

Comparison of Automated and Manual Nucleic Acid Extraction Methods for Detection of West Nile Virus RNA from Mosquito Specimens

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Background

Mosquito surveillance plays an important role in controlling the spread of West Nile Virus (WNV) through North America. Currently, the Taqman Real-time RT-PCR method has been used in many laboratories for the detection of WNV in Mosquitoes. However, the nucleic acid extraction method, a manual Qiagen Viral RNA extraction kit, is labour-intensive. An automated nucleic acid extraction, Qiagen BioRobot 9604, might be an attractive alternative to the manual method. We evaluated both methods by comparing their performances.

Methods

Mosquito pools, each with 50 mosquitoes, were homogenized in viral transport media, and were spiked with dilutions of Armored WNV RNA (Ambion, INC) containing 10000, 1000, 100 and 0 copies (n=9x2, for each dilution). Those pools spiked with Armored WNV RNA served as positive samples, while those without served as true negative samples. Standard controls were prepared with the Armored WNV RNA in the following concentrations, 10000, 5000, 1000, 500 and 100 copies. To evaluate precision for the methods, samples and standards were grouped into 4 different runs of 18 samples and 6 controls. The manual and robot RNA extractions were performed concurrently for each group. The nucleic acid extracts were amplified, in duplicate, by Taqman RT-PCR using a primer/probe set for the non-structural protein 5 (NS5-2). CT results were analyzed using the Taqman and Analyse-It statistical software.

Results

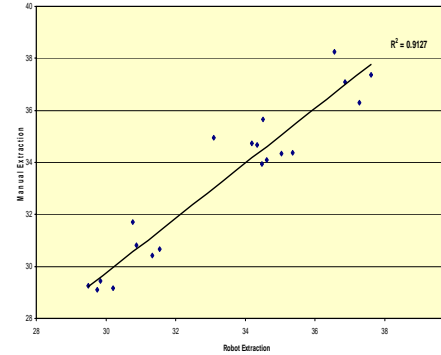
We obtained 53/54 Taqman RT-PCR positive results from the robot extraction, and 51/54 Taqman RT-PCR positive results from the manual extraction. For both extraction methods no false positive results (0/18) were obtained. The sensitivity for the robot extraction method was 98.15%, while the sensitivity for the manual extraction was 94.44%. For both extraction methods the specificity was 100%. Upon examining the CT results for the high positive samples (those spiked with 10000 and 1000) for the two methods, it was determined that the manual extraction performed slightly better than the robot extraction. However, when the CT results for the border line samples (the 100 spiked ones) were examined, the robot extraction produced slightly better results.

Precision results were obtained from the standards tested with each of the runs. Based on the values shown in Table 2, the coefficient of variation was determined to be a range from 0.46% to 2.16%. The results in Table 2 were also used to determine the correlation between the two methods. Figure 1 illustrates this correlation to be linear, with a R² (Pearson product moment correlation coefficient) of 0.91.

	Sensitivity	Specificity
Robot	98.15%	100 %
Manual	94.44%	100 %

Table 1. The sensitivity and specificity results for the manual and robot extractions.

Figure 1. The average CT results obtained for each of the standards performed over 4 runs, plotted in a XY scatter to show the correlation between the two methods. Linearity was observed, with a R² of 0.91.



	10000		5000		1000		500		100	
	Robot	Manual	Robot	Manual	Robot	Manual	Robot	Manual	Robot	Manual
Avg	29.82	29.24	31.13	30.90	34.06	34.61	34.85	34.58	37.08	37.26
SD	0.30	0.14	0.37	0.56	0.67	0.36	0.43	0.74	0.46	0.80
CV	0.99	0.46	1.19	1.83	1.97	1.05	1.24	2.14	1.25	2.16

Table 2. The precision results were observed in 5 different concentrations of standards over 4 different runs. The values shown were generated from the CT values obtained during each run. The coefficient of variation for the results ranged from 0.46% to 2.16%. This indicated both methods were very low in the measurement error.

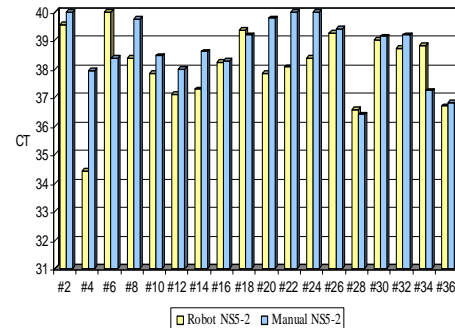


Figure 2. A comparison, of the CT results obtained for the pools containing 100 copies of spike, between the two extraction methods. This illustrates that the Robot extraction was producing better CT results than the manual extraction.

Discussion

Most method comparison studies are qualitative, as a number of samples are tested and the sensitivity and specificity are calculated based solely on those which resulted positive or negative. This could be misleading as it may not represent a wide variation of environmental sample types.

A quantitative approach was used here by spiking environmental samples containing the maximum number of mosquitoes, those with the highest probability of inhibition occurring, with known copies of an Armored WNV RNA. Various concentrations of spike were used to observe the performance across a wide range of sample types, and all of the results were used to determine the specificity and sensitivity of each method. It was determined that the robot's sensitivity was better, by 4%, than the manual extraction, however their specificities were identical at 100%. Since no false positive results were observed, it was concluded that no carry-over or contamination was occurring. A closer look was taken at the performance of each method by analyzing the standards for each run. The correlation between the methods and the precision was determined, as it was important to see how reproducible the robot extraction was. A coefficient of variation range, 0.46% to 2.16%, indicated that both methods were very low in measurement error.

From a cost perspective, the BioRobot and Qiagen Kit extraction differed minimally at approximately \$4/column each. In terms of time to perform the extraction, the BioRobot would take 3 hours to extract 96 specimens, while it could take 3-5 hours by manual extraction for one individual to complete (this is technician dependent).

Conclusion

Even though the Qiagen BioRobot was slightly more sensitive at detecting the Armored RNA in the low copy samples, we see the overall performance between the Qiagen BioRobot 9604 and the Qiagen Viral RNA kit to be statistically equal based on the R² value. From a cost and time perspective we also see the two methods to be equal. Therefore, it would be acceptable to use either the Viral RNA kit or the BioRobot for the extraction of WNV from mosquito pools.

References

1. Use of an internal positive control in a multiplex reverse transcription-PCR to detect West Nile virus RNA in mosquito pools. *J Clin Microbiol.* 2004 Feb;42(2):841-3.
2. Comparison of automated and manual nucleic acid extraction method for detection of Enterovirus RNA. *J Clin Microbiol.* 2003 Aug;41(8):3532-6.

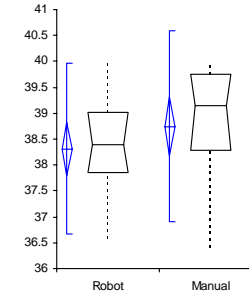


Figure 3. A comparative descriptive analysis of the data in figure 2, shows the robot worked slightly better (lower CT) statistically.