Evaluation of the Abbott RealTime HCV Genotype II kit

Introduction

The Abbott RealTime HCV Genotype II kit is designed for use in the transcription and amplification of the 5'UTR or NS5B regions of the hepatitis C virus (HCV) genome. RealTime-PCR amplification is conducted in three separate reaction mixtures with different fluorophores where genotype-specific probes are used to detect HCV genotypes 1, 1a, 1b, 2, 3, 4, 5 and 6 such that:

- Reaction A detects all HCV isolates, subtype 1a and genotype 3
- Reaction B detects genotype 2, subtype 1b and genotype 1
- Reaction C detects genotype 4, 5 and 6.

The kit was evaluated by NRL for registration as a reference test in the category of monitoring and management under the pre-July 2010 IVD regulatory framework.

Methods

Sensitivity and Accuracy

A total of 65 HCV RNA positive plasma specimens, representing genotypes 1-6 and subtypes 1a and 1b, with viral loads greater than 500 IU/ml were tested in the Abbott RealTime HCV Genotype II kit according to the manufacturer's instructions. All specimens had previously been assigned a genotype (and in some cases a subtype) by either nucleic acid (NA) sequencing of the HCV genome or by use of the Siemens Versant HCV Genotype 2.0 Assay (LiPA). RNA from 500 µl of each specimen was extracted using the manual sample preparation method of the Abbott Sample Preparation System. Extracted RNA, along with both negative and positive assay controls were amplified using the m2000rt instrument. Each specimen was tested once and the genotype/subtype determined by the Abbott RealTime HCV Genotype II assay compared with the previously determined genotype (reference).

Reproducibility

HCV RNA from each of 21 replicates of a single HCV RNA positive specimen of approximately 5000 IU/ml was analysed using the Abbott RealTime HCV Genotype II assay, as above. Eleven of these replicates were tested once each using a sample preparation volume of 500 µl, the remaining 10 replicates with a sample preparation volume of 200 µl.

Results

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Assay Sensitivity and Genotype and Subtype Accuracy

Assay Sensitivity and Genotype Accuracy

For the purposes of this evaluation, Assay Sensitivity was defined as the ability of the assay to detect as positive a HCV RNA positive specimen. Assay Sensitivity was calculated as:

> *(Number of Specimens for which HCV RNA detected / Total Number of valid positive specimen results) x 100*

Genotype Accuracy was defined as the ability of the assay to report the correct HCV genotype. Similarly, Subtype Accuracy is defined as the assay's ability to report the correct HCV subtype. Both were calculated as the number of correct results as a percentage of the total number of valid results. The numbers of each genotype/subtype tested in the assay are summarised in Table 1

Table 1: Summary of the number of each Genotype/subtype tested in this evaluation.

Genotype	1 [a/b]	2	3	4	5	6
Number of specimens tested	28 [16/9]	8	17	2	1	9

The results of specimen testing, including the numbers of concordant, discordant and indeterminate results are presented in Table 2. A concordant result is one where the result given by the assay is the identical to that determined by NA sequencing. Conversely, a discordant result is one where the result given by the assay is different to that given by NA sequencing. An indeterminate result is one where the assay reported that HCV was detected in a specimen but the HCV genotype was not able to be determined.

Table 2: Summary of Assay Sensitivity and Genotype Accuracy

Total number of specimens tested	65
Total number of specimens with valid results	64
Number of specimens where HCV not detected	0
Number of specimens with concordant genotype result	55*
Number of specimens with discordant genotype result	2*
Number of specimens with	8

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indeterminate result	

** One specimen was reported as both 1 and 6 by the assay, but was genotype 6 by NA sequencing. This result is counted twice: once as a concordant result and once as a discordant result.*

Of the total number of specimens tested, one specimen returned an invalid result because of a failure of the internal control to amplify and was excluded from subsequent calculations. Of the remaining 64 specimens, all returned results where HCV RNA was detected. Therefore the Assay Sensitivity, as defined above, was 100%.

The results from a total of 55 specimens were concordant, at the genotype level, with those previously determined by NA sequencing. A summary of the 10 specimens with either discordant (2) or indeterminate genotype (8) results is presented in Table 3. All but one of these specimens was genotype 6 by NA sequencing, the remaining specimen, genotype 3. Of the 2 specimens with discordant results, both of which had a reference genotype of 6, one was reported as genotype 1 by the assay, the other as both genotype 1 and genotype 6. Of the 8 specimens with indeterminate results, 7 were genotype 6 and one was genotype 3 by NA sequencing.

Table 3: Summary of discordant and indeterminate Genotype results

Reference Genotype	m2000rt Genotype
6	Indeterminate
3	Indeterminate
6	Indeterminate
6	Indeterminate
6	1
6	Indeterminate
6	Indeterminate
6	1/6
6	Indeterminate
6	Indeterminate

By calculating the number of concordant genotype results as a percentage of the total number of specimens tested, the assay's overall Genotype Accuracy is 85.9%. This value is clearly affected by the large number of specimens for which the genotype result was "indeterminate". It might be reasonable to argue that these do not constitute *discordant* results, as such. Therefore, by

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ignoring the 8 specimens with indeterminate results the assay's ability to report a correct result, for those specimens where a result can be reported, could be calculated as being 98.2%. This second value better reflects the fact that the assay was able to correctly identify most of the genotypes tested in this evaluation.

Two genotype 6 specimens produced discordant results (one of which also produced a concordant result, as described above). These results are consistent with evidence presented in the Sponsor's submission dossier that suggest there is considerable cross-reactivity between the assay's genotype 6 probes and genotype 1 NA sequences (page 45 of the package insert). The Sponsor also presented evidence that the assay was able to correctly identify only 8 of the 12 genotype 6 specimens tested (page 44 of the package insert). In this evaluation, of the 9 genotype 6 specimens tested only one gave a result concordant with the reference, albeit with an additional discordant result.

Subtype Accuracy

A total of 28 genotype 1 HCV specimens were tested, 25 of which had had subtypes determined by NA sequencing (Table 4). For the remaining three specimens the assay reported subtype information but none was available by NA sequencing as a reference result; these results were not included in calculation of Subtype Accuracy. Two specimens gave results in the assay that lacked subtype information (i.e. the results given were genotype 1), but for which the reference result included a subtype (1b). Consequently the assay's Subtype Accuracy was determined to be 92% (23/25).

Table 4: Summary of Subtype Accuracy for HCV Genotype 1, 1a and 1b specimens

Total number of valid Genotype 1 specimen results	28
Total number of Genotype 1 subtypes [1a + 1b]	25 [16, 1a; 9, 1b]
Number of concordant subtype results	23
Number of missing subtype results	2*
Number of subtypes for which reference subtype result not available	3

** Number of specimens for which a subtype was not reported by the assay*

Reproducibility

Replicates of a single HCV subtype 1a specimen were tested in the assay, at either 500 µl or 200 µl sample volume, to determine the assay's ability to reproducibly return genotype and subtype results. The results of replicate

testing are presented in Table 5. The results of all replicates were concordant with the reference at both the genotype and subtype level.

Table 5: Summary of assay reproducibility for replicates of a subtype 1a specimen at two separate sample input volumes

	Sample Input Volume	
	500 µl	200 µl
Total number of replicates tested	11	10
Total number of valid results	11	10
Number of concordant genotype results	11	10
Number of discordant genotype results	0	0
Number of indeterminate genotype results	0	0
Number of concordant subtype results	11	10
Number of discordant subtype results	0	0

The average threshold cycles for probes for HCV (which detects the presence of HCV RNA), Internal Control (IC), genotype 1 and subtype 1a for the results presented in Table 5 are summarised in Table 6 and demonstrate consistent, reproducible performance of the assay.

Table 6: Summary of the mean, standard deviation (s.d.) and coefficient of variation (%CV) for 500 µl, 200 µl or all replicates values of subtype 1a reproducibility specimen.

	Sample Input Volume 500 µl		Sample Input Volume 200 µl		All replicates	
	mean ± s.d.	%CV	mean ± s.d.	%CV	mean ± s.d.	%CV
HCV	24.68 ± 1.05	4.27	25.85 ± 0.15	0.57	25.21 ± 0.96	3.82
IC	18.98 ± 0.66	3.49	18.60 ± 0.21	1.11	25.07 ± 0.76	3.02
genotype 1	23.59 ± 1.23	5.21	24.61 ± 0.18	0.73	18.81 ± 0.53	2.80
subtype 1a	24.50 ± 0.55	2.24	25.76 ± 0.09	0.36	24.05 ± 1.02	4.25

Consistent with the Sponsor's claim, testing performed for this evaluation indicates that the assay can detect HCV genotypes 1, 2, 3, 4 and 5 and

subtypes 1a and 1b. In contrast, only one of the 9 genotype 6 specimens produced a correct, rather than indeterminate, result. Most of the genotype 6 specimens comprised non-a/non-b subtypes which the assay is not designed to type. The extent to which the assay did not report these genotypes correctly is unlikely to be of clinical relevance.

The assay has since been included on the Australian Register of Therapeutic Goods.

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