Enzyme-linked immunosorbent assay for the detection of group C rotavirus in children

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Background
Group C Rotavirus (GpC RV) causes sporadic cases and outbreaks of acute diarrhea in children and adults globally. GpC RV grows poorly in cell culture and because of lack of simple diagnostic tools, its prevalence and disease burden has not been established. With the implementation of group A rotavirus (GpA RV) vaccines and the reduction in GpA RV infection in humans worldwide, other viral agents like norovirus have emerged. The present study aimed to develop a monoclonal antibody (mAb)-based, enzyme-linked immunosorbent assay (ELISA) for detecting GpC RV antigen in stool specimens of children.

Methods
Anti-GpC mAbs were produced using 6-8 weeks-old female BALB/c mice which were immunized with baculovirus-expressed GpC virus-like particles (VLPs). mAbs in culture supernatants from individual hybridoma cells were screened and characterized with GpC ELISA, immunoprecipitation, and dot blot hybridization. The ELISA for GpC RV was developed with purified mAb as capture and HRP-conjugated mAb as detector. This new ELISA was evaluated for its specificity and sensitivity using a number of gastroenteritis viruses, including GpA RV (CDC-9, CDC-66, Wa, RRV, DS-1, WC3, L26, P, W161, Rotarix, RotaTeq G1 and P[8], MW333, ST3, NCDV and OSU), GpB RV ADRV (stool), GpC RV (Cowden, Shintoku), Adenovirus-40 and 41, Echovirus-EV71 and ECHO11, and Human astrovirus. For validation of the assay, 100 fecal specimens from infants with diarrhea in Taiwan were tested. ELISA-positive specimens were further tested and confirmed by nested PCR using primers specific to human GpC RV VP6 and VP7 genes.

Results
We isolated 2 mAbs (clones 475 and 918) with specific reactivity against GpC RV, but not with other gastroenteritis viruses. mAb 918 was confirmed to be specific for human GpC VLP only and mAb 475 was found to react with human GpC VLP and porcine Cowden strain by ELISA. mAb 475 was mapped by dot blot hybridization to specifically react with GpC VP6 peptides (amino acids 182-207 and amino acids 287-311). This mAb was used as capture and detector to develop a new ELISA specific for the detection of GpC RVs (GpC VLP, Cowden, Shintoku). This assay showed a high sensitivity, with detection limit to GpC VLP of less than 1ng/mL ; and a high specificity, with no detection for Sf9 cell supernatant. The sensitivity was further confirmed with a detection limit of TCID50 of Cowden and the high specificity was confirmed with MA104 cell supernatant. We evaluated and validated this ELISA by testing 100 fecal samples from children with diarrhea in Taiwan and found 12 (12 %) positive for RV C, 8 samples (8%) were further confirmed by nested RT-PCR for VP6 or VP7 GpC.

Conclusion
We developed a simple and reliable diagnostic test that can be utilized to determine the disease burden of GpC RV in post GpA vaccine introduction era.