A New Method for Sampling and Detection of Exhaled Respiratory Virus Aerosols

Kerrianne N. Huynh,1 Brian G. Oliver,2,3 Sacha Stelzer,7 William D. Rawlinson,2 and Euan R. Tovey1,3

1Faculty of Medicine, University of Sydney, 2South Eastern Area Laboratory Services, Prince of Wales Hospital, and 3Woolcock Institute of Medical Research, Sydney, Australia

We have developed a mask sampler for exhaled respiratory viruses. Among a group of 9 patients with cold symptoms who had virus-positive nasal mucus specimens, as analyzed by multiplexed polymerase chain reaction, virus-positive mask samples were obtained after coughing (20 times), talking (20 min), or breathing (20 min) from 6, 5, and 3 patients, respectively.

Despite >70 years of research, the modes of transmission of influenza and other common respiratory viruses remain the subject of debate. The recent heightened awareness of a possible human influenza pandemic has identified a number of research gaps in our understanding of these modes of transmission that affect precautionary planning for both clinical and public settings. These research gaps include questions regarding whether there is significant presymptomatic or asymptomatic spread, the relative contribution of differently sized aerosols to total transmission, and how a virus with an R0 of <2 spreads, despite numerous interventions [1–3].

Addressing these gaps requires better detection and characterization of exhaled virus aerosols produced by infected patients. To our knowledge, there are no reports on the quantification of personal virus aerosols generated by individual infected patients, and only a single recent report directly quantified the personal aerosols of infected patients, and only a single recent report directly quantified the personal virus aerosols generated by individual infected patients. To our knowledge, there are no reports on the quantification of exhaled virus aerosols produced by infected patients.

To explore the exhalation of virus aerosols generated by upper respiratory events, such as coughing, talking, and breathing, we developed a novel mask-like sampling device using electret, which is suitable for analysis by PCR.

Methods and materials. Twenty young adult volunteers (4 male and 16 female), with a mean duration of symptoms of 3.3 days, were selected using a modified common cold questionnaire (see online appendix[8]). They each provided a sample of nasal mucus and then wore a separate sampling mask on each of the following 3 occasions: 20 min of reading aloud, 20 min of quiet breathing, and 20 voluntary coughs over a 3–5-min period. These times were a compromise between the time that was anticipated to be sufficient and the time that would be tolerated by the patient. At no time during sampling did patients or researchers touch the collection surface of the mask with their gloved hands; only the ties used to hold the mask to the face were touched. Volunteers who had no symptoms of a cold for at least the previous 2 weeks (n = 3) were recruited as negative control subjects. The study was approved by the University of Sydney Human Ethics Committee.

The prototype sampling device, shown in figure 1, consisted of a handmade, close-fitting, half-face mask made from impermeable, stretchable PVC that contained a central section of electret (diameter, 25 mm; ETR115; Japan Vilene) that was located opposite of the nose and mouth region. This type of electret was chosen after comparison of >80 brands. It collected >80% of 0.52 μm latex particles (virus droplet proxy) and had low airflow resistance (<15 mm H2O) at normal respiratory flows. After sampling, the electret was immediately removed from the mask and was placed in 1 mL of RNA lysis buffer (Qiagen); it was then transported at −80°C to the laboratory (Faculty of Medicine, University of Sydney, Sydney, Australia), and RNA was isolated using the Qiagen RNeasy Mini Kit (Qiagen) according to the manufacturers instructions. Following
conversion to cDNA, rhinovirus and respiratory syncytial virus were assessed by an in-house PCR assay, as described elsewhere [9], using 50 cycles of amplification. Subsamples from 9 patients who had more-severe symptoms were also tested for the presence of influenza A and B virus, parainfluenza virus 1, 2, and 3, respiratory syncytial virus, and human metapneumovirus using a single-round, in-house multiplexed PCR assay, based on previously described primers [10, 11]. Results were confirmed using specific biotin-labelled oligonucleotides in a multiplexed assay, as described elsewhere [11, 12]. Additional details of PCR methods are provided in the online appendix.

**Results.** The detection of viruses in the different samples by PCR is shown in table 1. Of the 20 patients who had samples tested, 9 had virus-positive nasal mucus samples. Rhinovirus was detected in samples from 6 of 20 patients, influenza A virus was detected in samples from 2 of 9 patients, and parainfluenza virus 3 was detected in samples from 2 of 9 patients. Parainfluenza viruses 1 and 2, respiratory syncytial virus, and human metapneumovirus were not detected in any of the samples. Of the 9 patients who had virus-positive mucus samples, 6 had ≥1 virus-positive aerosol sample. Six patients had virus-positive samples from coughing, 5 from talking, and 3 from breathing. The aerosol sample from patient 6 tested positive for both rhinovirus and parainfluenza, although the mucus sample from this patient was only positive for rhinovirus. None of the asymptomatic subjects had a sample that tested positive for virus. There were no major differences in detection of viruses between male and female subjects.

**Discussion.** This small study is, to our knowledge, the first to report direct detection of virus aerosols exhaled by infected individuals. The study reveals that breathing alone, for some people, is sufficient to aerosolize rhinovirus. Although coughing, talking, and sneezing have conventionally been associated with the generation of virus aerosols [5]—an association that was confirmed in our study—generation by breathing alone has not been previously indicated for humans. Whether this is confined to rhinovirus is not known, because only a few samples were examined in our study. However, breathing by pigs has been recognized as generating the aerosols of the virus that is responsible for foot-and-mouth disease [13]. Other studies have revealed that normal breathing by many people generates a substantial aerosol of particles ∼150 nm in diameter [14]. This suggests that different respiratory events may generate different sized particles, which may carry virus, and provide different opportunities for transmission.

We evaluated electret to sample these bioaerosols, because it combines the characteristics of high collection efficiency for small droplets with little interference to breathing. The electrostatic attraction of electret that facilitates this collection is

<table>
<thead>
<tr>
<th>Patient</th>
<th>Virus detected</th>
<th>Nasal mucus</th>
<th>Coughing</th>
<th>Talking</th>
<th>Breathing</th>
<th>Day of illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rv</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Rv</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Rv</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Rv</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Rv</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Para</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Para</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Flu</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>Flu</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>...</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**NOTE.** Flu, influenza virus; –, negative; Para, parainfluenza 3 virus; +, positive; Rv, human rhinovirus.

<sup>a</sup> Mean value.
deactivated by the viral RNA lysis buffer, permitting recovery and analysis. Previous attempts to collect microbial aerosols have used a variety of methods, including impaction [4], impingers, and gelatine or nucleopore air filters attached to air pumps [15]. To our knowledge, electret has not previously been used for sampling. Our application provides a sampler that is intuitive to use, safe, simple, noninvasive, suitable for a wide range of age groups, easily stored, and inexpensive. Use of the sampler is safer for clinical staff than are current procedures that require handling of secretions. The combination of a mask with multiplexed PCR provides a tool with a wide range of applications in the diagnosis and management of respiratory infections.

A possible confounder of this study is that the collection devices may have been accidentally contaminated with viral RNA from contact with facial skin. However, this was unlikely, because current studies using a more rigid mask design in which the electret is held about 1 cm from the face, have also detected such aerosols (A. Blazey, personal communication). Although these results are preliminary, the potential applications of this method to explore aerosol transmission justify its early publication.

There are many unanswered questions. We do not know the size range of the particles that were produced and collected or the absolute quantities of virus that were expired over time by the different respiratory events, the extent that virus was shed via a nasal or oral route, the site within the respiratory tract from which aerosols were generated, or whether the positive PCR assay results represented infective virus. However, this study provides a framework within which these questions can be addressed.

In summary, we have revealed that a simple mask-like device can be used to sample exhaled respiratory bioaerosols for analysis by PCR. Preliminary experiments suggest that viral aerosols are common, and these findings support a greater role and diversity of opportunities for aerosol transmission. This study may provide a new method for screening populations for the presence of bioaerosols from the respiratory tract and permit new studies of microbial pathogenesis and transmission.

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Potential conflict of interest. E.R.T. is cited as an inventor of the sampler in a provisional patent application assigned to the Woolcock Institute of Medical Research. All other authors: no conflicts.

References