Evaluation of the Bio-Rad Monolisa HCV Ag-Ab ULTRA

Introduction

The Bio-Rad Monolisa HCV Ag-Ab ULTRA assay is an enzyme immunoassay for the detection of HCV infection, based on the detection of capsid antigen, and antibodies associated with an infection by Hepatitis C virus, in patient serum or plasma.

Methods

Specificity

5074 specimens from a single blood donor population and 150 specimens from a single diagnostic population were tested on the Bio-Rad Monolisa HCV Ag-Ag ULTRA assay. All samples initially reactive were retested in duplicate. Samples repeatedly reactive were tested in the NRL HCV confirmatory algorithm to determine the true status. Specimens that were confirmed as HCV antigen and/or antibody positive were excluded from the specificity and negative delta calculations.

Sensitivity

A total of 214 confirmed anti-HCV positive specimens were tested on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay. Sensitivity and positive delta values were calculated.

A total of 91 specimens, collected as a series of sequential samples from nine individuals undergoing HCV seroconversion were also tested. The results of testing these nine seroconversion panels on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay were compared with the results of testing the same panels on the benchmark assay (BMA). The mean difference in detecting a reactive sample was determined

False Reactivity

Forty-two specimens that had given falsely reactive results on previously evaluated anti-HCV assays were tested on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay. Data from testing these samples were used to determine the degree of common false reactivity between the Bio-Rad HCV Monolisa HCV Ag-Ab ULTRA assay and these other currently registered anti-HCV assays.

Reproducibility

A QC sample of appropriate antibody reactivity for the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay was selected and tested at prescribed intervals. A total of 140 replicates of the antibody QC sample was tested.

Due to the unavailability of a suitable HCV antigen QC sample the antigen control within the kit doubled as the antigen QC for this evaluation. In addition to the antigen control being used in accordance with the package insert for validation of the assay it was also tested at prescribed intervals in a similar manner to the antibody QC. A total of 134 replicates of the antigen control was tested.

Reproducibility was separately assessed for antigen and antibody by determining mean, standard deviation (S.D.) and coefficient of variation (C. V.). Outlying results were identified using Grubb's test.

Results

Specificity

Blood Donor Samples

Seventeen of 5074 specimens from a single blood donor population were repeatedly reactive on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay. None of the repeat reactors was confirmed positive. This gave a specificity of 99.67% and a negative delta value of 5.0.

Table 1: Results of testing anti-HCV negative blood donor samples on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay (with corresponding specificity and delta values).

Samples Tested (n)	Initial Reactors (n)	Repeat Reactors (n)	
		True Positives	False Positives
5074	23	0	17

Specificity	**99.67% (95%CI 99.45% - 99.80%)**
δ value	**– 4.99**

Anti-HCV Negative Diagnostic Samples

Three of 150 specimens from a single diagnostic population were repeatedly reactive on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay. All three of the repeatedly reactive samples were confirmed negative for HCV antibodies. Two of the repeatedly reactive specimens were tested by PCR: one gave a positive result and the second gave an invalid result. There was insufficient sample to test the third repeatedly reactive sample. All three of these samples were excluded from the calculations. This gave a specificity of 100% and a negative delta value of 5.79.

Table 2: Results of testing presumed anti-HCV negative diagnostic samples on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay (with corresponding specificity and delta values).

Samples Tested (n)	Initial Reactors (n)	Repeat Reactors			
		Antibody Positive (n)	Antibody Negative		
			PCR Positive (n)	PCR Invalid (n)	PCR Not Tested (n)
150	3	0	1	1	1

Specificity	**100% (95% CI 96.83 – 100%)**
δ value	**– 5.79**

Sensitivity

Confirmed anti HCV Positive Samples

All 214 confirmed anti-HCV positive samples were reactive on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay. This gave an estimated sensitivity of 100% and positive delta value of 6.0.

Table 3: Results of testing anti-HCV positive samples on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay (with corresponding sensitivity and delta values).

Number of anti-HCV Positive Samples Tested	Number Correct
214	214

Sensitivity	**100% (95%CI 97.8% - 100%)**
δ value	**+6.03**

Seroconversion Samples

In four of the nine seroconversion panels there was **no difference** in seroconversion sensitivity between the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay and the BMA; in two of the nine seroconversion panels the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay detected seroconversion a total of 9 samples **before** the BMA; in two seroconversion panels the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay detected seroconversion a total of three samples **after** the BMA. The mean difference in seroconversion sensitivity for these 8 panels was –0.63 samples (range -6 to +2 samples)

In one panel, reactivity was detected in samples 1 – 5 and also in samples 7 and 8 but not in sample 6 in the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay, compared with reactivity in samples 7 and 8 only in the BMA, i.e. in this panel there was a net increase of five samples where reactivity was detected in the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay, presumably due to the presence of HCV antigen in samples 1 –- 5. However it is very important to note that the S/CO ratio dropped below the cut-off value in sample 6 in the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay before becoming positive again in sample 7, the same sample at which the antibody only BMA became positive. This gap in the detection of reactivity in the HCV antigen-antibody assay may be explained by a slight decrease in viral load or by the fact that the virus might associate with antibodies thereby preventing its detection.[1]

1. S Laperche et al. TRANSFUSION Volume 45, December 2005.

False Reactivity
Forty-two falsely reactive samples in previously evaluated assays were tested on the Bio-Rad Monolisa Ag-Ab ULTRA assay. Thirty-nine samples were non-reactive. All three of the reactive samples were also falsely reactive in the Abbott Prism HCV ChLIA assay.

Table 4: Results of testing falsely reactive samples on the Bio-Rad Monolisa HCV Ag-Ab assay.

Assay Producing False Reactivity	Bio-Rad Monolisa HCV Ag-Ab ULTRA Assay		Samples Showing Common False Reactivity (n)
	Samples Tested (n)	Samples Non-reactive (n)	
Abbott Prism HCV ChLIA	27	24	3
Bio-Rad Monolisa Anti-HCV Plus	8	8	0
Innogenetics Innotest HCV Ab IV	3	3	0
Roche Cobas Core Anti-HCV EIA II	3	3	0
Abbott Murex Anti-HCV (version 4) EIA	1	1	0
TOTAL	**42**	**39**	**3**

Reproducibility
A total of 140 observations from 70 test runs for the antibody QC sample and 134 observations from 67 runs for the antigen QC sample demonstrated acceptable reproducibility (CV < 20%) for both antibody and antigen. No outliers were detected in either dataset.

Table 5: Results of testing the QC sample Pelispy Type 3 and the HCV ULTRA Ag control in the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay.

QC Sample	Test Runs (n)	Observations (n)	Mean (S/Co)	S.D.	C.V. (%)
Pelispy Type 3 (Ab)	70	140	2.365	0.161	6.82
HCV ULTRA Ag	67	134	3.872	0.309	7.99

This assay has since been registered on the Australian Register of Therapeutic Goods.

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