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

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10

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

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22 **ABSTRACT**

23 The performance of an in-house protocol for virus detection on commercialized electrostatic
24 wipes (EW) was assessed experimentally by impregnating them with suspensions of
25 cytomegalovirus, adenovirus and influenza virus, and by determining the recovery efficiency,
26 repeatability, and detection limit of the protocol. The protocol was sensitive enough to
27 detect 4 log₁₀ gene copies of virus. At room temperature, influenza RNA was stable on EW
28 during at least 4 days. When EW were placed high in 32 influenza-infected patients' rooms,
29 influenza RNA was detectable in 75% (n=24) of EW, suggesting that EW are simple and
30 reliable methods for influenza virus airborne detection.

31

32 **INTRODUCTION**

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

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

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

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

46

47 **METHODS**

48 **Virus preparation**

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

55 **Study population of the pilot study**

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

61 **Electrostatic wipes contamination and exposure**

62 Four types of commercialized EW (surface 315 cm²) were tested (brands APTA[®], CEDAR[®],
63 TOUS LES JOURS[®], and CASINO[®]) for virus detection. To validate the protocol, EW were

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

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

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

83

84

85

86 RESULTS AND DISCUSSION

87 Analytical performance of the protocol

88 To estimate the **recovery efficiency** and the **repeatability** of our protocol, 4 types of
89 commercialized EW were impregnated in quadruplicate with 100 μl of a suspension of CMV
90 ($5.87 \log_{10}$ copies/ml) or ADV ($5.86 \log_{10}$ copies/ml) and dried at room temperature for one
91 day until virus elution and DNA extraction. CMV and ADV were chosen as model for
92 enveloped and non-enveloped viruses, respectively, to validate the protocol due to their
93 ease of use, propagation in cell culture and quantification. The mean concentrations of CMV
94 DNA and ADV DNA eluted from these 16 wipes were $4.48 \log_{10}$ copies/ml (average loss of
95 CMV DNA by the elution protocol = $-1.39 \log_{10}$ copies/ml; concentration ratio = 76.3%) and
96 $4.21 \log_{10}$ copies/ml (average loss of ADV DNA = $-1.65 \log_{10}$ copies/ml; concentration ratio =
97 71.7%), respectively. Standard deviations (SD) between the 16 measures were $0.21 \log_{10}$
98 copies/ml (coefficient of variation CV = 4.79%) and $0.29 \log_{10}$ copies/ml (CV = 6.85%) for
99 CMV and ADV, respectively, resulting in a mean SD of $0.25 \log_{10}$ copies/ml (CV = 5.82%). To
100 confirm these results for IAV, 2 types of wipes were impregnated in triplicate with a
101 suspension of IAV ($5.11 \log_{10}$ copies/ml). The average loss of IAV RNA during the elution
102 protocol was $-1.27 \log_{10}$ copies/ml (concentration ratio = 75.1%), and SD between the
103 measures was $0.27 \log_{10}$ copies/ml (CV = 7.05%).

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

108 of nucleic acid detectable in 7/7 trials [6]. With our protocol, this limit was obtained at 4.7
109 \log_{10} gene copies (50 000 gene copies), for both CMV and ADV suspensions, and 4 \log_{10} gene
110 copies (10 000 gene copies) for IAV (Table I).

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

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

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

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

136 Thirty-two EW were placed in the hospital rooms of influenza-infected adult patients (mean
137 age = 85 years, range: 42 – 101 years), during the acute phase of infection (< 48h after
138 symptom onset), high enough to exclude any droplet contamination (1.80 m above the
139 headboard). After 48h of exposure, IAV RNA was detectable in 75% (n=24) of EW. The mean
140 concentration of IAV RNA eluted from these wipes was $3.36 \log_{10}$ copies/ml, ranging from
141 2.14 to $4.43 \log_{10}$ copies/ml. This result suggests that our method detects IAV-containing
142 aerosols in a hospital setting.

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

152

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

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157

158 **COMPETING INTERESTS:** None declared

159

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- 188

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191 **TABLES AND FIGURES**

192 **Table I: Detection limit of viruses on electrostatic wipes.** EW were impregnated with
193 suspensions of CMV, ADV and IAV, containing from 2 to 6 log₁₀ gene copies of viral nucleic
194 acid. The detection limit was defined as the smallest amount of DNA detectable in 7/7 trials
195 [6].

196

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

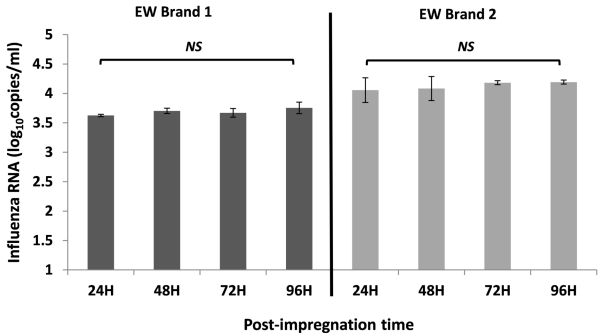


Table I: Detection limit of viruses on electrostatic wipes.

Viral DNA or RNA amount (log₁₀ copies)	Samples (n)	CMV-positive wipes (n ; %)	ADV-positive wipes (n ; %)	IAV-positive wipes (n ; %)
6	7	7 (100%)	7 (100%)	<i>Not tested</i>
5	7	7 (100%)	7 (100%)	7 (100%)
4.7*	7	7 (100%)	7 (100%)	7 (100%)
4	7	5 (71.4%)	6 (85.7%)	7 (100%)
3	7	2 (28.6%)	2 (28.6%)	3 (42.9%)
2	7	0 (0%)	0 (0%)	0 (0%)

*4.7 log₁₀ copies = 50 000 copies