Evaluation of Molecular Assays for Norovirus Detection

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INTRODUCTION

- Norovirus (NoV) exerts a considerable clinical and financial impact upon healthcare globally
- Laboratory diagnosis must be prompt and accurate to reduce the impact of NoV; molecular tests are best placed to provide this
- **AIM:** compare different molecular options and assess a prototype point-of-care assay for NoV detection

METHODS

- One hundred and fifty six patient samples (87 female, 69 male) were tested by 3 methods and compared to the in house reverse-transcriptase real-time PCR (RT-PCR)
- All samples were faeces from individuals with diarrhoea; 137 were from hospital locations and 19 from primary care
- Samples were tested retrospectively during January to April 2013 (n = 131) and a small subset were tested prospectively from July 2013 (n = 25)
- An aliquot of liquid sample was suspended in 1 mL sterile water, centrifuged and then 200 µL of the supernatant was extracted on the easyMag (bioMérieux) and eluted into 100 µL. RT-PCR was then performed on the ABi 7500 (Applied Biosystems)
- The three evaluated test methods were:
  - BD MAX™ RNA Extraction Kit (Becton Dickinson) with PCR performed with Express one-step qRT-PCR kit (Life Technologies). Samples were processed as above prior to testing
  - xTAG® Gastrointestinal Pathogen Panel (GPP; Luminex). An aliquot of sample was placed in a Burton bead tube, vortexed and centrifuged prior to easyMAG extraction
  - Prototype GeneXpert® Norovirus Assay (Cepheid). A swab was inserted into the liquid stool, homogenised in sample reagent then transferred to the reaction cartridge and loaded onto the GeneXpert

Figure 1 Results of testing diarrhoeal samples (n =156) on three platforms. In house real-time RT-PCR was considered gold standard

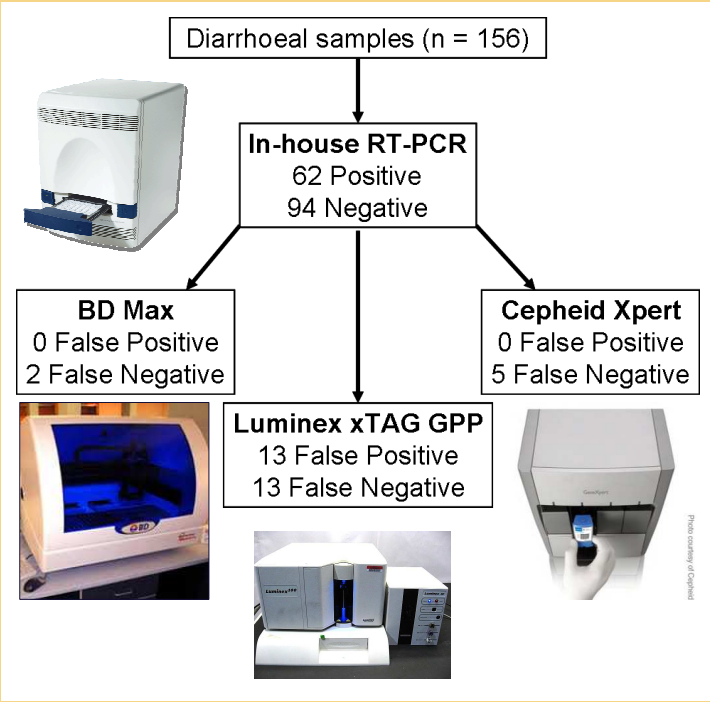

Table 1 Performance* of three commercial molecular Norovirus assays in patient samples (n = 156)

Test	Sensitivity	Specificity	NPV	PPV	Accuracy
BD MAX	96.8 (92.4 - 100)	100 (96.8 - 100)	97.9 (95.1 - 100)	100 (95.0 - 100)	98.7 (97.0 -100)
xTAG GPP	79.0 (68.9 – 89.2)	86.2 (79.2 – 93.1)	86.2 (79.2 – 93.1)	79.0 (68.9 – 89.2)	83.3 (77.5 – 89.2)
Xpert	91.9 (85.2 – 98.7)	100 (96.8 - 100)	94.9 (90.6 – 99.3)	100 (94.7 - 100)	96.8 (94.0 – 99.6)

*Presented as percentage with 95% confidence interval

RESULTS

- Results of laboratory testing are presented in Figure 1 and Table 1
- Of the 62 positive samples, 35 were GI and 27 were GII
- The BD Max had minimal hands-on-time, with results for 24 samples available 2h 30 min after receipt in the lab (2 h 10 min machine run time)
- The xTAG GPP required the most hands-on-time; results were available within 24 h for a full run of 86 test samples
- The Xpert required the least hands on time (3 min per sample), with machine run time of 1 h 30 min

CONCLUSIONS

- The BD Max and Xpert assays gave acceptable performance for routine diagnosis of NoV
- The xTAG GPP was not considered acceptable for routine use, although the wide range of pathogens detected may prove beneficial in specific situations
- The Xpert assay offered simple processing and timely results which may enable point-of-care use in patient areas
- Point-of-care testing will likely reduce the time to results in high-incidence clinical areas, allowing rapid initiation of infection control measures and outbreak response

ACKNOWLEDGEMENTS

We are very grateful to Becton Dickinson and Cepheid for providing the consumables for this work. Thanks to Juliet Kenicer, Alison Hardie and Li Lin for running the BD Max, xTAG GPP and Xpert systems. Thanks also to Pete McCulloch, Julie White and the RIE molecular virology staff for providing results of the in-house test.