Comparison of norovirus detection using RIDA®QUICK Norovirus Kit at Gold Coast Hospital Microbiology Department versus norovirus RT-PCR

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INTRODUCTION:

Worldwide, norovirus is one of the leading causes of acute gastroenteritis. (1,2) A major cause of gastroenteritis outbreaks in such institutions as hospitals and nursing homes, the virus is usually transmitted through person-to-person by the faecal-oral route. Environmental transmission in hospitals reportedly involves such areas as floors, work surfaces, light switches, door handles and medical equipment, with the virus surviving for prolonged periods on such surfaces. (3)

Outbreaks of norovirus can lead to ward closures and increased patient stay, at a high cost to hospitals. Hence, in combination with proper infection control precautions, there is the need for a test to detect norovirus which is both accurate and rapid, particularly for use in outbreaks. Presently, suspected norovirus cases at Gold Coast Hospital are referred to Scientific Services for RT-PCR. Whilst this test is a sensitive and specific gold standard, it is a time consuming, expensive and difficult assay and there is a high volume of work and frequent transport delays. Such factors may cause a delay in infection control measures and increase the risk of norovirus spreading through the hospital.

The purpose of this analysis was to evaluate the RIDA®QUICK (*R-Biopharm AG, Darmstadt, Germany*) rapid immunoassay kit for detection of norovirus compared to RT-PCR. We wished to establish, by determining the sensitivity and specificity, whether the kit could augment RT-PCR for rapid diagnosis during a norovirus outbreak.

METHOD:

A total of 51 known RT-PCR positive and negative stool samples were acquired from Scientific Services (reference laboratory) Coopers Plains, Brisbane. Each faecal sample was marked positive and negative in a way not obvious when testing was performed, so as not to influence the reading of results. Once marked, all specimens were combined and random negative and positive specimens tested by RIDA®QUICK. Testing was performed initially on single samples and then in groups of three maximum.

RIDA®QUICK NOROVIRUS PROTOCOL: (4)

Sample Preparation

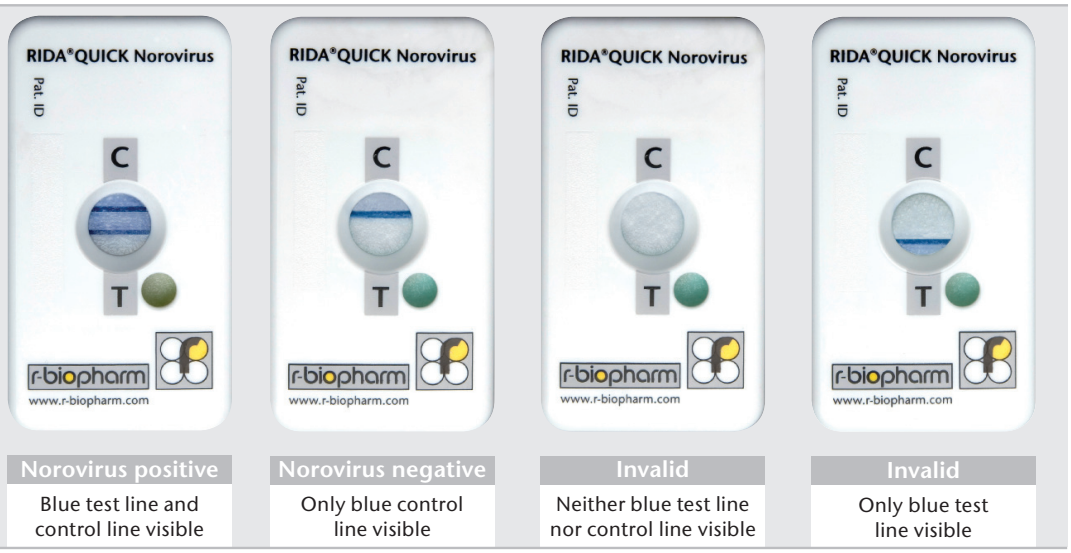
- Stool specimens were mixed thoroughly.
- 1ml of Diluent was added to each plastic Eppendorf reaction tube for each specimen.
- 100 µl specimen was transferred to the diluent buffer & vortexed for 10 seconds.
- Leave for 2 minutes to allow for sedimentation.

TEST PROCEDURE:

- 250 µl supernatant was transferred to a new reaction tube.
- 6 drops of Conjugate 1 were added to the reaction tube and mixed by aspiration.
- Supernatant-conjugate mixture was transferred with a slow continuous flow to the sample well.
- Incubation for 10 min at room temperature.
- 4 drops of Conjugate 2 were added to the reaction window
- Incubation for 1 min at room temperature.
- 10 drops of Wash Buffer were added to the reaction window.
- 6 drops of Substrate were added to the reaction window.
- Results were recorded and read within 3 minutes.

INTERPRETATION:

Figure. 1
Test cassette and interpretation of results.



T = blue, Norovirus positive
C= blue, control line

RESULTS:

Table 1. Sensitivities, specificities, and predictive values of RIDASCREEN®QUICK kit and RT-PCR assay

Sensitivity= 20/26 x 100= 77%,
Specificity= 25/25 x 100= 100%
PPV= 20/20 x 100= 100%,
NPV= 25/31 x 100 =81%

		RT-PCR		
		+	-	Total
RIDA®QUICK Norovirus	+	20	0	20
	-	6	25	31
Total		26	25	31

Most samples were the common genogroup GG 2 (25 out of 26 positive samples) with only one GG 3 (1 out of 26 positive samples) and one GG 1 (1 out of 26 positive samples) available for testing. A discrepant result was noted with GG1. Most reactions were immediate, clear, unambiguous and easy to read. One test was repeated due to an equivocal result with a faint incomplete line and was clearly positive on repeat testing.

DISCUSSION:

Previous immunoassays (RIDASCREEN® and IDEIA)

Previous work was done at the Gold Coast in 2007 on RIDASCREEN® (*R-Biopharm AG, Darmstadt, Germany*) and IDEIA (*OXOID Ltd., Basingstoke, UK*) norovirus immunoassays for sensitivity and specificity compared to RT-PCR (5).

The RIDASCREEN® Norovirus (3rd Generation) immunoassay demonstrated a 76.6% sensitivity and 97.5% specificity. The IDEIA immunoassay demonstrated a sensitivity of 66.6% and specificity of 95%. The sensitivity of these assays (particularly the RIDASCREEN®) was thought adequate for use as an adjunct to RT-PCR in the outbreak setting to facilitate a quicker result. However, these assays were both technically time consuming at 1hour 45 minutes with 2 washes and 1 hour 30 minutes with 1 wash for the RIDASCREEN® and IDEIA respectively. For this reason introduction of one of these immunoassays to Queensland laboratories was postponed until the release of the new rapid immunoassay (RIDA®QUICK).

RIDA®QUICK

The RIDA®QUICK was analysed for rapidity, simplicity, ease and sensitivity compared to known RT-PCR positive and negative samples. The results show similar sensitivity to the RIDASCREEN® assay at 77% with 100% specificity. The assay was simple to perform and rapid with a test time at 15 minutes. The results were clear and easy to read.

CONCLUSION:

On the basis of these results, we suggest that the RIDA®QUICK could be introduced into Queensland laboratories for the rapid diagnosis of norovirus in an outbreak setting in situations of clinical uncertainty as an adjunct to RT-PCR.

Reference List:

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3. **Gallimore CI, Taylor C, Gennery AR, et al.** Contamination of the Hospital Environment with Gastroenteric Viruses: Comparison of Two Pediatric Wards over a Winter Season. Journal of Clinical Microbiology, Sept 2008; 3112-3115.
4. **www.rapid-diagnostics.info/download.php**
5. **Derrington P & Schreiber F,** Evaluation of Two Commercial Assays on Known PCR Positive and Negative Faecal Samples. July 2007.