Evaluation of the Roche Elecsys Anti-HCV Assay

Introduction

The Roche Elecsys Anti-HCV assay is an electrochemiluminesce immunoassay for the qualitative determination of antibodies to Hepatitis C virus in human serum and plasma. It is intended for use on the Elecsys 2010, Modular Analytics E170, cobas e 411 and cobas e 601 analysers.

Methods

Specificity

5037 samples from a single blood donor population and 556 samples from a single diagnostic population were tested on the Elecsys Anti-HCV 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. Samples that were confirmed as positive were excluded from the specificity and negative delta calculations.

A total of 998 donor samples and all 556 diagnostic samples were tested on the cobas e 411 analyser; the remaining 4039 donor samples were tested on the Modular Analytics E170 analyser.

Sensitivity

A total of 318 confirmed anti-HCV positive samples were tested on the Elecsys Anti-HCV assay. Sensitivity and positive delta values were calculated.

A total of 91 samples, 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 Elecsys Anti-HCV 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.

All sensitivity tests were performed on the cobas e 411 analyser.

False Reactivity

Sixty-one samples that had given falsely reactive results on previously evaluated and registered anti-HCV or HCV Ag/Ab assays were tested on the Roche Elecsys Anti-HCV assay. Data from testing these “problem” samples were used to determine the degree of common false reactivity between the Elecsys Anti-HCV assay and these other currently registered anti-HCV or HCV Ag/Ab assays.

All 61 problem samples were tested on the cobas e 411 analyser.

Reproducibility

A QC sample of appropriate antibody reactivity for the Elecsys Anti-HCV assay was selected and tested at prescribed intervals on the cobas e 411 and the Modular Analytics E170 analysers. A total of 89 replicates of the QC sample were tested.

Reproducibility was assessed separately for each analyser by determining mean, standard deviation (SD) and coefficient of variation (CV). Outlying results were identified using Grubbs’ test.

Results

Specificity

Blood Donor Samples

Eleven of 5037 samples from a single blood donor population were repeatedly reactive on the Elecsys Anti-HCV assay. This gave a specificity of 99.78% and negative delta values of 5.3 for samples tested on the cobas e 411 analyser and 6.5 for samples tested on the Modular.Analytics E170 analyser.

Table 1: Results of testing antibody negative blood donor samples on the Elecsys Anti-HCV assay (with corresponding specificity and delta values).

Analyser	Samples Tested (n)	Initial Reactors (n)	Repeat Reactors (n)	
			True Positives	False Positives
Cobas e 411	998	2	0	2
Modular Analytics E170	4039	10	0	9
Total	**5037**	**12**	**0**	**11**

Specificity	**99.78% (95%CI 99.60% – 99.89%)**
δ value (cobas e 411)	**- 5.3**
δ value (E170)	**- 6.5**

Anti-HCV Negative Diagnostic Samples

Eight of 556 samples from a single low risk diagnostic population were repeatedly reactive on the Elecsys Anti-HCV assay. One of the repeat reactors confirmed positive, and an additional three repeat reactors gave indeterminate results on confirmatory testing: all four samples were excluded from the specificity and delta calculations. This gave a specificity of 99.3% and a negative delta value of 4.3.

Table 2: Results of testing presumed anti-HCV negative diagnostic samples on the Elecsys Anti-HCV assay (with corresponding specificity and delta values).

Samples Tested (n)	Initial Reactors (n)	Repeat Reactors (n)	
		True Positives	False Positives
556	11	1	4

Specificity	**99.3% (95%CI 98.0% - 99.8%)**
δ value	**- 4.29**

Sensitivity

Confirmed anti HCV Positive Samples

All 318 confirmed anti-HCV positive samples were reactive on the Elecsys Anti-HCV assay. This gave an estimated sensitivity of 100% and a positive delta value of 12.

Table 3: Results of testing anti-HCV positive samples on the Elecsys Anti-HCV assay (with corresponding sensitivity and delta values).

Number of anti-HCV Positive Samples Tested	Number Correct
318	**318**

Sensitivity	**100% (95%CI 98.55% - 100%)**
δ value	**11.97**

Seroconversion Samples

In seven seroconversion panels the Elecsys Anti-HCV assay detected reactivity in the same sample as the BMA. In two seroconversion panels the Elecsys Anti-HCV assay detected reactivity after the BMA. The mean difference in seroconversion sensitivity was + 0.22 samples (range 0 to +1 sample).

False Reactivity

Sixty-one falsely reactive samples in previously evaluated assays were tested on the Roche Elecsys Anti-HCV assay. Fifty-nine samples were non-reactive. One of 26 samples falsely reactive on the Abbott PRISM HCV ChLIA and one of 17 samples falsely reactive on the Bio-Rad Monolisa HCV Ag-Ab ULTRA assay were also falsely reactive on the Roche Elecsys HIV Combo assay.

Table 4: Results of testing falsely reactive samples on the Elecsys Anti-HCV assay.

Assay Producing False Reactivity	Roche Elecsys Anti-HCV assay		Samples Showing Common False Reactivity (n)
	Samples Tested (n)	Samples Non-reactive (n)	
Abbott Prism HCV ChLIA	26	25	1
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
Bio-Rad Monolisa HCV Ag-Ab ULTRA	17	16	1
Abbott Murex Anti-HCV (v 4) EIA	4	4	0
TOTAL	**61**	**59**	**2**

Reproducibility

Although some variation between the two analysers was noted, the CV of replicate results obtained from testing the QC sample Pelispy Type 38 (diluted 1/64) on the cobas e 411 and Modular Analytics E170 analysers demonstrated acceptability reproducibility (CV < 20%). Grubbs' test did not detect any outliers.

Table 5: Results of testing the QC sample Pelispy Type 38 (dilued 1/64) on the Elecsys Anti-HCV assay.

Analyser	t Runs (n)	ervations (n)	n (S/Co)	D.	%)
obas e 411	26	34	4.071	41	7
r Analytics E170	13	55	4.878	64	7

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

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