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## Influence of preanalytical parameters on influenza and respiratory syncytial virus molecular detection

Claire Grolhier, Charlotte Pronier, Zahida Mamou, Christopher Heslan,  
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1 **Influence of preanalytical parameters on influenza and respiratory syncytial virus**  
2 **molecular detection**

3 Claire Grolhier<sup>1</sup>, Charlotte Pronier<sup>2</sup>, Zahida Mamou<sup>1</sup>, Christopher Heslan<sup>1</sup>, Vincent Thibault<sup>2</sup>

4 <sup>1</sup> *Univ Rennes, Department of Virology, CHU Rennes, F-35000 Rennes, France*

5 <sup>2</sup> *Univ Rennes, Department of Virology, CHU Rennes, Inserm, EHESP, Irset (Institut de*  
6 *recherche en santé, environnement et travail) - UMR\_S 1085, F-35000 Rennes, France*

7 **Corresponding author:** Pr Vincent THIBAUT email: [vincent.thibault@chu-rennes.fr](mailto:vincent.thibault@chu-rennes.fr)

8 Laboratoire de Virologie

9 CHU de Rennes

10 2 rue Henri le Guilloux

11 35033 Rennes cedex 9

12 FRANCE

13 **Word count: 491**

14 **Highlights**

- 15 • Viral transport media are required for the molecular diagnosis of respiratory viral  
16 infections
- 17 • Due to high demands, shortage of viral transport media requires to switch from  
18 different manufacturers
- 19 • Performances of virus molecular detection may be influenced by preanalytical  
20 conditions such as storage time and temperature in different media

21 • Comparison of 3 different viral transport media indicates that storage up to 72h at  
22 room temperature decreases detection sensitivity and 4°C storage should be  
23 recommended

24 • **Conflict of interest**

25 • The authors report no conflict of interest related to this work.

26 • All reagents were provided by Cepheid. This work was conducted independently to  
27 the manufacturer.

28

29

30 **Letter to the editor,**

31 In the context of the COVID-19 pandemic, shortage of material forced laboratories to turn to  
32 diverse alternative solutions. Confronted to this situation for virus transport media (VTM), we  
33 conducted a study to evaluate the influence of pre-analytical parameters on respiratory virus  
34 molecular detection (1, 2). Fifteen specimens were identified through Allplex™ Respiratory  
35 panel-1 assay (Seegene Eurobio), 5 influenza A virus (H3:FA1-3 and H1:FA4-5 subtypes)  
36 positive, 5 influenza B virus positive (FB1-5), 3 RSV-A (VA1-3) and 2 RSV-B (VB1-2)  
37 positive. Samples were selected within a defined Ct range of 21-34 Ct. Three VTM were  
38 evaluated: ESwab®, Virocult® and Cepheid® according to the study design (Figure 1).  
39 Samples were kept under different temperature conditions for up to 72 hours and tested at  
40 each time point using Xpert Xpress Flu / RSV assay (Cepheid). For each VTM, the reference  
41 Ct value is the one obtained at initial testing (**T0**). The dCt was calculated by comparing the  
42 obtained values under each condition compared to the T0 values: H4-T0; H24 [+4°C]-T0;

43 H72 [+4°C]-T0 and H72 [AT]-T0 (figure 2 and table 1). A dCt over 2 was considering  
44 significant (3).

45 For FluA, the most divergent Ct were observed with Eswab (72h-AT) with 5 values over the  
46 defined cut-off of 2 Ct. Less divergent values were observed for shorter time points and lower  
47 temperatures. For FluB and RSV, the dispersion was less striking than for FluA and only one  
48 divergent point (FluB/Virocult and RSV/Cepheid) was observed for each virus, both after  
49 storage at AT for 72h. When comparing results for all detected viruses tested at H4, storage  
50 for 72h at 4°C or AT led to more results exceeding the defined cut-off (n=4 p=0.03 or 10  
51 p<0.001).

52 Qualitatively, the concordance was 100% whatever the VTM, the storage period and  
53 temperature, and no false negative was observed. While the sample Ct suffered only little  
54 variations over time for each VTM, significant differences were mostly observed after storage  
55 for 72 hours. It is therefore advisable to respect the supplier's recommendations and to  
56 analyze the sample before 72h. Noteworthy, the signal obtained for *influenza A* virus was the  
57 most sensitive to duration or temperature of storage but no evident explanation could be  
58 raised for this observation.

59 To our knowledge, this proof of concept study is the first to evaluate Xpert Flu/RSV  
60 performance to detect influenza virus and RSV under several temperatures and delay storage  
61 using E-swab® and Virocult® as alternative VTM for Xpert Xpress Flu/RSV testing.  
62 However, storage of samples for 72 hours could possibly lead to false negative results.

63 Due to the limited number of samples, covering a narrow range of viral loads, this study  
64 should be considered exploratory and be reinforced by complementary studies including virus  
65 inactivating VTM and SARS-CoV-2 detection (4). Indeed, the use of a VTM capable of viral  
66 inactivation, such as Copan eNat, constitutes a real asset (5). Preliminary data obtained using

67 the inactivating media Sun-Trine Red with the Xpert Xpress ® Flu/RSV/SARS-CoV-2 assay  
68 are encouraging.

69

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70 **Declarations**

71 **Ethics approval and consent to participate:**

72 Not applicable

73 **Consent to publish**

74 The corresponding author has obtained all author's approvals to publish this work.

75 **Availability of data and material**

76 Yes, upon request

77 **Conflict of interest**

78 The authors report no conflict of interest related to this work.

79 **Funding source**

80 All reagents were provided by Cepheid, Inc.

81 **Authors' contributions**

82 ZM, CH, CG performed the experiments. CG, CP and VT performed the literature review,  
83 and wrote the first draft of the article. All authors approved the submitted version of the  
84 manuscript.

85 **Acknowledgements:** not applicable

86

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88 **References**

- 89 1. Garnett L, Bello A, Tran KN, Audet J, Leung A, Schiffman Z, Griffin BD, Taylor N,  
90 Kobasa D, Strong JE. 2020. Comparison analysis of different swabs and transport  
91 mediums suitable for SARS-CoV-2 testing following shortages. *Journal of Virological*  
92 *Methods* 285:113947.
- 93 2. Charlton CL, Babady E, Ginocchio CC, Hatchette TF, Jerris RC, Li Y, Loeffelholz M,  
94 McCarter YS, Miller MB, Novak-Weekley S, Schuetz AN, Tang Y-W, Widen R, Drews  
95 SJ. 2018. Practical Guidance for Clinical Microbiology Laboratories: Viruses Causing  
96 Acute Respiratory Tract Infections. *Clin Microbiol Rev* 32.
- 97 3. Ferroni A, Izopet J, Miedougé M. Vérification d'une méthode qualitative d'amplification  
98 génique, p. 273–282. *In* QUAMIC, 2019th ed. Société Française de Microbiologie.
- 99 4. Wolters F, Grünberg M, Huber M, Kessler HH, Prüller F, Saleh L, Fébreau C, Rahamat-  
100 Langendoen J, Thibault V, Melchers WJG. 2021. European multicenter evaluation of  
101 Xpert® Xpress SARS-CoV-2/Flu/RSV test. *J Med Virol* 93:5798–5804.
- 102 5. Richard-Greenblatt M, Comar CE, Flevaud L, Berti M, Harris RM, Weiss SR, Glaser L.  
103 2021. Copan eNAT Transport System To Address Challenges in COVID-19 Diagnostics  
104 in Regions with Limited Testing Access. *J Clin Microbiol* 59:e00110-21.

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108 **Legend to figures**

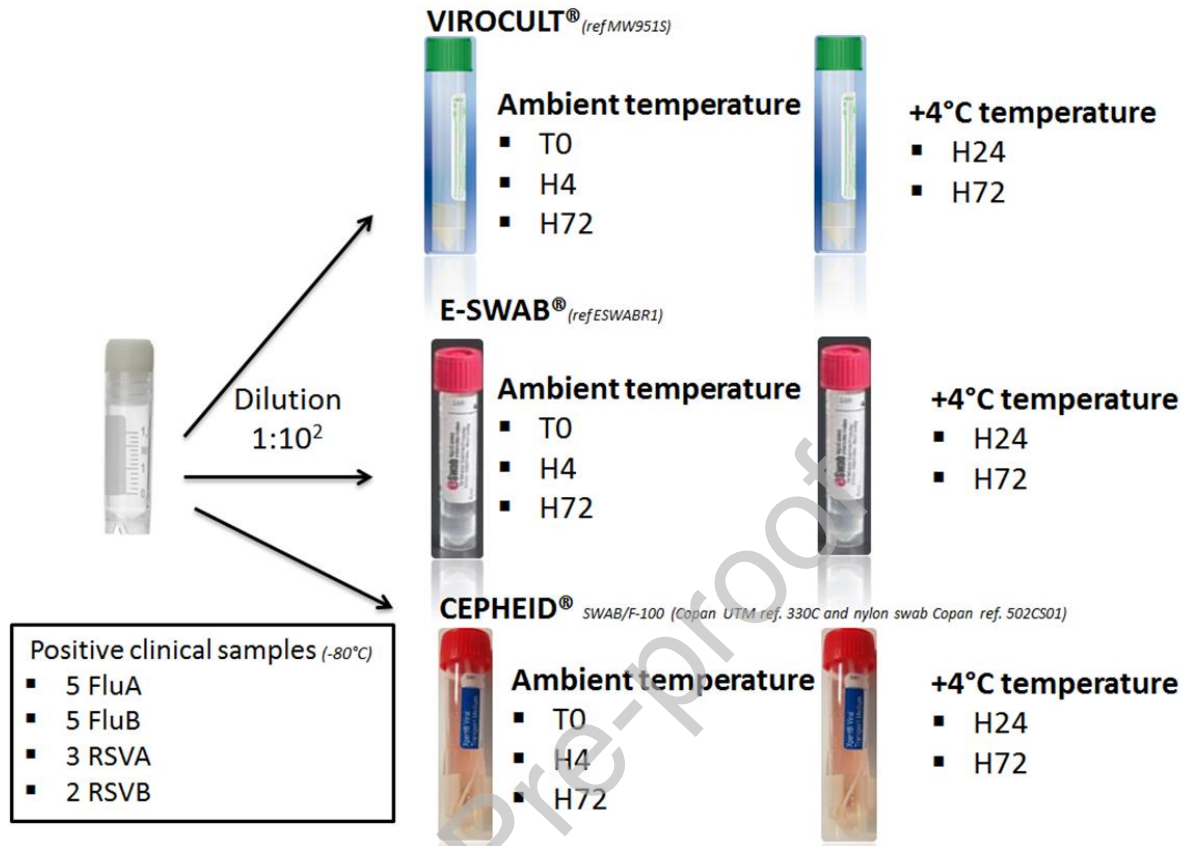
109 **Figure 1:** Experimental design for each tested VTM. Fifteen positive clinical samples were  
110 analyzed: influenza A virus (n=5), influenza B virus (n=5), RSV-A (n=3) or RSV-B (n=2).  
111 Three VTM were compared: Cepheid®, Virocult® and E swab® under several temperature  
112 storage (+4°C and ambient temperature (TA) and delay (T0, H4, H24 and H72).

113 **Figure 2:** Comparison of dCt for each sample stored for different periods of time, under  
114 different temperature conditions and gathered according to VTM: A: Cepheid®; B:  
115 Virocult®; C: E-swab®. Each virus family is represented by a different symbol: FluA, open  
116 squares; FluB, closed squares; RSV, open circles. The dotted horizontal lines represent the 2  
117 Ct cut-off.

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119 Figure 1



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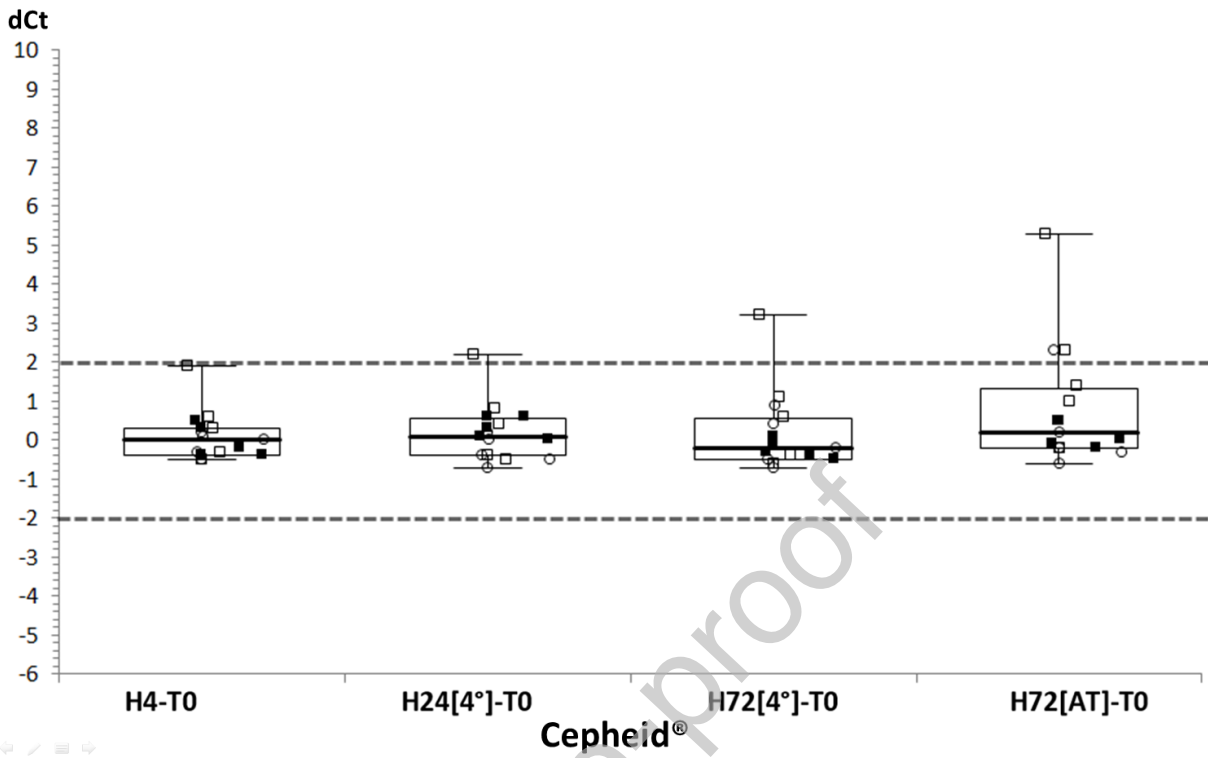
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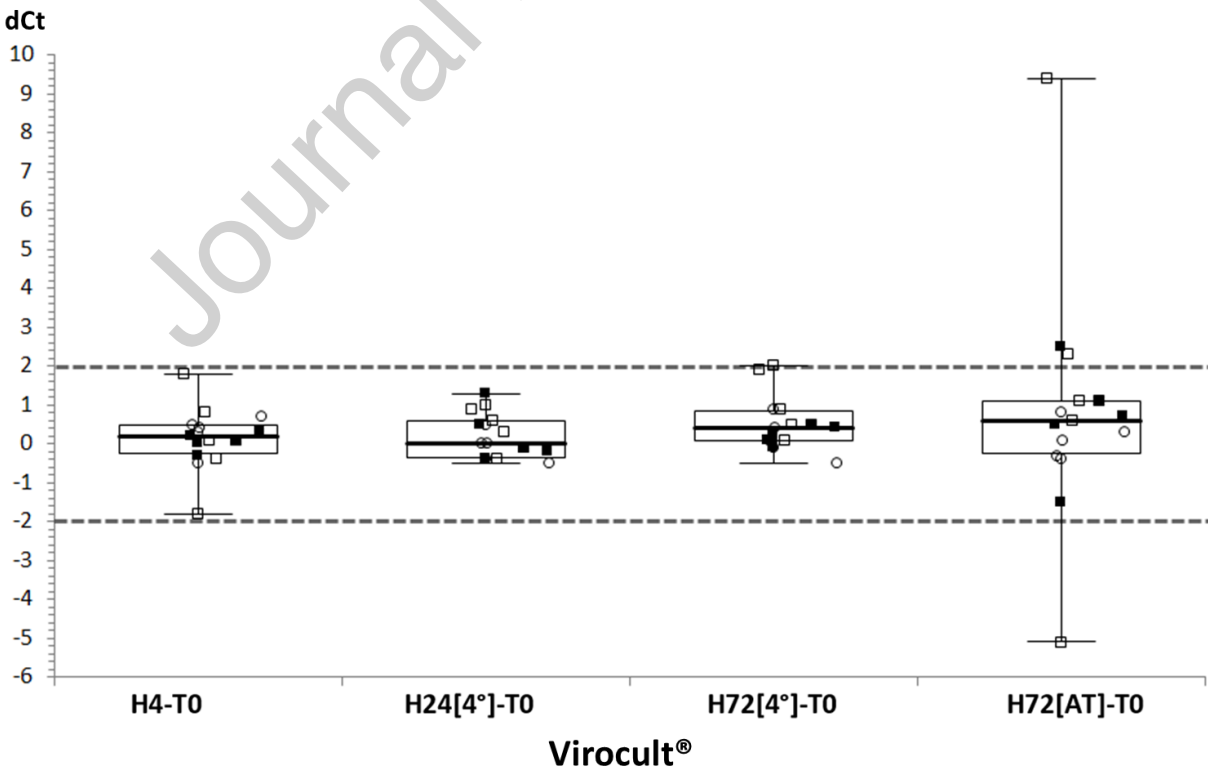
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127 Figure 2A



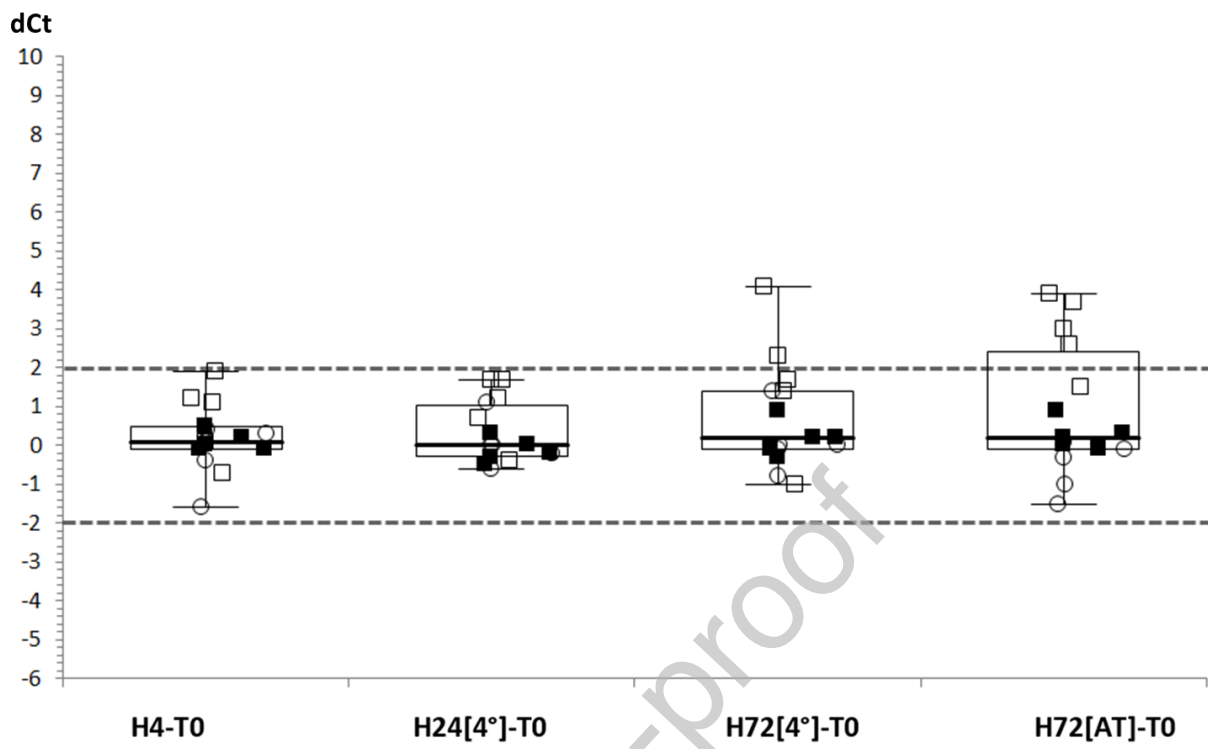
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129 Figure 2B



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131 Figure 2C



132

133

134 Table 1: **Delta Ct calculated against T0 values and obtained for each virus and storage condition** (*dCt values above 2 are represented on*  
135 *grey background*)

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	Cepheid®				Virocult®				E-swab®			
	H4-T0	H24 [4*]-T0	H72 [4*]-T0	H72 [AT]-T0	H4-T0	H24 [4*]-T0	H72 [4*]-T0	H72 [AT]-T0	H4-T0	H24 [4*]-T0	H72 [4*]-T0	H72 [AT]-T0
<b>FluA (n=5)</b>												
Minimum	-0.5	-0.5	-0.6	-0.2	-1.8	-0.4	0.1	-5.1	-0.7	-0.4	-1	1.5
Median	0.3	0.4	0.6	1.4	0.1	0.6	0.9	1.1	1.1	1.2	1.7	3
Maximum	1.9	2.2	3.2	5.3	1.8	1	2	9.4	1.9	1.7	4.1	3.9
Values over 2 dCt (n)	0	1	1	2	0	0	1	2	0	0	2	4
<b>FluB (n=5)</b>												
Minimum	-0.4	0	-0.5	-0.2	-0.3	-0.4	-0.1	-1.5	-0.1	-0.5	-0.3	-0.1
Median	-0.20	0.3	-0.3	0	0.1	-0.1	0.2	0.7	0	-0.2	0.2	0.2
Maximum	0.5	0.6	0.1	0.5	0.3	1.3	0.5	2.5	0.5	0.3	0.9	0.9
Values over 2 dCt (n)	0	0	0	0	0	0	0	1	0	0	0	0
<b>RSV (n=5)</b>												
Minimum	-0.5	-0.7	-0.7	-0.6	-0.5	-0.5	-0.5	-0.4	-1.6	-0.6	-0.8	-1.5
Median	0	-0.4	-0.2	-0.2	0.4	0	0.1	0.1	0.1	0	0	-0.3
Maximum	0.2	0.2	0.9	2.3	0.7	0.5	0.9	0.8	0.4	1.1	1.4	0.1
Values over 2 dCt (n)	0	0	0	1	0	0	0	0	0	0	0	0
<b>All viruses (n=15)</b>												
Values over 2 dCt (n)	0	1	1	3	0	0	1	3	0	0	2	4