Comparison of different sampling types for the detection of rhinovirus infections using quantitative RT-PCR

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Aim of the study

Obtaining nasal swabs from children is simple and well tolerated for repeated sampling. In this study, we made quantitative comparisons between different sampling types using RT-PCR with real-time amplification to detect human rhinoviruses (HRVs).

Specimen collection

The study was carried out during a peak incidence of HRV infections in the community in September–October 2006. Children attending the paediatric clinic of Turku University Hospital and manifesting symptoms of viral respiratory infection were eligible for the study. The protocol was approved by the Ethics Committee of Turku University Hospital. Informed consent was obtained from the parents of the children. Following samples (Figure 1) were collected in the indicated order:
1. Nasal swab using wood-shafted cotton swab.
2. Nasal swab using Copan Pernasal flocked swab (Copan Italia spa, Brescia, Italy).
3. Throat swab using wood-shafted cotton swab.
4. Nasopharyngeal aspirate (NPA).

Results

Of 39 children participating in the study, 29 had HRV infection (Table 1) and virus was detected in all but one of the samples obtained. Quantitative comparisons between sample types were made for 24 complete sets of samples obtained from children with HRV infection (Table 2 and Figure 2). The HRV1b extraction controls had a mean±SD value of 5.9±0.1 log(copies/ml) in 14 runs, indicating high repeatability.

In average, mailed aspirate or cotton nasal swab had 3-fold less copies/sample (p<0.01) than fresh aspirate, a minimal difference from the diagnostic point of view. The time in mail, 1–5 days (mean 2.7 days) did not correlate with the drop of viral copies. To our surprise, rhinovirus was detected in all but one of the 24 throat swabs, although the average amount of virus was 25-fold less (p<0.0001) than in fresh aspirate. There was no difference in virus yield between fresh aspirate and Copan swab.

Since the Copan “Pernasal” swab is smaller and softer with a flexible shaft as compared to the cotton swab, it was the most well tolerated of the sample types used.

Methods

All samples were tested in batches, including each sample from a patient in the same run of nucleic acid extraction, reverse transcription and real-time PCR.

Extraction of total nucleic acids. Swab was suspended into 1 ml of phosphate buffered saline by vortex mixing and 500 μl of the suspension was processed using Nuclisense easyMag automated extractor (BioMérieux) with an elution volume of 55 μl. An extraction control of HRV1b with a target copy number of 10^6 copies/ml was included in each run.

Reverse transcription (RT). RNA in 10 μl of the nucleic acid extract was reverse transcribed with MMLV RNase H− reverse transcriptase (Promega) and piconavirus antisense (−1) primer in a total volume of 40 μl as described by Löhrot et al. 1999 A standard preparation of HRV16 RNA (10^4 copies/reaction) was included doubly in each run of RT-reactions.

Real-time PCR. An aliquot of 5 μl of the cDNA from the RT reaction was amplified with QuantiTect SYBR Green master mix (Qiagen) and piconavirus sense (3+) and antisense (−1) primers using Rotor Gene 3000 instrument (Corbett Research) as described by Peltola et al. 2008.

Analysis. Melting curve analysis was used to confirm the initial specificity of the amplifications. Later, the HRV specificity of the cDNA from each patients NPA sample was confirmed by sequencing of a larger PCR fragment as described in Peltola et al. 2008. The threshold cycle (Ct) values were compared to those of the HRV16 RNA standard for copy number calculation using pre-determined amplification efficiency (93.3%). Paired two sample t-test for means of log(copies/RT-sample) was used for statistical analysis.

Conclusions

1. Nasal swab is a reliable sample for the diagnosis of HRV infections by RT-PCR.
2. The sample can be mailed in ambient temperature with a minimal loss of detectable virus.
3. HRV can be frequently detected in throat swab by RT-PCR.
4. The flocked Copan swab combines high sensitivity with patient friendliness for the optimal sampling method for HRV diagnostics.

References


Table 1. RT-PCR findings in the study children.

<table>
<thead>
<tr>
<th>Patient status</th>
<th>No.</th>
<th>Age (median [range])</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRV+, all samples obtained</td>
<td>24</td>
<td>1.4 (0.3–15.4)</td>
</tr>
<tr>
<td>HRV+, missing sample</td>
<td>5</td>
<td>1.4 (0.2–1.6)</td>
</tr>
<tr>
<td>Enterovirus-</td>
<td>4</td>
<td>1.2 (0.9–3.7)</td>
</tr>
<tr>
<td>Piconavirus-</td>
<td>6</td>
<td>1.4 (0.8–8.2)</td>
</tr>
<tr>
<td>All patients</td>
<td>39</td>
<td>1.4 (0.2–13.4)</td>
</tr>
</tbody>
</table>

Table 2. Average yields of HRV in different sample types (n=24).

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Mean±SD of calculated log(copies) in RT-PCR sample/swat</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPA</td>
<td>6.2±1.1</td>
</tr>
<tr>
<td>Mailed NPA</td>
<td>5.8±1.3</td>
</tr>
<tr>
<td>Cotton swab</td>
<td>5.8±1.2</td>
</tr>
<tr>
<td>Copan swab</td>
<td>6.3±1.0</td>
</tr>
<tr>
<td>Throat swab</td>
<td>4.8±1.4</td>
</tr>
</tbody>
</table>

Figure 1. Sample collection devices. From top: Copan swab, cotton swab, and NPA mucus extractor.

Figure 2. Box plot of RT-PCR results in different sample types. P values are given for significant differences in paired comparisons to NPA by T-test (2-tail).