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# Urine compared with serum for diagnostic rt-PCR in an Asian Zika Virus outbreak in Singapore in 2016

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## BACKGROUND

An Asian ZikaVirus caused an outbreak in Singapore in August 2016

The literature suggests that the *rt-PCR yield from urine is better than serum*. This is relevant to case detection as cases often present for healthcare several days after onset of symptoms

In contrast, some reports suggest *the opposite may be true in pregnancy*; that ZikaV could be detected for longer in serum than in urine

At the time of the outbreak in 2016 in Singapore, 'suspect' cases had both urine and serum tested

## PURPOSE

*To compare the yield from serum and urine for the detection of ZikaV by rt-PCR*

## METHODS

A comparison of results, from pairs of urine and serum samples collected on the same day, was based on an audit of routine laboratory data at Tan Tock Seng Hospital, Singapore

RNA was extracted with the EasyMag (Biomerieux) with 500uL input and 55uL eluate. The rt-PCR was based on a method used in 2007 (EID 2008 14,8; 1232 -1239)

*Pairs of sample types* were processed at the same time, on an urgent 24 hour basis, 7 days/week

Ct values were plotted as the difference in ct (urine ct - serum ct) against the average ct ((urine ct + serum ct)/2)

Values *above the 'zero' line*

= higher RNA load in the *serum*

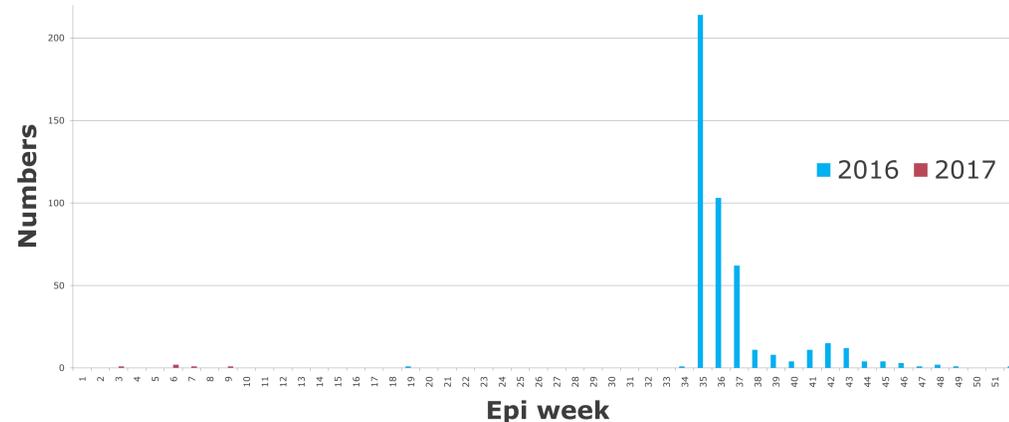
Values *below the 'zero' line*

= higher RNA load in the *urine*

Ct values are inversely related to the quantity of target

A ct difference of 3.3 translates approximately to a ten fold difference in RNA load.

## Zika Virus cases in Singapore



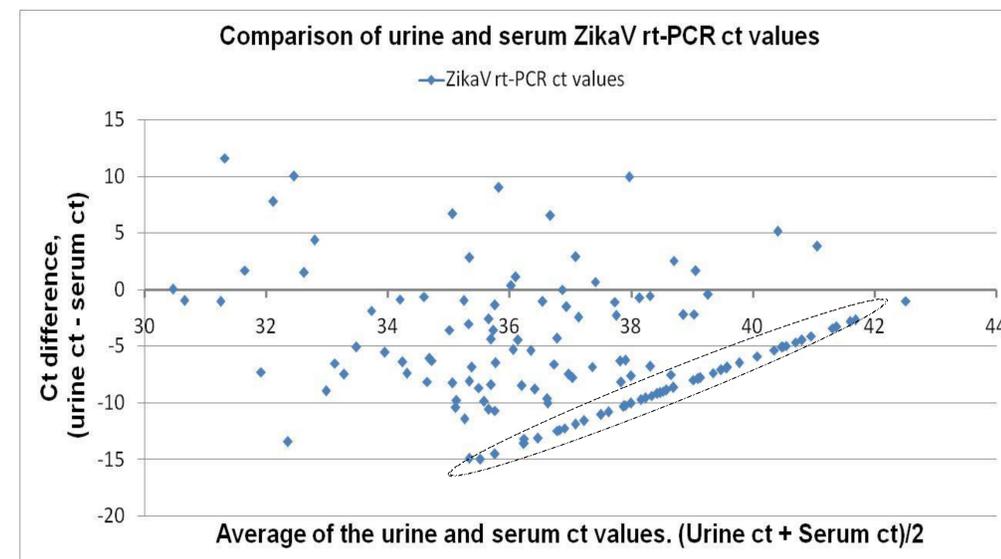
## RESULTS

See the figure below.

There were 135 paired samples from patients with ZikaV.

RNA was 'not detected' in 2 urine samples and 47 serum samples.

In order not to lose the urine partner data from this large group of 47 with negative serum samples and to allow the data to be represented on the figure, the 'not detected' serum samples were assigned ct values of 43 as the assay was run to 42 cycles. This allowed a difference and mean to be calculated; this subset appears on the figure as a linear group, highlighted with an interrupted oval.



## RESULTS and DISCUSSION

The urine yielded a higher RNA load for the majority of samples, as shown by lower ct values. Most urine samples had a ct at least 5 cts earlier than the partner serum sample.

The subset highlighted with an interrupted oval show that several urine samples were 10 to 15 cts earlier; this equates with a greater RNA concentration by a factor of thousands and tens of thousands.

In contrast, *in 22 of the 135 pairs (16%) the load was higher in the serum sample*; in several sera the signal was over a thousand fold higher than in the urine.

This was unexpected as the literature suggests urine is preferable to blood, although data for Asians and Asian strains of ZikaV are not available. There are some data from pregnant women showing that serum may be a better sample in pregnancy but the data are limited. Only two in our dataset were from females of child bearing age.

Preliminary analysis of our data does not reveal an association with gender, age, renal function or day of illness.

## CONCLUSION

The urine yielded a higher RNA load for the majority of patients by a factor of thousands and tens of thousands.

*However, in 16% the load was higher in the serum sample; in several sera the signal was over a thousand fold higher than in the urine.*

Testing urine alone might miss a considerable proportion of patients

'No disclosures'

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