MOLECULAR DETECTION OF ENTERIC VIRUSES: FAECAL SAMPLES VERSUS RECTAL SWABS

POSTER P1415
ECCMID 2014, BARCELONA

G. J. A. Eltringham

Newcastle Molecular Laboratory, Public Health England, Level 1 Medical School, Royal Victoria Infirmary, Newcastle-upon-Tyne, UK

INTRODUCTION

Acute gastroenteritis remains one of the most common human diseases and is a major cause of morbidity and mortality worldwide. Gastroenteritis can be caused by a variety of pathogens including bacteria, parasites and viruses, however, in around 40% of cases no microbiological cause can be identified.

Of the viruses that have been shown to cause diarrhoeal disease in humans, there are currently five that account for the majority of viral infections: rotaviruses, norovirus, sapovirus, astrovirus and enteric adenovirus.

The evolution of diagnostic methods has progressed through electron microscopy, cell-culture, and immunoassays to very sensitive molecular methods. Each has limitations such as sensitivity, some can be time consuming, and some require several steps or the use of potentially hazardous chemicals.

The increased sensitivity of molecular assays means that a greatly reduced initial sample volume may still be useful in pathogen detection. Therefore, investigations were undertaken to assess the suitability of a swab transport system rather than faeces for the diagnosis of viral gastrointestinal disease.

METHODS

Forty faeces samples (M01-M40), known to be positive for a variety of enteric viruses were tested using two triplex PCR assays. One targeting rotavirus, norovirus genogroup I and II, and the second, group F adenoviruses 40/41, sapovirus and astrovirus. A further singleplex assay was used to target an internal process control (MS2 bacteriophage). Positive and negative controls were included.

Each faeces sample was diluted 1:10 in phosphate buffered saline. A 200µl volume of each was inoculated into the Fecal Transwab® system (Medical Wire and Equipment), containing Cary Blair transport medium specifically developed for the detection of enteric bacteria. These samples were then held at room temperature for four hours, followed by overnight refrigerated storage in an attempt to simulate “real-life” specimen transportation and storage

A 200µl aliquot was then extracted using the bioMérieaux NucliSENS® easyMAG® automated extraction platform. A 5µl volume of each eluate was then applied to each PCR multiplex using the Applied Biosystems™ 7500 TaqMan Real-time PCR system (Life Technologies®). The results derived from these were compared with those of each faeces sample when previously tested using a routine pre-extraction and extraction method.

RESULTS

Following the completion of the PCR, the amplification traces and cycle threshold (Ct) values were examined for each of the targets, including the internal process control (IPC). Of the forty faeces samples that underwent the routine pre-extraction preparation, all forty gave a positive result: 10 rotavirus, 6 norovirus GI, 8 GII, 6 adenovirus, 4 astrovirus and 6 sapovirus. Thirty eight gave positive results for the same viral targets when using the Faecal Transwab® method with no pre-extraction preparation. Using the swab method, an astrovirus (Ct 27.8) and a sapovirus (Ct 20.6) were not detected. The MS2 IPC was detected in all forty samples, with both methods.

All samples that gave a positive result for a particular target, were identified by presence of a typical amplification curve. The positive and negative controls gave appropriate results, therefore, validating the PCR run. The Ct values are shown for each target in Tables 1. and 2., comparing the routine faeces sample protocol with that of the swab system.

Sample	Virus	Faeces Ct	Fecal Transwab® Ct
M12	Rota	17.9	16.6
M19	Rota	20.3	16.9
M20	Rota	16.5	22.2
M23	Rota	17.2	15.7
M24	Rota	17.2	15.6
M26	Rota	15.2	20.1
M29	Rota	18.2	19.1
M30	Rota	19.8	21.0
M32	Rota	18.4	17.7
M33	Rota	20.7	22.4
M06	Noro GI	26.4	20.1
M10	Noro GI	24.1	29.6
M22	Noro GI	20.2	32.5
M31	Noro GI	18.1	26.9
M34	Noro GI	29.1	33.1
M39	Noro GI	18.3	23.9
M01	Noro GII	15.7	25.8
M08	Noro GII	19.0	25.3
M11	Noro GII	24.2	18.7
M27	Noro GII	18.1	19.0
M35	Noro GII	18.2	27.2
M36	Noro GII	27.1	35.9
M37	Noro GII	24.4	28.5
M38	Noro GII	18.1	20.1

Table 1. Comparative PCR results for Rotavirus, Norovirus GI and Norovirus GII

Sample	Virus	Faeces Ct	Fecal Transwab® Ct
M02	Adeno	24.8	28.1
M04	Adeno	14.5	14.5
M07	Adeno	14.5	15.4
M14	Adeno	14.4	14.7
M15	Adeno	14.0	14.3
M16	Adeno	25.8	24.6
M05	Astro	15.4	17.0
M09	Astro	17.7	18.3
M18	Astro	20.8	20.9
M40	Astro	27.8	Undetected
M03	Sapo	25.1	25.1
M13	Sapo	20.6	Undetected
M17	Sapo	18.3	26.0
M21	Sapo	27.7	34.1
M25	Sapo	16.6	35.3
M28	Sapo	18.4	30.0

Table 2. Comparative PCR results for Adenovirus 40/41, Astrovirus and Sapovirus

DISCUSSION

On examination of the results using either the routine faecal extraction method or the Fecal Transwab® method, the same viral targets were detected in 38/40 samples. The two detected following the routine method only, one astrovirus and one sapovirus, gave Ct values (27.8 and 20.6 respectively) indicating a reasonably high concentration of virus in the sample.

The comparative Ct values of the 38 samples giving concordant positive results does not reflect any sensitivity issues when using the Faecal Transwab® system compared to the routine method of processing.

In two cases no target viruses were detected when using the swab method of sampling. It may be that any differences could be as a result of either virion distribution in non-homogeneous sample types, or be down to the fact that prolonged storage between the application of the two extraction methods may have led to sample degradation, rather than a reduction in sensitivity. A lack of sample material prevented any further investigation of this anomaly.

The detection of the internal process control in all samples, including those two where no virus could be detected, provides evidence that PCR inhibition was not encountered and that extracted template was added to each PCR reaction.

CONCLUSIONS

The use of molecular detection methods has greatly improved our ability to diagnose viral causes of enteric infections, with increased sensitivity allowing for sample processing to be rationalised. The use of swab based systems reduces the amount of sample required and can negate the need to perform pre-extraction procedures that may be time consuming, costly and involve hazardous chemicals.

Although sensitivity may look to be slightly compromised when compared to routine methods, this may be due to the nature of the sample, or inappropriate storage of the positive material containing original samples used to simulate real-life specimens, rather than the sampling process.

ACKNOWLEDGEMENTS

Professor John Magee – Public Health England, Newcastle Laboratory
Dr Andrew Sails – Public Health England, Newcastle Laboratory
Medical Wire and Equipment