Immunoassay

The procedure followed was that of the manufacturer. Briefly, strips coated with HCV recombinant proteins and synthetic peptides (representing NS1, NS3, NS4 & NS5) regions were placed in rack compartments with coated surface upwards. One ml of sample diluent was dispensed into each sample trough followed by 10ul of sample. One ml of negative and positive control were pipetted into the respective control troughs. The strips were then incubated on a shaker for 14-18 hours at 20°C-25°C. The strips were washed, and 1ml of conjugate added. After 30 minutes at room temperature the strips were again washed and 1ml of substrate added. They were then incubated on a shaker for 30 minutes at 20°C-25°C. The substrate was then aspirated and 1 ml of sulphuric acid was added. The strips were then incubated on a shaker at 20°C-25°C and then dried. A test serum was considered to be positive for HCV antibodies if there were (i) at least one HCV antigen lines with a 2+ or higher rating or (ii) at least two HCV antigen lines with 1+ or higher rating, while a negative result will show no band.

RESULT

Of the 150 sera tested, 80 (53.3%) were Anti-HCV positive by both EIA and PA. These were tested using the line immunoassay which also gave positive results (Table 1). Fifty-nine (39.3%) sera were found to be Anti-HCV negative on both EIA and PA. These were considered to be

TABLE 1: Anti-HCV line irnmunoassay confirmation results with PA/EIA positive samples

	Line immunoassay positive	Line immunoassay negative
EIA+/PA+ (n = 80)	80	0
EIA+/PA- (n = 11)	1	10
Total	81	10

Anti-HCV negative and were not further tested on the line immunoassay. There were 11 (7.4%) samples which showed discordant results viz EIA positive but PA negative; these were further tested by the line immunoassay wherein 10 were negative and 1 was positive (Table 1). The sensitivity and specificity of the tests are shown in Table 2.

DISCUSSION

In acute hepatitis C infection the earliest serum marker is HCV-RNA which appears some weeks before specific antibodies to HCV.[5] Therefore, in acute hepatitis C infection, detection of HCV RNA by the PCR technique is a valuable tool for the diagnosis of the acute illness since a mean period of 22 weeks has been observed between infection and specific antibody seroconversion.[5] HCV antibody tends to persist in chronic hepatitis C patients.[6] This study sought to test such patients for HCV antibodies using the EIA and PA assays.

The Anti-HCV Enzyme Immunoassay technique is a widely used test in most laboratories for the detection of antibodies to HCV. All non-reactive samples by EIA were considered to be negative for anti-HCV. Repeatedly reactive sera by EIA were then confirmed by using the immunoblot or line immunoassay technique.

In recent years, newer and improved tests have been established such as the Particle Agglutination Assay which uses gelatin particles coated with c22-3 and c200 HCV antigens. The PA assay is simple to perform with fewer steps in the procedure since no washes are required. The PA unlike EIA, requires no special, sophisticated equipment and is therefore suitable even for small laboratories having little or no instrumentation. One disadvantage of the PA is that the agglutination patterns are read visually and therefore may be subjective to the laboratory operator's interpretation. Hence, the laboratory personnel need adequate training and experience for the PA test.

The EIA on account of its high sensitivity may give false reactives/positive results. Of the 11 specimens which were EIA reactive, the line immunoassay indicated ten to be anti-HCV false positive and one to be anti-HCV true positive; this true positive failed to be detected by the PA. Failure of the PA to detect this positive serum meant that a small proportion of HCV antibody positives (1.2%) may be missed by the PA test. Our laboratory is mainly a referral diagnostic laboratory for clinical cases with acute or chronic hepatitis, therefore accuracy is of utmost importance in the diagnosis of viral hepatitis as well as to influence clinical management. The PA technique with its lower sensitivity is therefore not suitable in our laboratory as a first line screen. However the PA may be recommended for use as a screening test in small laboratories which do not have the equipment/facilities to perform the EIA technique.

The sensitivities of PA and EIA are 98.8% and 100% respectively while the specificity of PA is 100% as compared to that of EIA (85.5%). All sera positive on EIA and PA are to be regarded as confiied to contain HCV antibodies. However, EIA reactive sera which give PA

TABLE 2: Sensitivity and specificity of Anti-HCV EIA and PA test kits

	UBI HCV EIA	Serodia-HCV PA
Sensitivity (%)	100	98.8
Specificity (%)	85.5	100.0

All sera were tested by the UBI HCV EIA and Serodia-HCV PA. Sera reactive on one/both assays were confirmed by anti-HCV line immunoassay as the "gold" standard.

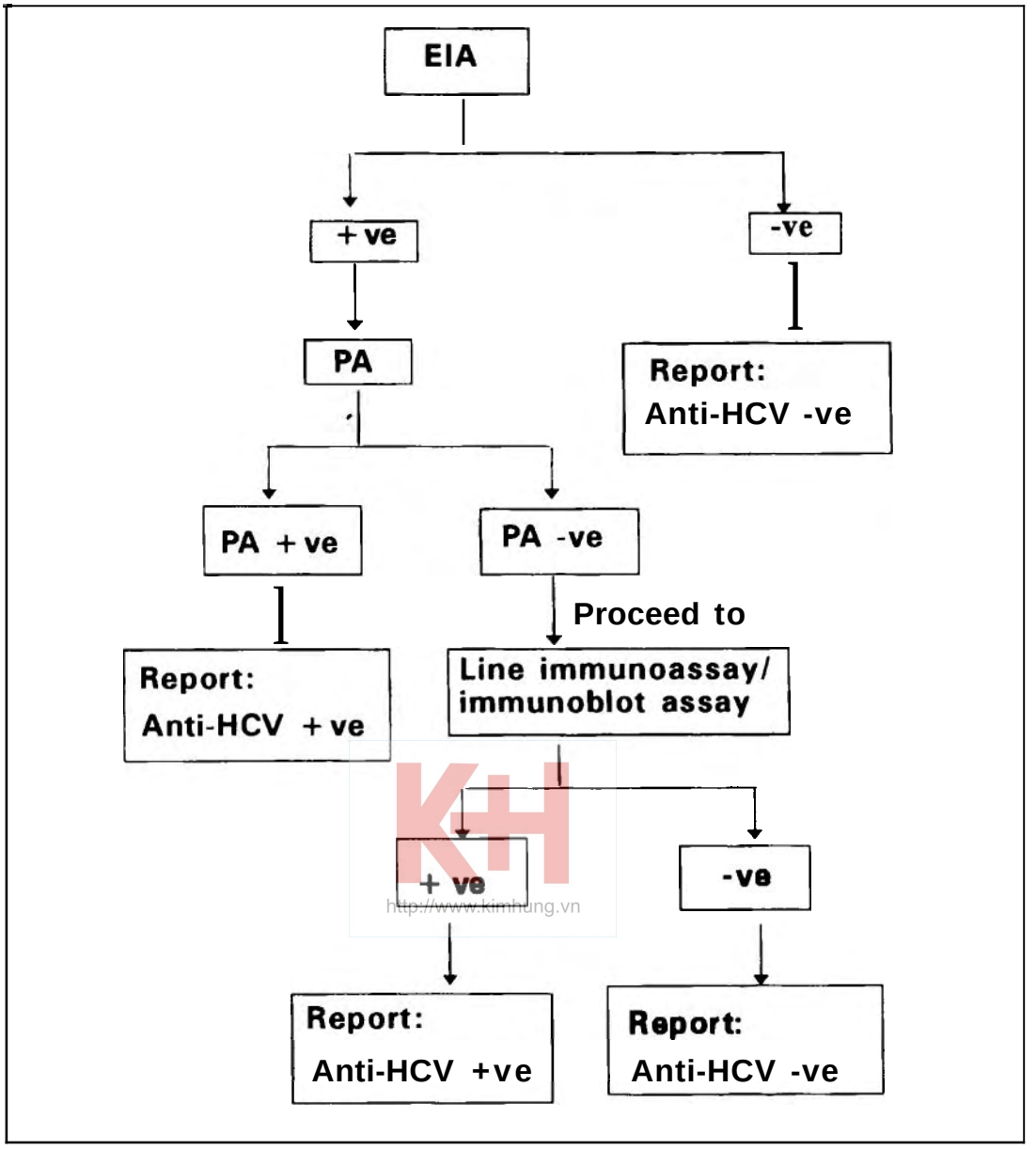

FIG. 2: Recommended flow chart for screening of anti-HCV using HCV UBI EIA and Serodia-HCV PA.

negative results are not to be regarded as excluding HCV infection; such sera should be tested by a third assay such as line immunoassay/ immunoblot before excluding infection.

The cost of **PA** (about RM12.50/test) is more economical than the line immunoassay (about RM98.00/test). Hence it is more cost effective to employ the **PA** as the second line screening test.

As shown in Fig. 2, we conclude that (i) **EIA** is most suitable as a first line screening test for anti-HCV, (ii) **PA** could be a used as a second/ supplementary screen to **EIA** on account of its cost-effectiveness and (iii) Line immunoassay is most suitable as a confirmatory test to verify the true anti-HCV status of any sera giving discordant results in the **EIA** and **PA** tests.

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