

Comparison of Rapid Anti-HCV Multi-sure Kit with Gold Standard ELISA

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ABSTRACT

Objective: To compare the diagnostic yield of Multi-sure rapid HCV (hepatitis C virus) kit with ELISA.

Study Design: Comparative study.

Place and Duration of Study: Pakistan Health Research Council, specialised research center for gastroenterology and hepatology, from August 2016 to January 2017.

Methodology: A modified rapid anti-HCV kit was compared with ELISA. This rapid kit is multi-parameter qualitative immune chromatographic kit for the *in-vitro* detection of antibodies to HCV in human blood. Patients who came to PHRC, were tested using anti-HCV ELISA, and their test was run simultaneously on multi-sure HCV rapid kit were included in the study. Each positive and negative sample was included in this study. SPSS software was adapted for data analysis.

Results: A total of 420 samples were collected. Among them, 255 (61%) were of male and 165 (39%) were of female patients. Mean age was 35 ±14.33 years. All the samples run for anti-HCV on ELISA were also run on multi-sure rapid kit. It is evident that 22.4% were reactive on ELISA and 23.6% were reactive on rapid kit, while 75.5% were non-reactive on ELISA and 68.1% were non-reactive on rapid kit. Borderline positive results were seen in 2.1% on ELISA and 5.0% on rapid kit. Sensitivity of rapid kit was 87.2%, specificity 89.3% with 82.8% positive predictive value and 98.9% negative predictive value.

Conclusion: Multi-sure kit showed significantly, less non-reactive and more borderline results as compared to ELISA. Comparison of multi-sure rapid kit with ELISA showed that core antibody can be used as an alternate marker for ELISA. Other non-structural proteins including NS3, NS4 and NS5 were found to be less significant. So, it is concluded that this rapid kit may not be recommended as an alternative of ELISA, except for places where ELISA is not available.

Key Words: Hepatitis C-virus, Rapid kit, Sensitivity, Specificity, Structural proteins, Anti-HCV antibodies.

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INTRODUCTION

Hepatitis C virus is a RNA virus which has four structural and six non-structural proteins including NS2, NS3, NS4A, NS4B, NS5A and NS5B.¹ An estimated 3% of world population is infected with hepatitis C virus.² In Pakistan, a national survey showed an overall HCV prevalence of 5%.³ According to WHO classification, Pakistan falls in the intermediate zone of infection for HCV.⁴ As no vaccine is available for hepatitis C, it is necessary to diagnose and treat the infected person after appropriate screening.⁵ Numerous immunoassays have been developed for detecting HCV infection and most of these assays are principally based on the detection of antibodies against recombinant HCV polypeptide.⁶ These assays include rapid ICT, ELISA

and EIA. Anti-HCV is typically identified by ELISA method, which is good screening assay⁷, and proved to be more sensitive.⁸ Four generations of ELISA have been developed, which detect different structural and non-structural proteins. Fourth generation simultaneously detects HCV capsid antigen as well as antibodies to the Core, NS3, NS4 and NS5 region of the virus.⁹ Rapid diagnostic ICT kits are less sensitive as compared to ELISA.¹⁰ Within rapid tests, some are sensitive while others are less sensitive. A recent study reported that the highest antibody response is observed against core HCV protein (85%) followed by NS4 (54%), NS5 (50%) and NS3 (41%);¹¹ whereas, antibodies against HCV non-structural proteins (NS3, NS4 and NS5) weakens consecutively.¹²

A new rapid kit by the name of MP diagnostic multi-sure anti-HCV kit has recently been introduced in Pakistan. It has four bands, one for the core and three for non-structural proteins (NS3, NS4 and NS5). All these bands appear separately on the device. According to kit's literature, this kit has 99.28% sensitivity and 97.96% specificity with 99.25% diagnostic accuracy. The present

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Table I: Comparison of multi-sure rapid kit and ELISA.

| | Multi-sure (n=420) | ELISA (n=420) | Multisure | Reactive | ELISA Non-reactive | Borderline |
|--------------|--------------------|---------------|-----------|------------|-----------------------|------------|
| Reactive | 99 (23.6%) | 94 (22.4%) | 99 | 82 (87.2%) | 11 (3.5%) | 6 (66.7%) |
| Non-reactive | 286 (68.1%) * | 317 (75.5%) | 286 | 1 (1.1%) | 283 (89.3%) | 2 (22.2%) |
| Borderline | 21 (5.0%) * | 9 (2.1%) | 21 | 2 (2.1%) | 18 (5.6%) | 1 (11.1%) |
| Invalid | 14 (3.3%) | - | 14 | 9 (9.6%) | 5 (1.6%) | - |
| Total | 420 (100%) | 420 (100%) | 420 | 94 | 317 | 9 |

*Statistically significant $p < 0.05$, Border Line results appear as grey zone. Multi-sure were significantly less non-reactive 286 (68.1%) and more borderline 21 (5.0%) as compared to ELISA 317 (75.5%), 9 (2.1%) ($p < 0.05$).

Table II: Comparison of core, NS3, NS4 and NS5 with ELISA.

| Multi-sure | ELISA | | | Total |
|------------|------------|--------------|------------|-------|
| | Reactive | Non-reactive | Borderline | |
| Core | | | | |
| 1-3 | 82 (87.2%) | 7 (7.4%) | 5 (5.3%) | 94 |
| 0 | 12 (3.7%) | 310 (95.1%) | 4 (1.2%) | 326 |
| NS3 | | | | |
| 1-3 | 39 (78.0%) | 7 (14.0%) | 4 (8.0%) | 50 |
| 0 | 55 (14.9%) | 310 (83.8%) | 5 (1.4%) | 370 |
| NS4 | | | | |
| 1-3 | 48 (71.6%) | 14 (20.9%) | 5 (7.5%) | 67 |
| 0 | 46 (13.0%) | 303 (85.8%) | 4 (1.1%) | 353 |
| NS5 | | | | |
| 0.5-2.5 | 22 (68.8%) | 10 (31.2%) | - | 32 |
| 0 | 72 (18.6%) | 307 (79.1%) | 9 (2.3%) | 388 |
| Total | 94 | 317 | 9 | 420 |

*0.5, 1, 1.5, 2, 2.5 and 3 are the values provided on reference identity

study was done to compare the diagnostic accuracy of MP diagnostic multi-sure anti-HCV rapid kit with ELISA. This study may define the sensitivity of multi-sure rapid kit.

METHODOLOGY

The study was conducted from August 2016 to January 2017 at Pakistan Health Research Council (PHRC). The study was comparative and inducted 420 blood samples as research object *via* convenient sampling technique using 95% confidence level and absolute precision 2% and prevalence of disease¹³ as 5%, in general population. Inclusion criteria were patients who come to PHRC, JPMC, specialised center for gastroenterology and hepatology, for screening of anti-HCV were included. An informed consent was taken from study participants and the study was done after approval of ethical committee of the institute.

In this study, a modified rapid kit of anti-HCV (multi-sure-MP/Diagnostic) was compared with gold standard ELISA (4th generation - Murex) for sensitivity and specificity. This rapid kit is multi-parameter qualitative immune chromatographic kit for the *in-vitro* detection of antibodies to HCV in human blood. Five ml blood samples of patients coming for the screening of hepatitis C were collected and serum was separated and tested for anti-HCV using ELISA and multi-sure rapid kit, simultaneously. According to kit literature, 25 microliter serum was poured in well A of kit, sample was allowed to flow on the membrane, until it reached the blue line then three drops

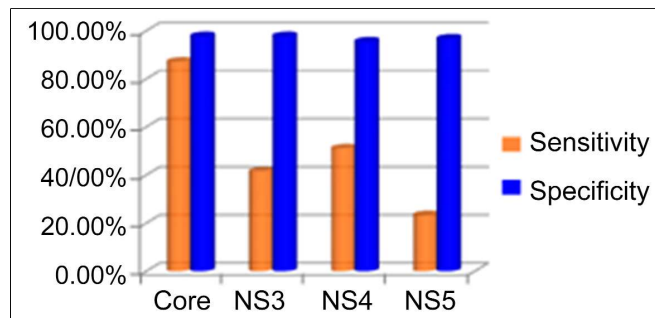


Figure 1: Graphical representation of sensitivity and specificity of core, NS3, NS4 and NS5. X-axis: Structural and non-structural proteins. Y-axis: Percentage of sensitivity and specificity.

of chase buffer (provided with kit) was added to the well B. Tab was pulled to allow the sample and buffer to mix and flow for 15 minutes. Control lines and test lines (bands) was observed and read with the help of reference identity scale (RIS) provided in the kit. Results were compared for sensitivity and specificity.

SPSS software was adapted for data analysis. The sensitivity and specificity of antibodies against each core, NS3, NS4 and NS5, were also analysed separately in comparison to ELISA.

RESULTS

A total of 420 samples were collected. Among them, 255 (61%) were of males and 165 (39%) were of females. Mean age of participants was 35 ± 14.33 years. Overall comparison of ELISA and rapid kit is shown in Table I. Multi-sure kit showed 99 (23.6%) reactive samples and ELISA showed 94 (22.4%). Sensitivity of the rapid kit had 87.2% (82 out of 94), and specificity 89.3% (283 out of 317) with 82.8% positive predictive value (82 out of 99) and 98.9% negative predictive value (283 out of 286). Fourteen samples on rapid kit showed invalid results, *i.e.* no color band appeared on the control line. Twenty-one samples were borderline on multi-sure kit, *i.e.* single NS4 or NS5 test line visible with intensity ≥ 1.0 , according to reference scale of kit. Multi-sure were significantly less non reactive 286 (68.1%) and more borderline 21 (5.0%) as compared to ELISA 317 (75.5%), 9 (2.1%) ($p < 0.05$).

Table II shows that out of 94 ELISA reactive, 82 (87.2%) were core antibody reactive. The positivity of NS3 was 78%, NS4 was 71.6%, and NS5 was 68.8%.

Comparison of the sensitivity and specificity of antibodies against core, NS3, NS4 and NS5 with ELISA is shown *via* graph in Figure 1. It showed that 87.2% of core positive cases were ELISA positive and 95.1% core negative were ELISA negative.

DISCUSSION

This study showed that overall multi-sure rapid kit had lower sensitivity (87.2%) and specificity (89.3%), which is much lower than the sensitivity (99.28%) and specificity (97.96%) claimed by the manufacturer. Another study conducted in the same research centre (PHRC) compared the sensitivity and specificity of three rapid kits with ELISA.¹⁰ That study reported 93% sensitivity of ACON USA and 89% for membrane Canada, while Novis Germany had 86% sensitivity. If we compare present results of rapid MP diagnostic multi-sure rapid kit with the rapid tests reported in the earlier study, the results showed that rapid MP diagnostic multi-sure kit is less sensitive as compare to most of the other rapid tests.

Multi-sure rapid kit, according to its literature, can detect structural proteins core and non-structural proteins NS3, NS4 and NS5 of the virus, but few studies have shown that antibody responses against HCV non-structural protein (NS3, NS4 and NS5) were erratic¹⁴ and NS3 and core antibody seems to be predominant.¹⁵ Therefore, core antibody may be significant to interpret.

In the present study, core antibody positivity was 87.2% for those who were reactive to ELISA, while core antibody negativity was 95.1% which was also negative on ELISA. This suggests that core antibody results are closer to the results of ELISA. Other non-structural proteins including NS3, NS4 and NS5 were found to be less significant as described in Table II. International research also proved that core antibody was more immune reactive protein reacting with 78.8% and 99.3% of acute and chronic samples, respectively¹⁶; and core antigen based testing has a sensitivity ranging from 80 to 99% and specificity ranging from 96% to 100%.¹⁷

In another comparative study of multi-sure kit and ELISA, the authors also hesitate to declare this kit as reliable in high incidence areas, and poor migration of serum was also reported on the flow device.⁵ Furthermore, its interpretation is more complex as intensity of the various lines is used and there is inter observer variation. However, research should be continued in search of cost-effective and more precise and simpler methods of detection for HCV, which can be performed at remote areas for screening purposes where basic necessities of life is not available.

CONCLUSION

Rapid kit named multi-sure kit showed significantly less non-reactive and more borderline results as compared

to ELISA. Comparison of multi-sure rapid kit with ELISA showed that core antibody can be used as an alternate marker for ELISA. Other non-structural proteins including NS3, NS4 and NS5 were found to be less significant. So, it is concluded that this rapid kit may not be recommended as an alternative of ELISA, except for places where ELISA is not available.

FUNDING:

This study was funded by Pakistan Health Research Council.

ETHICAL APPROVAL:

Study was done after approval of Ethical Review Committee of Pakistan Health Research Council.

PATIENTS' CONSENT:

Informed consents were taken from study participants.

CONFLICT OF INTEREST:

Multi-sure rapid kits were provided by the manufacturer (MP-Diagnostics).

AUTHORS' CONTRIBUTION:

RI: Research idea, data collection, laboratory/bench work, manuscript writing.

WA: Supervision, manuscript review.

SEA: Data analysis, sample size calculation.

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