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**TITLE: Electrostatic wipes as simple and reliable methods for influenza virus airborne detection**

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**Running title:** airborne flu detected by electrostatic wipes

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## ABSTRACT

The performance of an in-house protocol for virus detection on commercialized electrostatic wipes (EW) was assessed experimentally by impregnating them with suspensions of cytomegalovirus, adenovirus and influenza virus, and by determining the recovery efficiency, repeatability, and detection limit of the protocol. The protocol was sensitive enough to detect 4 log<sub>10</sub> gene copies of virus. At room temperature, influenza RNA was stable on EW during at least 4 days. When EW were placed high in 32 influenza-infected patients' rooms, influenza RNA was detectable in 75% (n=24) of EW, suggesting that EW are simple and reliable methods for influenza virus airborne detection.

## INTRODUCTION

Transmission of respiratory viruses is mediated by large secreted droplets (> 5 µm), released by cough, sneeze, talk or breath, that fall rapidly by gravitational settling in front of the infected subject, and by small aerosol particles (also called *droplet nuclei*, < 5 µm) which remain suspended longer in air and spread over greater distances [1].

Aerosol transmission of common respiratory viruses, such as influenza virus, is often neglected in healthcare setting compared to droplet transmission, although growing evidence supports its role in epidemic and/or nosocomial spread of viruses [2–4].

One hurdle to overcome for identifying and managing aerosol transmission is the lack of simple and inexpensive samplers for airborne viruses. Here, we assessed the relevance of commercialized electrostatic wipes (EW) as simple and reliable supports for the detection of

virus-containing aerosols. We particularly characterized the performance of an in-house protocol for virus elution and detection from this support and investigated its possible application during a seasonal flu outbreak through a pilot study.

## **METHODS**

### **Virus preparation**

Viral stocks of cytomegalovirus (CMV) and adenovirus (ADV), dedicated to validate the performance of our protocol for virus detection on EW, were obtained by virus propagation on MRC5 fibroblasts as previously described [5]. Influenza A virus (IAV) was obtained from a respiratory sample, collected for routine molecular analysis in our institution. Viral nucleic acid extraction and amplification by (RT)-PCR were performed using commercial kits listed below.

### **Study population of the pilot study**

Detection of IAV-containing aerosols on EW was performed in the hospital rooms of influenza-infected patients in the Departments of Geriatrics and Internal Medicine, Besançon University Hospital, France. All patients were informed of the presence of the wipes and agreed. Since the presence of the wipes did not interfere with patient care, the local ethics committee was not consulted for this pilot study.

### **Electrostatic wipes contamination and exposure**

Four types of commercialized EW (surface 315 cm<sup>2</sup>) were tested (brands APTA®, CEDAR®, TOUS LES JOURS®, and CASINO®) for virus detection. To validate the protocol, EW were

impregnated with suspensions of CMV, ADV or IAV, dried at room temperature from 1 to 4 days depending on the experiment, and stored at -80°C until viral nucleic acid extraction. For the pilot study, EW were placed in open plastic boxes, 1.80 m above the headboard, in influenza-infected patients' rooms during the acute phase of infection (< 48h after symptom onset). After 48h of exposure, EW were collected and stored in their plastic boxes at -80°C. Plastic boxes were disinfected (Surfanios®, Anios®, Lille-Hellemmes, France) after each use and residual contamination by IAV RNA was checked by swabbing the plastic surface and performing an influenza-specific RT-PCR.

#### **Viral elution from electrostatic wipes and nucleic acid amplification**

After viral exposure or experimental contamination, each wipe was put in a sealed plastic bag with 5 mL of a washing solution (PBS) containing 0.1% Tween 80 (Merck®, Darmstadt, Germany) and shaken for 10 minutes in a Stomacher® (AES®, Combourg, France). Two (± 0.5) mL of washing solution was collected and centrifuged (60 minutes; 9500 g; 4°C). Viral RNA and DNA were extracted from 500 µL of the pellet using the Nucleospin® RNA virus and the NucleoSpin® DNA blood (Macherey-Nagel) kits, respectively, and amplified by (RT)-PCR using the R-DiaFlu® (Diagenode), CMV R-gene® (Biomérieux) and Adenovirus R-gene® (Biomérieux) kits. Since our protocol includes a detergent solution (washing solution) that may greatly impact the infectivity of viruses (especially in the case of enveloped viruses), we did not try to isolate the biologically active viruses by cell culture after the elution step.

## RESULTS AND DISCUSSION

### Analytical performance of the protocol

To estimate the **recovery efficiency** and the **repeatability** of our protocol, 4 types of commercialized EW were impregnated in quadruplicate with 100  $\mu$ l of a suspension of CMV ( $5.87 \log_{10}$  copies/ml) or ADV ( $5.86 \log_{10}$  copies/ml) and dried at room temperature for one day until virus elution and DNA extraction. CMV and ADV were chosen as model for enveloped and non-enveloped viruses, respectively, to validate the protocol due to their ease of use, propagation in cell culture and quantification. The mean concentrations of CMV DNA and ADV DNA eluted from these 16 wipes were  $4.48 \log_{10}$  copies/ml (average loss of CMV DNA by the elution protocol =  $-1.39 \log_{10}$  copies/ml; concentration ratio = 76.3%) and  $4.21 \log_{10}$  copies/ml (average loss of ADV DNA =  $-1.65 \log_{10}$  copies/ml; concentration ratio = 71.7%), respectively. Standard deviations (SD) between the 16 measures were  $0.21 \log_{10}$  copies/ml (coefficient of variation CV = 4.79%) and  $0.29 \log_{10}$  copies/ml (CV = 6.85%) for CMV and ADV, respectively, resulting in a mean SD of  $0.25 \log_{10}$  copies/ml (CV = 5.82%). To confirm these results for IAV, 2 types of wipes were impregnated in triplicate with a suspension of IAV ( $5.11 \log_{10}$  copies/ml). The average loss of IAV RNA during the elution protocol was  $-1.27 \log_{10}$  copies/ml (concentration ratio = 75.1%), and SD between the measures was  $0.27 \log_{10}$  copies/ml (CV = 7.05%).

To estimate the **detection limit** of our protocol, EW were impregnated with serial dilutions of CMV, ADV or IAV suspensions, with amounts ranging from 2 to 6  $\log_{10}$  gene copies of viral nucleic acid, in 7 distinct trials, and dried for one day at room temperature prior to viral elution and nucleic acid extraction. The detection limit was defined as the smallest amount

of nucleic acid detectable in 7/7 trials [6]. With our protocol, this limit was obtained at 4.7 log<sub>10</sub> gene copies (50 000 gene copies), for both CMV and ADV suspensions, and 4 log<sub>10</sub> gene copies (10 000 gene copies) for IAV (Table I).

Since IAV has only a brief survival on porous surfaces [7], we aimed to estimate the duration of influenza RNA **stability** on the EW. For that purpose, two types of EW (brands APTA® and CEDAR®) were impregnated with a suspension of IAV (5.11 log<sub>10</sub> copies/ml) and dried at room temperature from 1 to 4 days. The amount of influenza RNA eluted from the EW did not significantly differ during this period, suggesting a good stability of the viral RNA on EW during at least 4 days (Figure 1).

EW have been proposed as interesting surrogates for the assessment of indoor airborne microbial exposure (i.e., with bacteria and fungi) [8]. However, no data is currently available for virus detection on EW and no comparison with other sampling methods has been performed. Comparing the performance between available samplers for airborne viruses is impeded by the variety of their designs (mainly based on impaction, liquid impingement, filtration, and electrostatic precipitation of viral particles [1]) and by the lack of standardized performance criteria. Repeatability, recovery efficiency, detection limit, and RNA stability, were chosen as main parameters to validate the performance of our in-house protocol for virus detection on EW. Since the repeatability of our protocol was close to the required repeatability of PCR assays dedicated to human diagnostic (SD < 0.25 log<sub>10</sub> copies/ml according to the French guidelines [9]) we conclude in suitable repeatability of our technique. A relevant sampler for virus-containing airborne particles should be sensitive enough to detect the infectious doses of virus inhaled by exposed subjects. Previous work has estimated that the inhalation doses of IAV ranged from 1.35 x 10<sup>4</sup> to 1.07 x 10<sup>5</sup> gene

copies of virus for exposures of 1h to 8h [10]. With an observed detection limit at  $1 \times 10^4$  gene copies of viral RNA, we suggest that our protocol is sensitive enough to detect airborne infectious doses of IAV. This suggestion was further confirmed through a pilot study in influenza-infected patients' rooms.

#### **Pilot study in influenza-infected patients' rooms**

Thirty-two EW were placed in the hospital rooms of influenza-infected adult patients (mean age = 85 years, range: 42 – 101 years), during the acute phase of infection (< 48h after symptom onset), high enough to exclude any droplet contamination (1.80 m above the headboard). After 48h of exposure, IAV RNA was detectable in 75% (n=24) of EW. The mean concentration of IAV RNA eluted from these wipes was  $3.36 \log_{10}$  copies/ml, ranging from 2.14 to  $4.43 \log_{10}$  copies/ml. This result suggests that our method detects IAV-containing aerosols in a hospital setting.

Several methods have been proposed to detect airborne influenza in healthcare settings. By using NIOSH cyclone or 6-stage Andersen samplers, IAV was detectable in 17.4% to 50% of samples obtained in emergency departments or in influenza-infected patients' rooms [2–4]. Most of these studies highlighted a high proportion of influenza RNA in aerosols < 4  $\mu\text{m}$ , suggesting an important part of small aerosols during IAV transmission. Our technique does not measure the size distribution of virus-containing airborne particles or the fraction of biologically active virus inside, and it requires a longer time of exposure than cyclone-based methods. However, its low cost and ease of use make it an attractive alternative to traditional samplers for monitoring airborne IAV contaminations.



In summary, experimental performance of virus detection on EW argue in favor of their use as simple, reliable and useful methods to detect and monitor airborne respiratory virus contaminations in healthcare settings.

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**COMPETING INTERESTS:** None declared

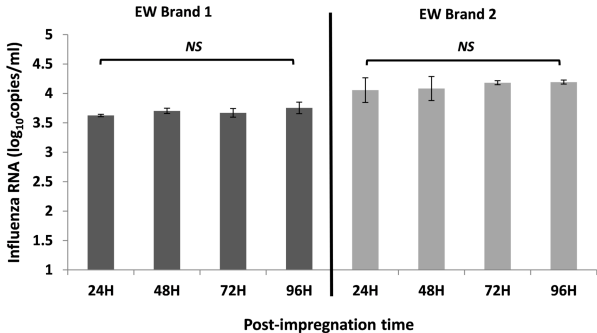
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## TABLES AND FIGURES

**Table I: Detection limit of viruses on electrostatic wipes.** EW were impregnated with suspensions of CMV, ADV and IAV, containing from 2 to 6 log<sub>10</sub> gene copies of viral nucleic acid. The detection limit was defined as the smallest amount of DNA detectable in 7/7 trials [6].

**Figure 1: Stability of influenza RNA on electrostatic wipes.** Two brands of EW were impregnated with a suspension of IAV and stored at room temperature from 24 to 96h prior to IAV RNA detection.



**Table I: Detection limit of viruses on electrostatic wipes.**

| <b>Viral DNA or RNA amount<br/>(log<sub>10</sub> copies)</b> | <b>Samples<br/>(n)</b> | <b>CMV-positive wipes<br/>(n ; %)</b> | <b>ADV-positive wipes<br/>(n ; %)</b> | <b>IAV-positive wipes<br/>(n ; %)</b> |
|--|------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| <b>6</b>   | 7                      | 7 (100%)                              | 7 (100%)                              | <i>Not tested</i>                     |
| <b>5</b>   | 7                      | 7 (100%)                              | 7 (100%)                              | 7 (100%)                              |
| <b>4.7*</b>  | <b>7</b>               | <b>7 (100%)</b>                       | <b>7 (100%)</b>                       | 7 (100%)                              |
| <b>4</b>   | 7                      | 5 (71.4%)                             | 6 (85.7%)                             | <b>7 (100%)</b>                       |
| <b>3</b>   | 7                      | 2 (28.6%)                             | 2 (28.6%)                             | 3 (42.9%)                             |
| <b>2</b>   | 7                      | 0 (0%)                                | 0 (0%)                                | 0 (0%)                                |

\*4.7 log<sub>10</sub> copies = 50 000 copies