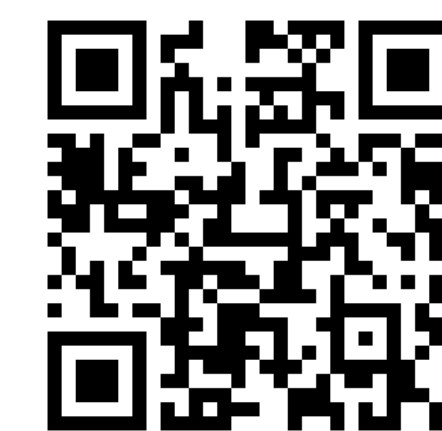


# Reduced Diagnostic Performance of Two Commercial Norovirus Antigen Enzyme Immunoassays against the Emergent Genogroup II Genotype 17 Kawasaki 2014 Variant

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## Background

- During the 2014-15 winter, an emergent norovirus genogroup II genotype 17 (GII.17) Kawasaki 2014 variant predominated in several Asian countries.
- The diagnostic performance of commonly used commercial enzyme immunoassays (EIAs) for the detection of this novel variant is unknown.

## Aim

- To evaluate diagnostic performance of two commercial norovirus EIAs against the emergent GII.17 Kawasaki 2014 variant.

## Methods

- A total of 90 stool samples tested positive by a norovirus real-time RT-PCR assay with Ct values were used.
- The samples were collected from patients hospitalized for norovirus gastroenteritis between December 2014 and March 2015 in Hong Kong. They consisted of 25 GII.4 Sydney 2012 and 65 GII.17 Kawasaki 2014 cases.
- Two commercial norovirus EIAs were evaluated: **RIDASCREEN® Norovirus 3rd Generation Antigen EIA (r-biopharm)** and **IDEIA™ Norovirus EIA (Oxoid)**.
- Clinical sensitivity of the 2 EIAs were calculated. Clinical and virologic factors (age, sex, virus genotype, and fecal noroviral load) that might associate with diagnostic performance of the 2 EIAs were analyzed using multivariate binary logistic regression.

## Results

- The clinical sensitivity of both EIAs were significantly lower for norovirus GII.17 Kawasaki 2014 compared with GII.4 Sydney 2012 (**Table 1**).
- Overall, optical density readings of both EIAs correlated negatively and significantly with Ct values. Notably, the strength of correlation was stronger for GII.4 Sydney 2012 (Spearman's  $\rho$  -0.88 and -0.81) than for GII.17 Kawasaki 2014 (Spearman's  $\rho$  -0.58 and -0.39) (**Figure 1**).
- Among high noroviral load samples, defined as having a Ct value less than an arbitrary cut-off of 20, false-negativity of both EIAs was more commonly observed in GII.17 Kawasaki 2014 compared with GII.4 Sydney 2012 (RIDASCREEN®: 26% versus 6%,  $P=0.2055$ ; IDEIA™: 70% versus 18%,  $P=0.0016$ ; Fisher's exact test).
- Lower fecal noroviral load** and **norovirus genotype GII.17 Kawasaki 2014** were independently associated with increased likelihood of obtaining negative results for both EIAs ( $p$  range:  $<0.0001$  to 0.003).

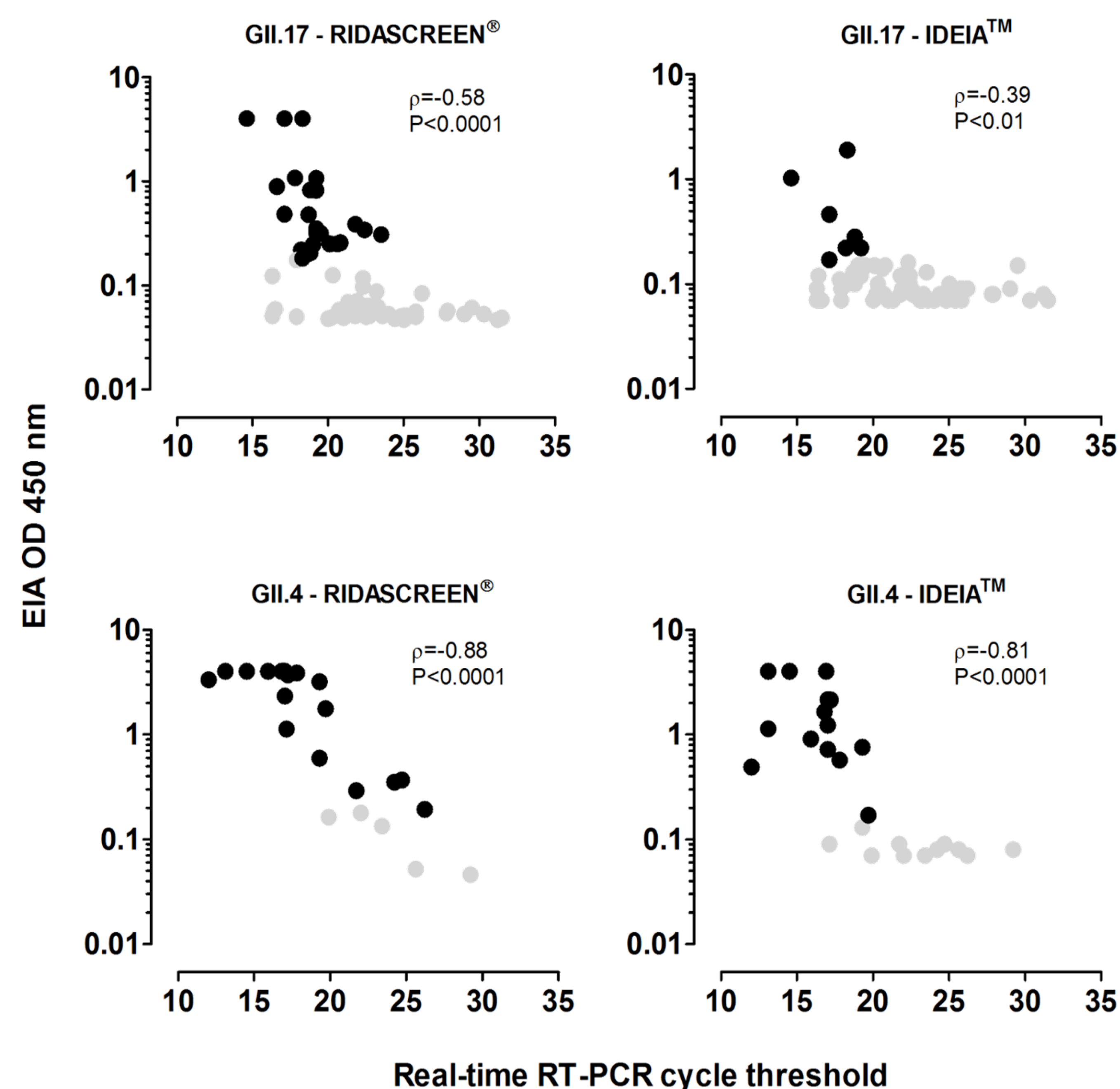
## Conclusions

Both RIDASCREEN® and IDEIA™ have reduced diagnostic performance against the emergent norovirus GII.17 Kawasaki 2014 variant. Emergence of novel norovirus variant necessitates validation of antigen detection assays.

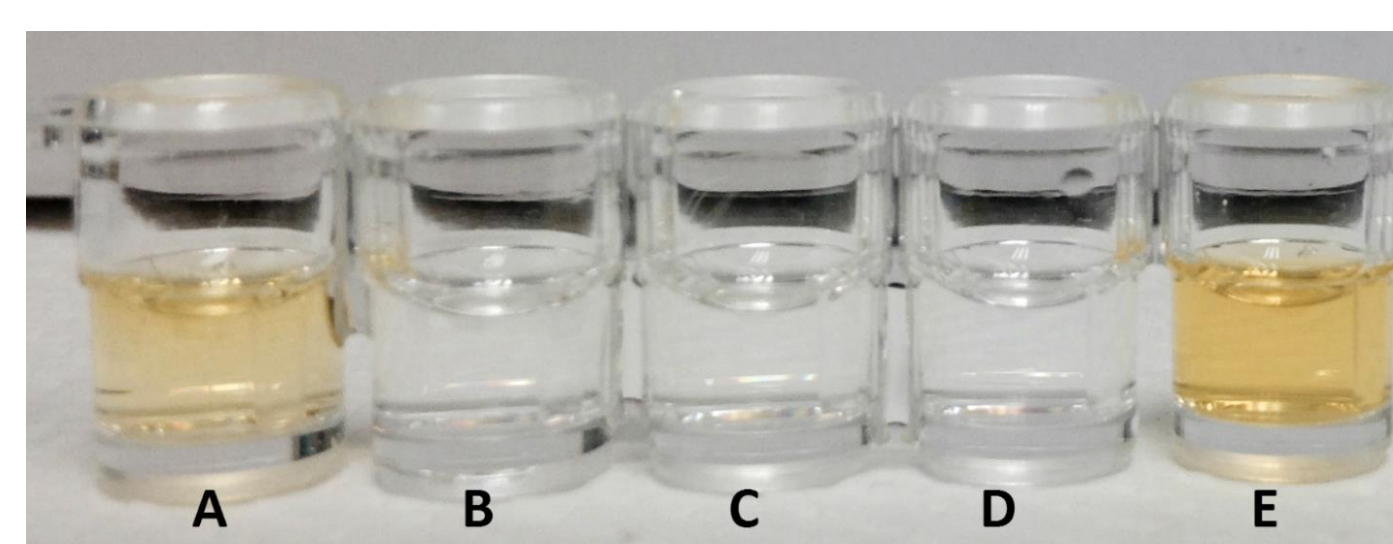
**Table 1.** Percentage of stool samples tested positive by two norovirus EIAs.

Norovirus EIAs	% stool samples tested positive	
	GII. 17 (n=65)	GII. 4 (n=25)
RIDASCREEN®	35	80
IDEIA™	11	56

(GII.17 versus GII.4: RIDASCREEN®,  $p=0.0002$ ; IDEIA™,  $p<0.0001$ ; Fisher's exact test)



**Figure 1.** Correlation of optical density readings of 2 norovirus EIAs with fecal noroviral loads in terms of cycle threshold values. Black and grey dots denote positive and false-negative test results, respectively.



	A	B	C	D	E
Ct	18	20	18	20	17

**Figure 2.** One representative picture from one of the EIAs demonstrating false-negative EIA results despite of high viral load of GII.17 Kawasaki 2014 (low real-time RT-PCR cycle threshold [Ct] values, lower panel).

## Acknowledgement

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