Supplemental Digital Content 1

PCR methods used in the study

Parechovirus was tested with a one-tube RT-PCR using the Quantitect Probe chemistry (Qiagen, Hilden, Germany), primers adopted from Corless et al., (1) and a redesigned probe (to better detect novel parechovirus types). The primers detected with an equal sensitivity Parechovirus A species 1-6 but did not show unwanted cross-reactivity with Parechovirus B (i.e. Ljungan virus). Parechovirus primers and probe were compared *in silico* with parechovirus sequences from GenBank, and tested against our earlier parechovirus real-time PCR using a variety of samples known to contain parechovirus types 1, 3, 5 and 6, which showed that the redesigned probe was as effective (data not shown) as the PCR methods used in our previous publications.(2, 3)

Anellovirus was tested with a real-time PCR using Qiagen HotStar chemistry (Qiagen, Hilden, Germany) adopted from Thom et al.(4) The assay was changed from nested PCR to one round PCR. Anellovirus primers were tested with anellovirus references found in a metagenomics virome survey (data not shown), detecting most of the *Alphatorquevirus* and *Betatorquevirus,* and some *Gammatorquevirus*, references.

References

1. Corless CE, Guiver M, Borrow R, et al. Development and evaluation of a 'real-time' RT-PCR for the detection of enterovirus and parechovirus RNA in CSF and throat swab samples. J Med Virol 2002;67:555-62.
2. Tapia G, Cinek O, Rasmussen T, et al. Longitudinal study of parechovirus infection in infancy and risk of repeated positivity for multiple islet autoantibodies: the MIDIA study. Pediatr Diabetes 2011;12:58-62.
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4. Thom K, Morrison C, Lewis JC, et al. Distribution of TT virus (TTV), TTV-like minivirus, and related viruses in humans and nonhuman primates. Virology 2003;306:324-33.