Evaluation of Two Commercial Assays on Known PCR Positive and Negative Faecal Samples

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INTRODUCTION:

A range of in-house molecular amplification techniques are in use for the diagnosis of norovirus but these are not well standardized and are time consuming and labour intensive. Real time technology and automated extraction methods should improve the turnaround time (TATs) and decrease contamination. However, presently there are unacceptably long TATs involved with both transport and processing of specimens for norovirus. This has important implications for Infection Control management of suspected outbreaks and prevents early outbreak control.

Gengogroup and genotype specific antibodies have been generated and shown to be applicable for use in rapid enzyme immunoassays. Presently there are two major EIA tests for norovirus in faeces:

The Oxoid IDEIA kit uses a combination of both genogroup 1 and 2 specific monoclonal and polyclonal antibodies in a solid phase sandwich enzyme immunoassay. This is a two step two hour procedure. The R-Biopharm RIDASCREEN® Norovirus kit uses specific monoclonal antibodies to several different genotypes in a sandwich type method. This is a 3 step two and half hour procedure.

The manufacturers of both assays advise the use of a spectrophotometer to optimally read the results. The manufacturers of the IDEIA kit suggest that visual reading is acceptable but it is recommended that any wells that are difficult to read are also read spectrophotometrically. The manufacturers of the RIDASCREEN® advise use of a spectrophotometer, but report several laboratories successfully using the kit in Australia by visual reading only (personal correspondence). The need to install a spectrophotometer has important cost and infrastructure implications for introduction of an assay into Queensland Health Laboratories.

It is therefore important to elicit both the sensitivity and specificity of the above assays as well as the ease of performing the assay and the sensitivity of visual versus photometric reading of results.

CLINICAL TRIALS:

Most trials testing the commercial ELISA kits give sensitivity and specificity results on single specimens versus molecular testing, usually RT-PCR, which is currently the accepted reference method. A range of in-house molecular amplification techniques are in use of many clinical studies with immunoassays. See table below for a summary of clinical trials.

No cross reactivity was found with either kit when tested against cultures of 106- 109 of viable organisms commonly found in faecal specimens.

CLINICAL STUDIES:

Reference	Location	EIA method and evaluation	Reference standard	Single specimen sensitivity %	Single specimen specificity %	Number of specimens	Detection method
Package ins	UK	IDEIA	RT-PCR	55	100	120	photometric
Sanz 2006	Spain	IDEIA	RT-PCR	80	100	260	photometric
De Cal 2007	Spain	IDEIA	RT-PCR	77	86	117	photometric
Castricinano 2007	Canada	IDEIA	RT-PCR EM	60 (36)	100 (94)	228	
Dimitriadis 2006	Australia	IDEIA	RT-PCR	66 63	85 88	130	Visual
Richards 2002	UK	IDEIA	RT-PCR EM	56 (24)	98 (99)	479	photometric
Rabenau 2003	Germany	IDEIA	RT-PCR	31	94	244	photometric
Okitsu-Negishi 2006	Japan	RIDASCREEN Gen 2	RT-PCR	76	95	503	photometric
Package ins 2004	Germany	RIDASCREEN Gen 2	RT-PCR	93	100	60	photometric
Package ins 2006	Germany	RIDASCREEN Gen 3	RT-PCR	79	100	123	photometric
Sanz 2006	Spain	RIDASCREEN Gen 2	RT-PCT	80	90	260	photometric
De Cal 2007	Spain	RIDASCREEN Gen 3	RT-PCR	59	73	117	photometric
Gonzalez 2006	Venezuela	RIDASCREEN Gen 2	RT-PCR	60	97	92	photometric
Castricinano 2007	Canada	RIDASCREEN Gen 3	RT-PCR EM	80	100	228	photometric
Dimitriadis 2005	Australia	RIDASCREEN Gen 2	RT-PCR	71	42	130	photometric

AIM:

The aim of the present study was to investigate the sensitivity of the two commercially available rapid tests for norovirus screening of faecal samples compared to RT-PCR. The study specifically addressed the questions whether the ELISA kits can be used for investigations of outbreaks and can be read visually. The answer to this question should help scientists in public health laboratories and hospitals who might be interested in using ELISA kits in the rapid diagnosis of norovirus in suspected outbreaks to assist with targeted and timely infection control.

MATERIAL AND METHODS:

Panel of stool samples. Known positive and negative faecal samples were collected from Scientific Services (Reference Laboratory) Coopers Plains, Brisbane. These samples had previously been tested by RT-PCR and frozen.

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 visual results. Once marked, all specimens were combined and random negative and positive specimens tested by ELISA.

Testing was performed in lots of six specimens, representative of a norovirus outbreak. Each run included a positive and negative controls. In total 100 samples were tested. Both kits were tested with the same faecal samples simultaneously according to manufacturers instructions. Wells from both kits were read photometricaly by a Titertek Multiskan MCC/340 spectrophotometer at 450 nm and results evaluated by calculations outlined in the instructions of each kit.

RESULTS:

100 tests were performed in batches of six, and apart from the first 12 tests before the photometer was available, all tests results were compared both visually and photometrically.

CONTROLS:

16 controls were run, one with each 6 test cycle, and the controls were acceptable.

SENSITIVITY AND SPECIFICITY:

Tables 1 and 2 show the sensitivity, specificity and agreement of the two tests read visually versus RT-PCR and details of the comparison between the two assays.

When compared to RT-PCR, RIDASCREEN® has the highest sensitivity and specificity (76.6 % and 97.5 % respectively) compared to IDEIA (66.6 % and 95 % respectively).

Table 3 shows comparison result in more detail. Of the known positive specimens by RT-PCR, 36 specimens were positive by both EIA assays and RT- PCR. 10 specimens tested positive by RIDASCREEN® and not by the IDEIA kit and only 4 tested positive by IDEIA and not RIDASCREEN®. Neither IDEIA nor RIDASCREEN® reacted to 10 specimens positive by RT-PCR.

The specificity of both tests was very high. In total, three "false positive" results were initially obtained, three with the IDEIA kit and two of those three were also positive with the RIDASCREEN®. However, one of these samples was retested by RT-PCR and was subsequently found to be positive. Unfortunately, the other two samples were discarded in error, so we are unable to confirm the result by RT-PCR*. This error may have resulted in falsely low sensitivity and specificity for both assays.

		IDEIA		RIDASCREEN®	
		+	-	+	-
RT-PCR	+	40	20	46	14
	-	2	38	1	39

Table 1 Sensitivity and specificity of the tests read visually versus RT-PCR.

Parameter	IDEIA	RIDASCREEN
Sensitivity	40/60 (66.6 %)	46/60 (76.6 %)
Specificity	38/40 (95%)	39/40 (97.5 %)
Agreement	78/100 (79%)	85/100 (85 %)

Table 2: Sensitivity, Specificity and Agreement of EIA kits read visually compared to RT-PCR

RT-PCR	IDEIA	RIDASCREEN	Number of Samples, (Total =100)
+	+	+	36
+	+	-	4
+	-	+	10
+	-	-	10
			Total 60
-	-	-	38
-	-	+	0
-	+	+	1*
-	+	-	1*
			Total 40

Table 3: Detail of results comparing the visually read ELISA tests and RT-PCR
* Tests unable to be confirmed

		Visual reading	Photometric reading	Sensitivity/ Specificity
Controls	+	16	16	100 %
	-	16	16	100 %
IDEIA	+	37	37	100 %
	-	51	48 2 Equivocal 1 Positive	94 % 4 % 2 %
RIDASCREEN®	+	39	36 2 Equivocal 1 Positive	92 % 5 % 3 %
	-	49	49	100 %

Table 4: Sensitivity of photometric readings compared to visual readings, of the 88 specimens tested.

VISUAL VERSUS PHOTOMETRIC READINGS:

Photometric readings were only recorded for 88 samples and 16 controls read by both methods are summarized in Table 4.

16 control cycles showed 100 % concordance between visual and photometric analysis. The sensitivity of the IDEIA kit for visual results versus photometric analysis was 100 % and that for the RIDASCREEN® was 92 %. The specificity was high at 94 and 100%.

EASE OF VISUAL ANALYSIS:

During the course of testing, a further observation was made. A marked difference in reaction strength was noticed between IDEIA and RIDASCREEN®. These variations were noted for the remaining 60 samples analyzed. Addition of stop solution increased ease of visual reading.

A strong color change was noted in 61 % of tests with the RIDASCREEN® versus 14 % with the IDEIA, and a weak result was noted in only 7 % with the RIDASCREEN vs. 29 % with the IDEIA.

	Positive IDEIA results		Positive RIDASCREEN® results	
	Number	Percent	Number	Percent
Strong visual result	4	14	17	61
Average visual result	16	57	9	32
Weak visual result	8	29	2	7
TOTAL	28	100	28	100

Table 5: Strength of reaction compared between positive IDEIA and RIDASCREEN®.

DISCUSSION:

This project was undertaken at the Gold Coast laboratory in response to the long turnaround time for results for norovirus in faecal samples at the time of an outbreak. During outbreaks, a timely and targeted Infection Control strategy is important, and this is difficult when the result of RT-PCR may take up to three days due to a combination of transport and processing delays.

We performed analysis of two rapid commercial EIA assays for norovirus detection in order to elucidate the sensitivity, specificity and performance of these assays. We plan to use these results to advise on the introduction of one of these assays into Queensland Health laboratories for rapid diagnosis of norovirus infection in an outbreak situation. For the analysis the IDEIA and RIDASCREEN® enzyme immunoassays were compared to known samples found to be negative or positive by RT-PCR assay at Scientific Services, Brisbane.

The RIDASCREEN® showed higher sensitivity and specificity (76,6 and 97,5%) than the IDEIA assay (66,6 and 95 %). It is unfortunate that two specimens in which false positive results were obtained were erroneously discarded and so could not be confirmed by repeat RT-PCR. One further specimen that had a false positive result was subsequently retested by RT-PCR and found to be positive on retesting, thus casting doubt on the false positive results.

The manufacturer's advice for the requirement of a spectrophotometer for reading the result in routine testing would be an enormous drawback in the introduction of one of these assays into small routine laboratories. The extra time taken up in further processing, calculations of results and expense of installing a spectrophotometer would result in a time consuming and inefficient process. Thus it was important to show that the visual reading was equivalent to the photometric reading and the results show that the sensitivity of visual readings for the IDEIA was 100 % and for the RIDASCREEN® was 92 %.

It was also noted that the RIDASCREEN® assay was easier to read and more frequently showed a very strong color reaction versus the IDEIA kit. This strong color reaction was seen in 61 versus 14 % of cases respectively. Although this is a subjective result, it does directly relate to the study's goal to determine ease of testing and significantly assist in determining which kit may be more suitable in routine testing without the use of a spectrophotometer.

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