JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

True-positive reflex threshold value for HCV antibody screening test

Ainulkhir Hussin[1,2], Rusmah Yusof[1], Mohd Yusof Nor Rahim[1], Frederick Dalusim[1], Nor Insyirah Othman[1], Nazlina Ibrahim[2], Muhammad Ashraf Shahidan[2]

[1] Department of Pathology, Queen Elizabeth Hospital, Ministry of Health, Kota Kinabalu, Sabah, Malaysia
[2] Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, The National University of Malaysia, Bangi, Selangor, Malaysia

Abstract

Introduction: The use of a signal-to-cut-off ratio has been recommended by the Centre for Disease Control and Prevention to determine the need for further validation using a supplemental test. In this study, we aimed to determine the optimal true-positive signal-to-cut-off ratio for the ABBOTT ARCHITECT i2000SR immunoassay (Abbott Laboratories, Illinois, USA), using the Serodia® HCV particle agglutination (HCV-PA) assay (Fujirebio Inc, Tokyo, Japan) as the reference test for anti-HCV screening.

Methodology: We analysed a total of 13,240 specimens using the ARCHITECT i2000SR immunoassay and subsequently subjected all the reactive specimens with a signal-to-cut-off ratio ≥ 1.00 (n = 267) to the Serodia® HCV-PA reference assay. Receiver operating characteristic (ROC) curve analysis was carried out and performance characteristics for each signal-to-cut-off ratio were determined. The selected signal-to-cut-off ratio value was then assessed using a line immunoassay (LIA) test.

Results: ROC curve analysis determined that the optimal signal-to-cut-off ratio was 5.05, which gave the highest Youden's Index (J) value of 0.89, with a sensitivity of 93.1% (88.9-97.2), a specificity of 96.0% (92.4-99.4), a positive predictive value of 96.4% (93.3-99.5), and a negative predictive value of 92.2% (87.5-96.8). Validation of the optimal S/Co value using the LIA test yielded an accuracy of 91.8%, with sensitivity and specificity values of 92.0% and 91.7%, respectively.

Conclusions: The optimal signal-to-cut-off ratio value for the ARCHITECT i2000SR immunoassay, which was determined using HCV-PA assay as the reference test and validated using a HCV-LIA assay, showed high sensitivity and specificity, and may be used in routine anti-HCV screening.

Key words: HCV; immunoassay; threshold; S/Co; true-positive.

J Infect Dev Ctries 2022; 16(3):522-527. doi:10.3855/jidc.13750

(Received 26 August 2020 – Accepted 18 April 2021)

Copyright © 2022 Hussin *et al*. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Hepatitis C virus (HCV) infection causes 700,000 deaths annually worldwide, and this number is expected to increase over the next 20 years [1]. Due to the asymptomatic nature of the disease, people infected with HCV may be unaware of their infection status and can easily progress to chronic HCV infection, which leads to liver fibrosis, cirrhosis, hepatocellular carcinoma and death [2]. Screening programs for early patient identification, and the availability of effective therapies such as direct antiviral agents, are crucial to prevent the silent progression of HCV [3,4].

Routine laboratory screening tests for HCV are principally based on HCV antibody (Ab) detection by immunoassay. There are two main types of anti-HCV immunoassays: enzyme immunoassays (EIA) and chemiluminescence immunoassays (CMIA). Although the clinical performance of HCV Ab screening tests has improved, false-positive results where an individual determined to be HCV reactive is actually not infected may occur, particularly in populations with low disease prevalence (< 10%) [5]. Therefore, more specific Ab tests such as particle agglutination (PA) and line immunoassay (LIA) are usually conducted after the initial reactive first line screening test to improve screening test result reliability.

The use of signal-to-cut off (S/Co) ratios, with optimal sensitivity and specificity percentages, has been recommended by Alter *et al.* [6] as an alternative option to the supplementary tests for true-positive confirmation and improved test reliability. This approach predicts ≥ 95% of true Ab positive results for several immunoassays, regardless of anti-HCV prevalence and characteristics of the tested population. In the present study, with the aim to reduce the unnecessary supplemental testing based on our population, we aimed to determine the optimal S/Co ratio for the ABBOTT ARCHITECT i2000SR

immunoassay system (Abbott Laboratories, Illinois, USA) using Receiver Operating Characteristic (ROC) curve analysis. The ARCHITECT i2000SR immunoassay results were compared to the Serodia® HCV-particle agglutination (PA) assay (Fujirebio, Tokyo, Japan). The optimal S/Co ratio was then determined using the HCV-LIA test to evaluate concordance between the HCV positive and negative results.

Methodology

Clinical Specimens

A database of 13,240 samples was collected from hospitals and health clinics in the state of Sabah, Malaysia from 1 July 2016 to 31 December 2017. Samples were subjected to the HCV screening test and HCV-PA test to determine the true-positive reflex threshold. Following this, a retrospective evaluation study was performed on 42,291 specimens screened for HCV using the HCV-LIA test from 25 February 2018 to 25 February 2020. The present study was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-17-2971-39094).

Anti-HCV screening test

For S/Co determination, the HCV screening test was performed using the ARCHITECT i2000SR immunoassay system (Abbott Laboratories, Illinois, USA), using the CMIA method as recommended by the manufacturer [7]. Reactive specimens (S/Co ratio ≥ 1.00) were further subjected to the Serodia® HCV-PA assay (Fujirebio Inc., Tokyo, Japan) whilst non-reactive specimens (S/Co < 1.00) were excluded from the study.

For S/Co ratio evaluation, the HCV screening test was performed according to the aforementioned methods. Similarly, non-reactive specimens (S/Co < 1.00) were excluded from the study. Reactive anti-HCV specimens were divided into two groups based on the S/Co value: i) group 1, specimens with S/Co between 1.00 and 5.05; and ii) group 2, specimens with S/Co ≥ 5.05. Specimens in group 1 were considered as true-negatives for HCV antibodies, while group 2 specimens were considered as true-positives. Both specimen groups were subjected to the INNO-LIA® HCV Score assay (Innogenetics, Zwijnaarde, Belgium).

HCV-PA test

All anti-HCV reactive specimens (n = 267) with an S/Co ratio from 1.00 to 20.00 were subjected to the Serodia® HCV-PA (Fujirebio, Tokyo, Japan) assay according to the manufacturer's protocol [8] for true-positivity verification. Inconclusive results were excluded from the study. True-positive specimens were defined as reactive anti-HCV specimens with a positive HCV-PA result, whereas false-positive specimens were defined as reactive anti-HCV specimens with a negative HCV-PA result. The optimal cut-off value (S/Co) was then determined using ROC curve analysis.

HCV-LIA test

The INNO-LIA® HCV Score (Innogenetics, Zwijnaarde, Belgium) confirmation test was conducted using an Auto-LIATM 48 analyser (Fujirebio Europe, Gent, Belgium) according to the protocol recommended by the manufacturer. Specimens with inconclusive results were excluded from the study. The specificity rate was determined for group 1 specimens that had a negative HCV-LIA test, whereas the sensitivity rate was calculated for group 2 specimens that had a positive HCV-LIA test.

Data analysis

Processing and analysis of raw data was carried out using Microsoft Excel (Microsoft Inc., Washington, USA). Statistical analysis was performed using SPSS 23.0 (IBM, New York, USA). ROC curve analysis and performance characteristics such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated to determine the optimal S/Co ratio for the assays.

Results

Overall, 267 of 13,240 specimens tested reactive (S/Co ≥ 1.0) for anti-HCV antibodies and were subjected to the HCV-PA assay for further validation. Of the 267 reactive specimens, only 144 specimens (53.9%) had a positive HCV-PA assay result (Table 1).

Table 1. Results for the HCV-PA test for S/Co value ≥ 1.0.

S/Co Value	Total Specimens	Positive HCV-PA (%)	Negative HCV-PA (%)
1.00-4.99	127	10 (7.9)	117 (92.1)
5.00-9.99	28	22 (78.6)	6 (21.4)
10.00-14.99	77	77 (100.0	0 (0.0)
15.00-19.99	35	35 (100.0	0 (0.0)
Total	267	144 (53.9)	123 (46.1)

HCV-PA: HCV Particle agglutination test.

Reactive specimens with an S/Co ratio of < 10.00 had smaller true-positive percentages, particularly at an S/Co ratio between 1.0 and 4.99 (7.9% of 127 specimens). Reactive specimens with an S/Co ratio value ≥ 10.00 were shown to be more reliable, with 100% true-positivity (Table 1).

ROC curve analysis calculated the area under the curve (AUC) as 0.96 (95% CI: 0.93-0.98) (Figure 1) with a maximal Youden's index (J) of 0.89 at S/Co = 5.05. Therefore, the S/Co ratio value of 5.05 was chosen as the optimal S/Co ratio value, due to the highest balanced sensitivity and specificity for the anti-HCV detection, at a rate of 93.1% and 96.0%, respectively (Table 2).

When evaluating the optimal S/Co value using the HCV-LIA assay, the sensitivity was 92.0% (Figure 2), indicating that there was substantial concordance between group 1 and the HCV-LIA results in predicting the absence of HCV antibodies, with minimal false-negative results. Likewise, group 2 specimens showed a good correlation with the HCV-LIA assay in determining positive results with a specificity of 91.7%, and a minimal number of false-positive results.

Discussion

A key component of accurately determining serological evidence of HCV infection is the application of an accurate true-positive S/Co value. This ensures that no patients are misdiagnosed, which could lead to unnecessary treatment, psychological harm, false notification of disease to the authorities, and

Figure 1. ROC curve for the optimal S/Co ratio value determination using PA test (n = 267).

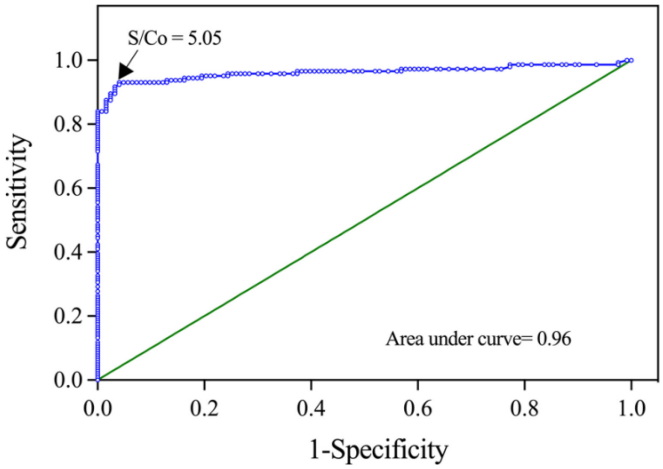

Figure 2. Evaluation of the optimal S/Co ratio value using HCV-LIA.

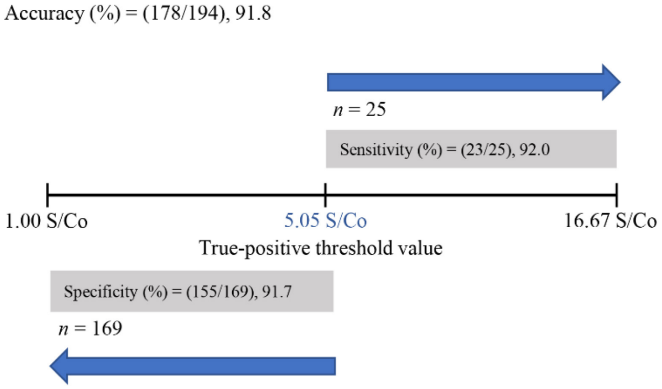

Table 2. Performance characteristics and Youden's Index values for the selected S/Co ratio from the ROC analysis.

S/Co	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Youden's Index *(J)*
1.99	96.5 (93.5-99.5)	62.6 (54.1-71.2)	75.1 (68.9-81.4)	93.9 (88.7-99.1)	0.60
2.99	93.8 (89.8-97.7)	84.6 (78.2-90.9)	87.7 (82.5-92.9)	92.0 (87.0-97.0)	0.78
3.97	93.1 (88.9-97.2)	92.7 (88.1-97.3)	93.7 (89.7-97.7)	91.9 (87.1-96.7)	0.86
4.89	93.1 (88.9-97.2)	95.1 (91.3-98.9)	95.7 (92.4-99.1)	92.1 (87.4-96.8)	0.88
5.05	93.1 (88.9-97.2)	96.0 (92.4-99.4)	96.4 (93.3-99.5)	92.2 (87.5-96.8)	0.89
5.91	89.6 (84.6-94.6)	96.7 (93.6-99.9)	97.0 (94.1-99.9)	88.8 (83.5-94.1)	0.86
6.75	87.5 (82.1-92.9)	97.6 (94.8-100.0)	97.7 (95.1-100)	87.0 (81.3-92.6)	0.85
7.88	84.0 (78.0-90.0)	100.0	100.0	84.2 (78.3-90.1)	0.84
8.77	81.2 (74.9-87.6)	100.0	100.0	82.0 (75.9-88.1)	0.81
9.91	77.8 (71.0-84.6)	100.0	100.0	79.4 (73.0-85.7)	0.78
10.96	71.5 (64.1-78.9)	100.0	100.0	75.0 (68.4-81.6)	0.72
11.91	62.5 (54.6-70.4)	100.0	100.0	69.5 (62.7-76.3)	0.63
12.99	54.2 (46.0-62.3)	100.0	100.0	65.1 (58.3-71.9)	0.54
13.99	38.9 (30.9-46.9)	100.0	100.0	58.3 (51.6-64.9)	0.39
14.99	25.0 (17.9-32.1)	100.0	100.0	53.2 (46.8-59.7)	0.25
15.88	10.4 (5.4-15.4)	100.0	100.0	48.8 (42.6-55.0)	0.10
16.99	4.2 (0.9-7.4)	100.0	100.0	47.1 (41.0-53.2)	0.04
17.87	1.4 (0.0-3.3)	100.0	100.0	46.4 (40.4-52.4)	0.01
18.86	0.69 (0.0-2.1)	100.0	100.0	46.2 (40.2-52.2)	0.01
20.56	0.0	100.0	100.0	46.1 (40.0-52.0)	0.00

PPV: Positive predictive value; NPV: Negative predictive value.

premature discharge of patients from treatment wards by clinicians. Therefore, in the present study, the optimal S/Co ratio for the ARCHITECT i2000SR immunoassay was determined using ROC curve analysis, with the aim to improve accuracy of anti-HCV screening tests for HCV diagnosis. Determination of the optimal S/Co cut-off value for the ARCHITECT i2000SR immunoassay was compared with the HCV-PA test, a supplementary test used for anti-HCV screening. This test utilises HCV-coated gelatin particles to detect the presence of anti-HCV antibodies in the serum of an infected individual and is widely used as a supplementary test for HCV. The HCV-PA assay has previously been used as a comparator test to determine the sensitivity and specificity of Elecsys Anti-HCV II assay for routine screening in the Asia Pacific region [9]. Additionally, the HCV-PA assay is able to detect HCV antibodies in dried blood spot (DBS) samples with 94.1% sensitivity and 100% specificity [10]. Furthermore, the HCV-PA assay shows similar performance to the Chiron HCV RIBA 3.0 assay, which has a sensitivity of 99.5% and a specificity of 100% [11]. Therefore, this assay was used as the reference test in the current study to determine the optimal S/Co ratio for HCV screening.

False-positive results are common in anti-HCV screening, especially in low-risk populations, and additional supplemental tests are therefore required. The present study showed that 46.1% of the reactive samples (S/Co ≥ 1.0) were negative using the supplemental HCV-PA test. This value was within the negative outcome range (40-50%) reported by Majid and Gretch for the reactive samples tested by supplemental immunoblot tests [12], indicating the need for S/Co ratio value optimisation.

In ROC curve analysis, generally an AUC of 0.5 suggests no discrimination, whilst AUC of 0.7 to 0.8 is considered acceptable, 0.8 to 0.9 is considered good, and > 0.9 is considered excellent in the differentiation between patients with and without disease [13,14]. Based on this definition, our ROC curve analysis of the S/Co values showed an outstanding result in differentiating the presence of HCV antibodies in the patient's serum, in which the distributions of test results for patients with and without HCV do not overlap. In the current study, S/Co values of 7.88 and above had specificity and a positive predictive value of 100%. However, their sensitivity was compromised and decreased from 84.0% to 0.0% as the S/Co value increased from 7.88 to 20.56. Therefore, it was concluded that an S/Co value of ≥ 7.88 was not suitable as a true-positive S/Co value, since a screening test should be highly sensitive [15]. An S/Co threshold value of < 7.88 showed promising sensitivity. However, 5.05 was selected as the optimal true-positive S/Co value due to its higher Youden's Index (J: 0.89) value and higher sensitivity (93.1%), whilst maintaining a good specificity (96.0%) (Table 2) to reduce the occurrence of false-negative and false-positive results. This optimal cut-off value was lower than the optimal cut-off values for the ARCHITECT i2000SR immunoassay reported by Bischoff *et al.* [16], Oh *et al.* [17] and Ha *et al.* [18], but similar to the true-positive reflex threshold value published by Kamili *et al* from the Centre for Disease Prevention (CDC) and the Malaysian Hepatitis C Screening, Testing and Treatment Guidelines [19,20]. Moreover, results from the present study were also in line with a previous study by Alter *et al.* [6], which recommended that the true-positive threshold value should be > 3.8 for anti-HCV screening tests, specifically the HCV EIA 2.0 and HCV Version 3.0 ELISA.

Evaluation of the optimal threshold value was performed in comparison with HCV-LIA, which is a confirmatory test for anti-HCV [21]. Compared to the HCV-PA assay, which covers C22-23 (core region) and C200 (NS3-NS4 region), the HCV-LIA test covers a different and wider range of antigens, namely the E2 hypervariable region (core region), as well as the NS3, NS4A, NS4B and NS5A regions [22,23]. Evaluation of the HCV-LIA test in the present study yielded sensitivity and specificity values of 92.0% and 91.7%, respectively, indicating that the optimal S/Co value resulted from the ROC curve analysis was consistent with the HCV-PA test results. This confirmed that the implementation of this optimal threshold value, instead of the suggested manufacturer's cut-off value in the HCV diagnosis algorithm, could reduce the occurrence of false-positive results (n = 155). However, the occurrence of S/Co values of > 5.05 in two specimens that were confirmed as negative for HCV antibodies by HCV-LIA test suggests that a cautious diagnosis should be made to confirm the presence of HCV antibodies, particularly for the specimens with S/Co values that are slightly higher than 5.05, which is defined as a borderline result.

The use of an optimal S/Co ratio value in the HCV screening algorithm could significantly reduce overall screening costs, by eliminating unnecessary supplemental testing, without compromising test performance. In a cost comparison study using 517 samples performed by Barreto *et al.* [24], it was reported that use of a HCV algorithm with an optimal S/Co ratio value and immunoblot anti-HCV

supplemental test for confirming the false-negative was 43.4% more economical than the conventional HCV algorithm, which requires all reactive anti-HCV samples to be tested with the supplemental test, whilst maintaining high concordance. Therefore, considering the capability of this optimal threshold value to reduce false positivity and predict the presence of HCV antibodies, we strongly suggest that the optimal S/Co value for the ARCHITECT i2000SR immunoassay that was identified in the current study should be integrated into the HCV diagnostic algorithm.

Conclusions

Using a HCV-PA assay as the reference test and performing validation with HCV-LIA, an optimal S/Co ratio of 5.05 was identified for the ARCHITECT i2000SR immunoassay, with high sensitivity and specificity. Therefore, we recommend that this S/Co ratio value is used in routine HCV Ab screening.

Acknowledgements

The authors would like to thank the Director General of Health Malaysia for the permission to publish the study findings. We are thankful to Dr. William Gotulis, Director of Queen Elizabeth Hospital, Sabah, Malaysia for his support for this study. We acknowledge the support and assistance from Dr. Ahmad Toha Samsudin, Mr. Azlan M'hamd Ajali and all the staff members at the Serology Laboratory, Queen Elizabeth Hospital, Sabah, Malaysia.

Author's contributions

AK was involved in the design of experiment, data collection, data interpretation, data analysis and drafting the manuscript. RY, FD, NIO and MYNR participated in the design of experiment and data interpretation. They were also involved in drafting the manuscript. NI and MAS were involved in data analysis, data interpretation and manuscript drafting, and in reviewing the design of the experiment. All authors were involved in reading, editing, counterchecking the final manuscript.

References

1. World Health Organization (WHO) (2021) Global Hepatitis Report. Available: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c. Accessed: 22 December 2021.
2. Manns MP, Buti M, Gane E, Pawlotsky JM, Razavi H, Terrault N, Younossi Z (2017) Hepatitis C virus infection. Nat Rev Dis Primers 2: 17006.
3. Hanafiah KM, Groeger J, Flaxman D, Wiersma AT (2013) Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatol 57: 1333-1342.
4. Spengler U (2017) Direct antiviral agents (DAAs) - a new age in the treatment of hepatitis C virus infection. Pharmacol Therapeut 183: 118-126.
5. Kesli R (2011) Evaluation of assay methods and false positive results in the laboratory diagnosis of hepatitis C virus infection. Arch Clin Microbiol 2: 1-4.
6. Alter MJ, Kuhnert WL, Finelli L (2003) Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. MMWR Recomm Rep 52: 1-13.
7. Abbott (2016) ARCHITECT i2000SR. Available: https://www.corelaboratory.abbott/us/en/offerings/brands/architect/architect-i2000SR. Accessed: 11 April 2017.
8. Fujirebio (2017) Serodia-HCV. Available: https://www.fujirebio-europe.com/user/login?destination=product/detail/10888#documentation. Accessed: 11 April 2017.
9. Yoo SJ, Wang LL, Ning H, Tao CM, Hirankarn N, Kuakarn S, Yang R, Han TH, Chan RC, Hussain BM, Hussin H, Muliaty D, Shen L, Liu H, Wei L (2015) Evaluation of the Elecsys® Anti-HCV II assay for routine hepatitis C virus screening of different Asian Pacific populations and detection of early infection. J Clin Virol 64: 20-27.
10. Cortina-Borja M, William D, Peckham CS, Bailey H, Thorne C (2016) Hepatitis C virus seroprevalence in pregnant women delivering live-born infants in North Thames, England in 2012. Epidemiol Infect 144: 627-634.
11. World Health Organization (WHO) (2001) Hepatitis C assay: operational characteristic (Phase 1), report 2. Available: https://apps.who.int/iris/bitstream/handle/10665/67061/WHO_BCT_BTS_01.5.pdf?sequence=1&isAllowed=y. Accessed: 22 December 2021.
12. Majid AM, Gretch DR (2002) Current and future hepatitis C virus diagnostic testing: problems and advancements. Microbes Infect 4: 1227-1236.
13. Hajian-Tilaki K (2013) Receiver Operating Characteristic (ROC) curve analysis for medical diagnostic test evaluation. Caspian J Intern Med 4: 627-635.
14. Hosmer DW, Lemeshow S (2000) Applied logistic regression. New York: John Wiley and Sons. 160-164.
15. Long SS, Prober CG, Fischer M (2018) Part-1: Understanding, controlling, and preventing infectious diseases. In Long SS, Prober CG, Fischer M, editors. Principles and Practice of Pediatric Infectious Diseases (Fifth Edition): Elsevier. 1-9.
16. Bischoff M, Koch MTSDT, Araujo FMF (2013) Decision on conducting HCV immunoblot and HCV viral load tests dependent upon the result of the screening tests. J Virol Microbiol: 1-7.
17. Oh E, Chang J, Yang J, Kim Y, Park Y, Han K (2013) Different signal-to-cut-off ratios from three automated anti-hepatitis C virus chemiluminescence immunoassays in relation to results of recombinant immunoblot assays and nucleic acid testing. Blood Transfus 11: 471-473.

18. Ha J, Park Y, Kim H (2019) Signal-to-cutoff ratios of current anti-HCV assays and a suggestion of new algorithm of supplementary testing. Clin Chim Acta 498: 11-15.
19. Ministry of Health Malaysia (MOH) (2017) Hepatitis C screening, testing and treatment guidelines.
20. Kamili S, Drobeniuc J, Araujo AC, Hayden TM (2012) Laboratory diagnostics for hepatitis C virus infection. Clin Infect Dis 55: S43-S48.
21. Rafik M, Bakr S, Soliman D, Mohammed N, Ragab D, ElHady WA, Samir N (2016) Characterization of differential antibody production against hepatitis C virus in different HCV infection status. Virol J 13: 116.
22. Ooi BG, Sinniah M, Ismail S, Baharuddin R (1996) Application of the Serodia-HCV particle agglutination for the detection of antibodies to hepatitis C virus. Malays J Pathol 18: 89-93.
23. Fujirebio (2017) INNO-LIA HCV score. Available: https://www.fujirebio.com/en/products-solutions/innolia-hcv-score. Accessed: 11 April 2007.
24. Barreto AMEC, Takei K, E.C S, Bellesa MAO, Salles NA, Barreto CC, Nishiya AS, Chamone DF (2008) Cost-effective analysis of different algorithms for the diagnosis of hepatitis C virus infection. Braz J Med Biol Res 41: 126-134.

Corresponding author
Muhammad Ashraf Shahidan, PhD.
Department of Biological Sciences & Biotechnology, Faculty of Science and Technology, The National University of Malaysia, Bangi, Selangor, Malaysia.
Tel: +60389215996
Fax: +60389252698
Email: mashraf@ukm.edu.my

Conflict of interests: No conflict of interests is declared.