

Evaluation of the Abbott ARCHITECT HCV Ag Assay

Introduction

The Abbott ARCHITECT HCV Ag assay is a two-step chemiluminescent microparticle Immunoassay (CMIA) for the quantitative determination of core antigen to Hepatitis C virus in human serum and plasma.

The assay uses acridinium labeled murine anti-HCV antibodies in the liquid phase and monoclonal anti-HCV coated paramagnetic microparticles as the solid phase. It is dedicated to the Abbott ARCHITECT *i* System (i2000/i2000SR modules only) with ARCHITECT System Software version 5.0 or higher.

The evaluation of the ARCHITECT HCV Ag assay was conducted by NRL at two sites using two different Architect modules: the i2000SR module at Site 1 and the i2000 module at Site 2.

Methods

Specificity

Eight hundred and three samples from a single HCV antibody negative diagnostic population were tested on the Abbott ARCHITECT HCV Ag assay. All samples initially reactive were retested in duplicate. Samples repeatedly reactive were tested for HCV RNA to determine the true status. Samples that were confirmed as positive were excluded from the specificity and negative delta calculations. A total of 620 samples were tested on the ARCHITECT i2000SR at Site 1; and 183 samples were tested on the ARCHITECT i2000 at Site 2.

Sensitivity

A total of 41 samples positive for both anti-HCV and HCV RNA were tested on the Abbott ARCHITECT HCV Ag assay. Sensitivity and positive delta values were calculated. A total of 71 samples, collected as a series of sequential samples from seven individuals undergoing HCV seroconversion, were also tested. The results of testing these seven seroconversion panels on the Abbott ARCHITECT HCV Ag assay were compared with the results of testing the same panels on the Abbott *Real-Time* HCV RNA assay. The mean difference in detecting a reactive sample was determined. All sensitivity tests were performed on the ARCHITECT i2000 analyser at Site 2. *Real-Time* HCV RNA testing was performed at Site 1.

Linearity

Linearity was evaluated by testing five replicates of each dilution of two 14 member dilution series of (i) HCV recombinant core protein (0.23 mg/ml) and (ii) a high HCV viral load patient sample, on the ARCHITECT HCV Ag assay. Panel members ranged from 2.4 – 19,430 fmol/L for core protein and 2.1 – 26,102 fmol/L for the patient sample.

Reproducibility

Owing to the unavailability of a suitable HCV antigen QC sample, the same HCV core protein as used for evaluating linearity was diluted and used as the QC sample for this evaluation, and tested at prescribed intervals. A total of 16 replicates of one dilution of the QC sample were tested on the ARCHITECT i2000SR at Site 1. A further 94 replicates of a second dilution of the QC sample were tested on the ARCHITECT i2000 at Site 2. Reproducibility was assessed separately for each dilution of QC sample by determining mean, standard deviation (SD) and coefficient of variation (CV). Outlying results were identified using Grubbs' test.

Results

Specificity

One of 803 samples from a single diagnostic population was repeatedly reactive on the Abbott ARCHITECT HCV Ag assay. This gave an estimated specificity of 99.88% and negative delta values of 1.6 for samples tested on the ARCHITECT i2000SR analyser and 3.0 for samples tested on the ARCHITECT i2000 analyser.

Table 1: Results of testing antibody negative diagnostic samples on the Abbott ARCHITECT HCV Ag assay (with corresponding specificity and delta values).

Analyser	Samples Tested (n)	Initial Reactors (n)	Non Repeat Reactors (n)	Repeat Reactors (n)	
				True Positives	False Positives
ARCHITECT i2000SR	620	5	4	0	1
ARCHITECT i2000	183	3	3	0	0
Total	803	8	7	0	1

Specificity	99.88% (95%CI 99.19% – 99.99%)
δ value (iS2000SR))	- 1.6
δ value (iS2000)	- 3.0

Sensitivity

Confirmed anti-HCV and HCV RNA positive samples

Thirty-eight of 40 samples confirmed positive for both anti-HCV and HCV RNA were positive on the ARCHITECT HCV Ag assay. This gave an estimated sensitivity of 95% and a positive delta value of 2.4.

Table 2: Results of testing samples confirmed positive on both anti-HCV and HCV RNA on the Abbott ARCHITECT HCV Ag assay (with corresponding sensitivity and delta values).

Number of anti-HCV and HCV RNA Positive Samples Tested	Number Positive on the ARCHITECT HCV Ag Assay
40	38

Sensitivity	95% (95%CI 81.8% - 99.1%)
δ value	2.4

The two samples that were negative on the ARCHITECT HCV Ag test had HCV RNA concentrations of 37 IU/mL (log transformed = 1.57) and 103 IU/mL (log transformed = 2.01) respectively, on the Abbott *RealTime* HCV RNA assay. The frequency and range of HCV RNA concentrations for the 40 samples tested on the Architect HCV Ag assay are shown in Figure 1.

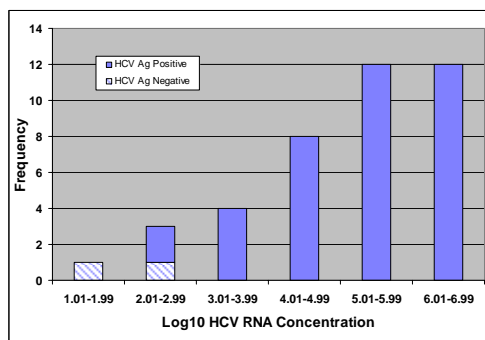


Figure 1. Frequency and range of HCV RNA concentrations for the 40 samples tested on the ARCHITECT HCV Ag assay.

Seroconversion Samples

The reactivity of the ARCHITECT HCV Ag assay (pink diamonds) compared with the Abbott *RealTime* HCV RNA assay (blue squares), for each of seven seroconversion panels, is shown in Figures 2 – 8.

For Panel 7 the ARCHITECT HCV Ag detected reactivity one sample earlier than the *RealTime* HCV RNA assay, but there was no apparent difference in performance between the assays in the remaining six panels. The mean difference in reactivity for these seven panels is – 0.2 samples (range –1 to 0 samples).

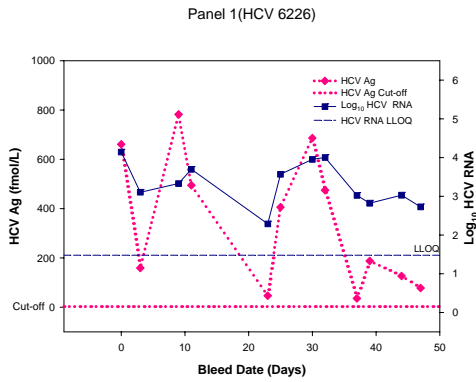


Fig. 2 Reactivity was detected in all samples on the HCV Ag and HCV RNA assays.

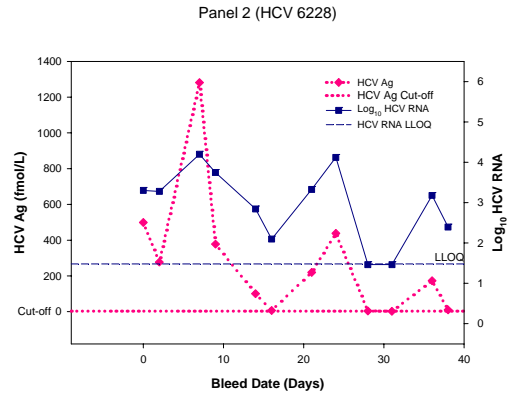


Fig. 3 Reactivity was detected in the same samples on the HCV Ag and HCV RNA assays.

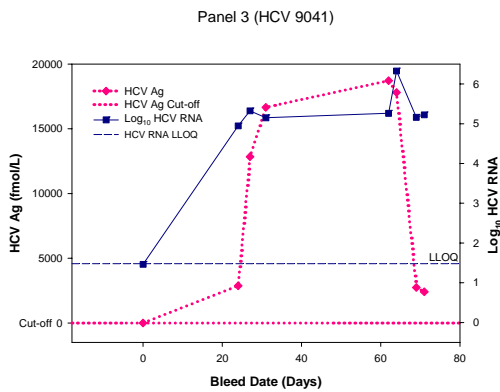


Fig. 4 Reactivity was detected in all samples on the HCV Ag and HCV RNA assays.

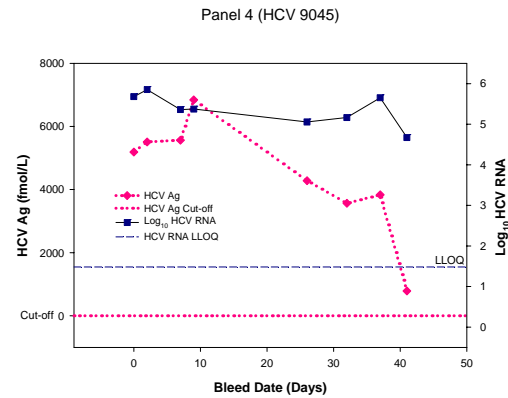


Fig. 5 Reactivity was detected in all samples on the HCV Ag and HCV RNA assays.

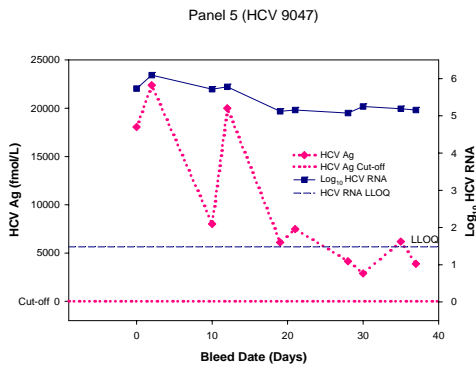


Fig. 6 Reactivity was detected in all samples on the HCV Ag and HCV RNA assays.

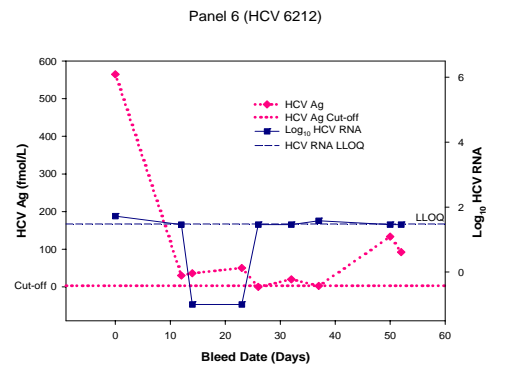


Fig. 7 Reactivity was detected in sample 1 on both the HCV Ag and HCV RNA assays and in samples 2, 3, 4, 8 & 9 on the HCV Ag assay only.

Panel 7 (HCV 6216)

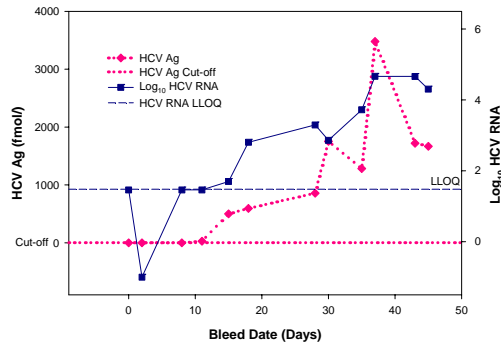


Fig. 8 Reactivity was detected one bleed earlier on the HCV Ag assay compared to the HCV RNA assay.

Figures 2 - 8: Performance of the Abbott Architect HCV Ag assay relative to the performance of the Abbott HCV RNA-assay in seven seroconversion panels.

Linearity

Greater variation between results of the five replicates of each dilution was seen with the high viral load patient sample compared with those obtained for the HCV core protein. There was also greater variation between the observed and expected results for the patient sample dilution series compared with the core protein dilution series. Overall the results were acceptable, demonstrating that linearity is obtained over the calibration range for the assay, viz 0 – 20,000 fmol/L.

Reproducibility

Greater variation in results was obtained for tests performed on the ARCHITECT i2000 module (coefficient of variation 20.1%) compared with those performed on the ARCHITECT i2000SR module (coefficient of variation 11.5%).

Table 3: Results of testing the QC sample HCV Core Protein (CP) on the ARCHITECT HCV Ag assay.

Analyser	QC ID	Test Runs (n)	Observations (n)	Mean (fmol/L)	SD	CV (%)
iS2000SR	CP (a)	8	16	9.975	1.148	11.5
iS2000	CP (b)	5	94	15.779	3.176	20.1

Comments on Assay Performance

The reagents, calibrators and controls all come ready for use and the Abbott HCV Ag assay is straightforward and easy to use. However, the evaluation was complicated by the logistics of conducting the testing at two different locations using two different ARCHITECT i Systems: the i2000SR at Site 1 and the i2000 at Site 2. In addition, during the evaluation, a Product Correction was received from the Sponsor, which impacted on all the results obtained at Site 1 necessitating retesting of some of the samples at Site 2.

The Product Correction stated that ARCHITECT HCV Ag results may be falsely elevated if the ARCHITECT anti-HCV and ARCHITECT HCV Ag assays are both run on the same module. As a preventive measure the Correction recommended that anti-HCV and HCV Ag tests are performed on separate ARCHITECT modules; or, if only one module is available, that HCV Ag tests are run immediately after the daily maintenance, after which a second daily maintenance is performed. Thereafter all other ARCHITECT tests can be performed in random access mode.

Before the Product Correction was received many of the evaluation samples had been run on an ARCHITECT i2000SR at Site 1. This was the only Architect module housed by the host laboratory, which performs a range of Architect assays including anti-HCV. Throughout this testing period HCV Ag controls were higher than expected and sometimes out of range, and many high negative results were obtained for the diagnostic negative samples (reflected in the low delta value of -1.6 obtained for these samples). The Product Correction provided a likely explanation for these observations.

Although anti-HCV testing was not a complicating factor on the ARCHITECT i2000 module at Site 2, high negative control results continued to be seen. This generally occurred if the HCV Ag controls were run immediately after the daily maintenance. To overcome this problem and on the advice of the sponsor, each day's run that followed immediately after the daily maintenance commenced with a known negative sample; the negative control being used for this purpose. Thereafter the controls were processed as normal and the results were usually within the expected range. It was not possible to conclude whether the high negative control values that occurred immediately following the daily maintenance on the i2000 were instrument related or specifically an issue relating to the HCV Ag assay. In NRL's opinion the former is more likely.

The Abbott ARCHITECT HCV Ag assay was recommended for registration on the Australian Register of Therapeutic Goods.

Discussions with Abbott have confirmed that the product correction letter was distributed globally prior to the ARCHITECT HCV Ag assay being registered on the ARTG. It is understood that Abbott have since determined the root cause of these issues and will implement permanent corrective actions that will be notified to all users of the HCV Ag assay forthwith. Further, an Abbott Applications Specialist will provide training on site for any laboratory in Australia wishing to implement this assay, to ensure awareness of the need for optimal ARCHITECT maintenance.

NRL would be pleased to provide appropriate samples to laboratories implementing this assay to assist with familiarisation of its operational characteristics.

Acknowledgements

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