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Improved efficiency of a hepatitis C virus antibody testing algorithm in blood donors from Saudi Arabia

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Laboratory diagnosis of hepatitis C virus (HCV) infection began a decade ago with the introduction of an enzyme-linked immunosorbent assay (ELISA) technique.[1] Now, highly sensitive third-generation immunoassays that detect antibodies to structural and non-structural proteins in serum are available.

Diagnosis of HCV infection cannot be made on the basis of ELISA alone, as this technique is not sufficiently specific, especially when testing blood donors, and confirmatory testing by recombinant immunoblot assay (RIBA) is required.[2]

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Diagnosis is also possible by nucleic acid testing (NAT). Reverse-transcription polymerase chain reaction (RT-PCR) assays are available for the detection of viral RNA in plasma or serum. However, some cases of HCV infection can produce variable NAT results,[3] and this initiated the production of new Centers for Disease Control and Prevention (CDC) guidelines to confirm infection with HCV.[4]

The guidelines suggest an initial ELISA screen followed by confirmatory RIBA and/or NAT testing, and use of the signal-to-cut-off (S/Co) value of third-generation screening methods to help guide the need for supplemental testing. The S/Co results are divided into negative, weak positive and strong positive. The guidelines suggest that samples which test positive by the screening test with a high S/Co ratio do not need a confirmatory test.

This retrospective study, which looked at all Saudi blood donors who tested HCV-positive on screening immunoassay, aims to evaluate the new guidelines in relation to detecting true HCV-positive Saudi donors by confirmatory RIBA testing.

Blood donors at King Fahad National Guard Hospital undergo strict selection based on criteria laid down by the American Association of Blood Banks (AABB) and College of American Pathologists (CAP). Donors complete a questionnaire and are interviewed before donation.

Recently, a third-generation micro-particle enzyme immunoassay (MEIA) for HCV antibody testing was introduced on the Axsym system (Abbott). The assay detects antibodies to structural and non-structural HCV recombinant proteins. This test is US Food and Drug Administration (FDA) approved for the screening of donors for HCV antibodies.

A total of 208 blood donors were recruited for this study. Results were expressed as the S/Co ratio, which is calculated by dividing the sample rate by the cut-off rate. Cut-off rate is calculated from the mean of two index calibrators. The kit uses S/Co <1.0 as negative and ≥ 1.0 as initially positive. All positive samples were repeated in duplicate.

All 208 donors were tested by RIBA (Chiron V3.0 strip blot assay) to confirm the presence of HCV antibodies. Specificity and sensitivity were calculated using the following equations: specificity=true negative x 100/(true negative + false positive); sensitivity=true positive x 100/(true positive + false negative).

Of the 208 blood donors studied, 111 were positive by screening assay (S/Co ≥ 1.0). Supplementary RIBA testing was carried out on all 208 samples; however, none of the 97 donors that were HCV-negative by ELISA gave a positive RIBA result. The results presented in Table 1 suggest that a cut-off value of 1.0 was non-specific and that the majority of positive screening results were false positives.

All 16 samples positive by RIBA gave very high ELISA readings (S/Co >16). In the Saudi population studied, an S/Co value of 1.0 for the MEIA screening assay resulted in specificity of 50.5% and sensitivity of 100%. When an S/Co of 16 was applied to the 111 positive screening assay results, all 16 donors with an S/Co value >16 were positive by RIBA (specificity 100%, sensitivity 100%).

The results presented here suggest a poor correlation between the MEIA screening assay and RIBA results. The Abbott third-generation MEIA assay, run on the Axsym system (Abbott), showed a low specificity for the detection of HCV-infected Saudi blood donors. Furthermore, the

Table 1. Comparison of ELISA screening with RIBA results in Saudi blood donors.

ELISA results	RIBA results			Total
	Positive	Indeterminate	Negative	
Positive (S/Co ≥ 1.0)	16 (14.4%)	39 (35.1%)	56 (50.5%)	111(100%)
Negative (S/Co <1.0)	0 (0.0%)	21 (21.6%)	76 (78.4%)	97(100%)
Strong positive (S/Co>16)	16 (100%)	0 (0%)	0 (0%)	16 (100%)
Weak positive (S/Co 1–16)	0 (0.0%)	39 (41.1%)	56 (58.9%)	95(100%)

majority (86%) of donors who tested positive by the screening assay were not positive by RIBA.

Defour *et al*.[5] investigated 17,418 high-risk veterans retrospectively and found that the majority (86%) of subjects with low S/Co HCV results were negative by RIBA, while 90% of the high S/Co results were positive by RNA testing. They suggested that low S/Co results in HCV screening indicates that infection is unlikely. Sookoian and Castano[6] investigated serum samples from patients treated in a liver unit, and their results suggested that use of a high S/Co value (26) predicted viraemic status in HCV-infected persons. In the present study, use of an S/Co value of 16 proved to be a strong predictor of HCV infection.

Although the third-generation HCV screening assay appears to have low specificity when testing blood donors, high specificity and sensitivity for detecting HCV infection in chronic liver disease has been reported.[7] Recently, study of a third-generation MEIA for the detection of HCV antibodies suggested that laboratories should evaluate screening assays and establish their own cut-off values, below which a positive result can be regarded as non-specific.[8]

The results of the present study suggest that the cut-off value could vary from one country to another, due to variations in the prevalence of different cross-reacting agents. In the Saudi population studied, an S/Co value >16 proved a better predictor of HCV positivity. These data suggest that the percentage of blood donors who are actually positive for HCV antibodies is less than has been reported[9] with the screening method. Thus, the use of S/Co values in reporting HCV antibody results (whether negative [S/Co <1.0], weak positive [S/Co 1.0–16.0] or strong positive [S/Co >16]) is recommended, and it is suggested that the use of RIBA should be limited to those samples that give weak positive results. □

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