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

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Highlights

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

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

- **Conflict of interest**
- The authors report no conflict of interest related to this work.
- All reagents were provided by Cepheid. This work was conducted independently to the manufacturer.

Letter to the editor,

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

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

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

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

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

Due to the limited number of samples, covering a narrow range of viral loads, this study should be considered exploratory and be reinforced by complementary studies including virus inactivating VTM and SARS-CoV-2 detection (4). Indeed, the use of a VTM capable of viral 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.

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Declarations

Ethics approval and consent to participate:

Not applicable

Consent to publish

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

Availability of data and material

Yes, upon request

Conflict of interest

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

Funding source

All reagents were provided by Cepheid, Inc.

Authors' contributions

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

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Legend to figures

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

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

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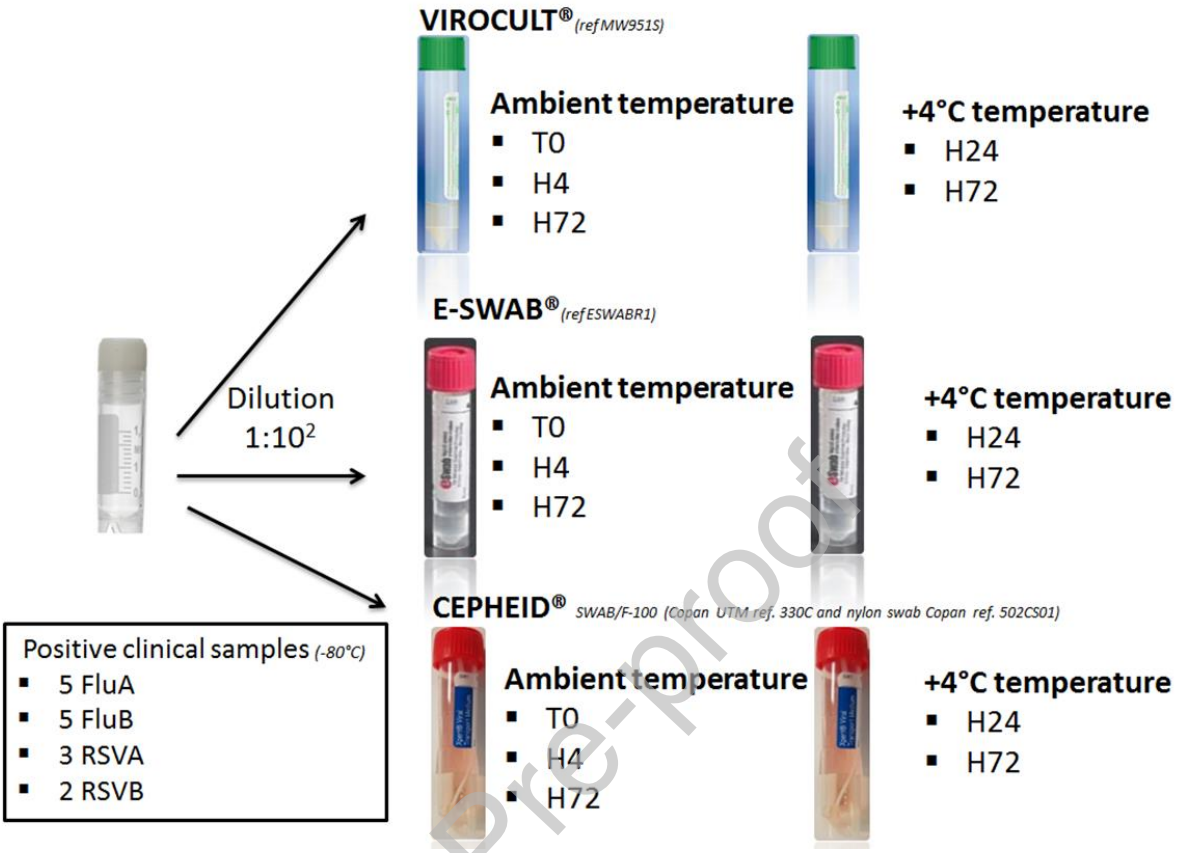
119 Figure 1



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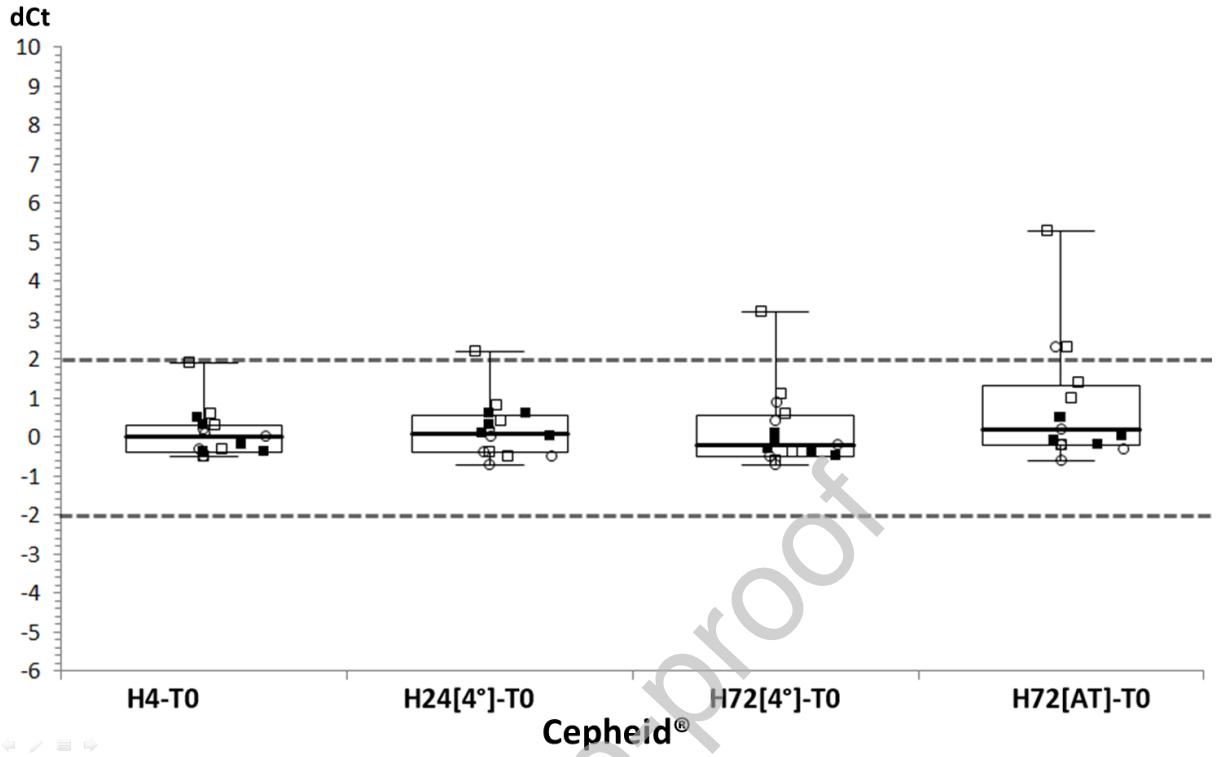
127 Figure 2A



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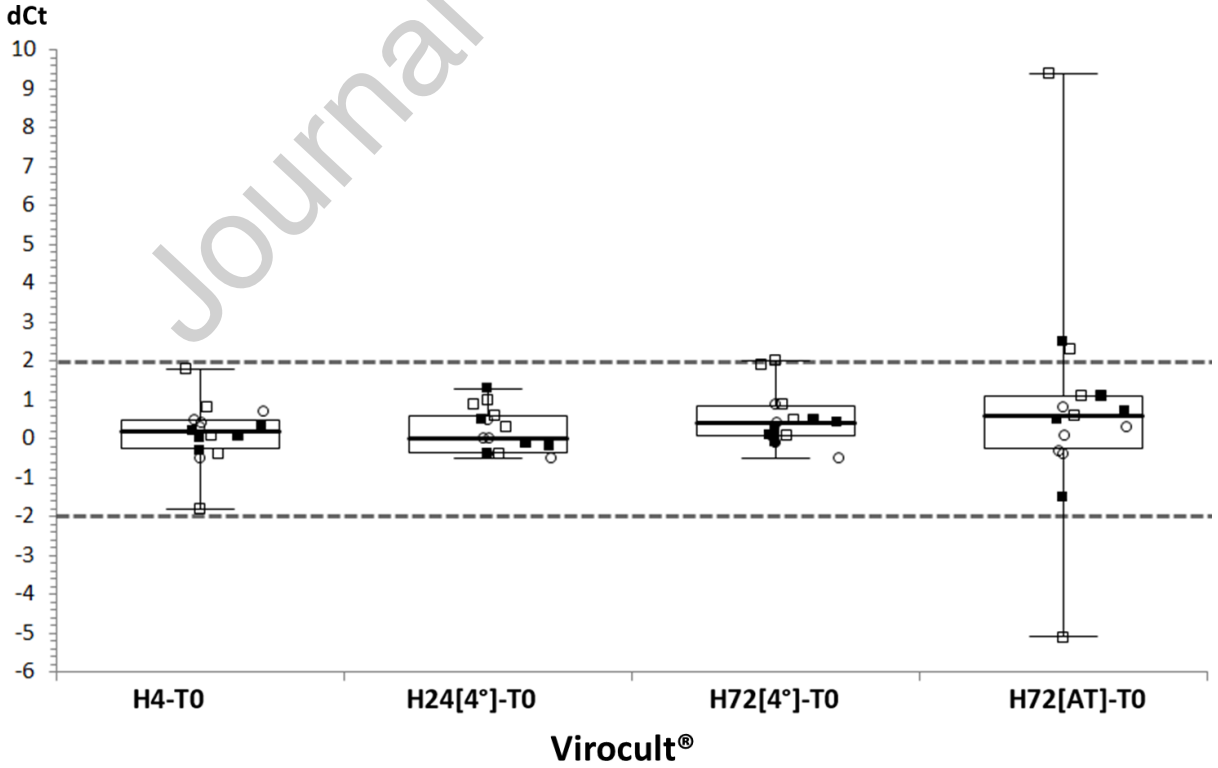
129 Figure 2B



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131 Figure 2C

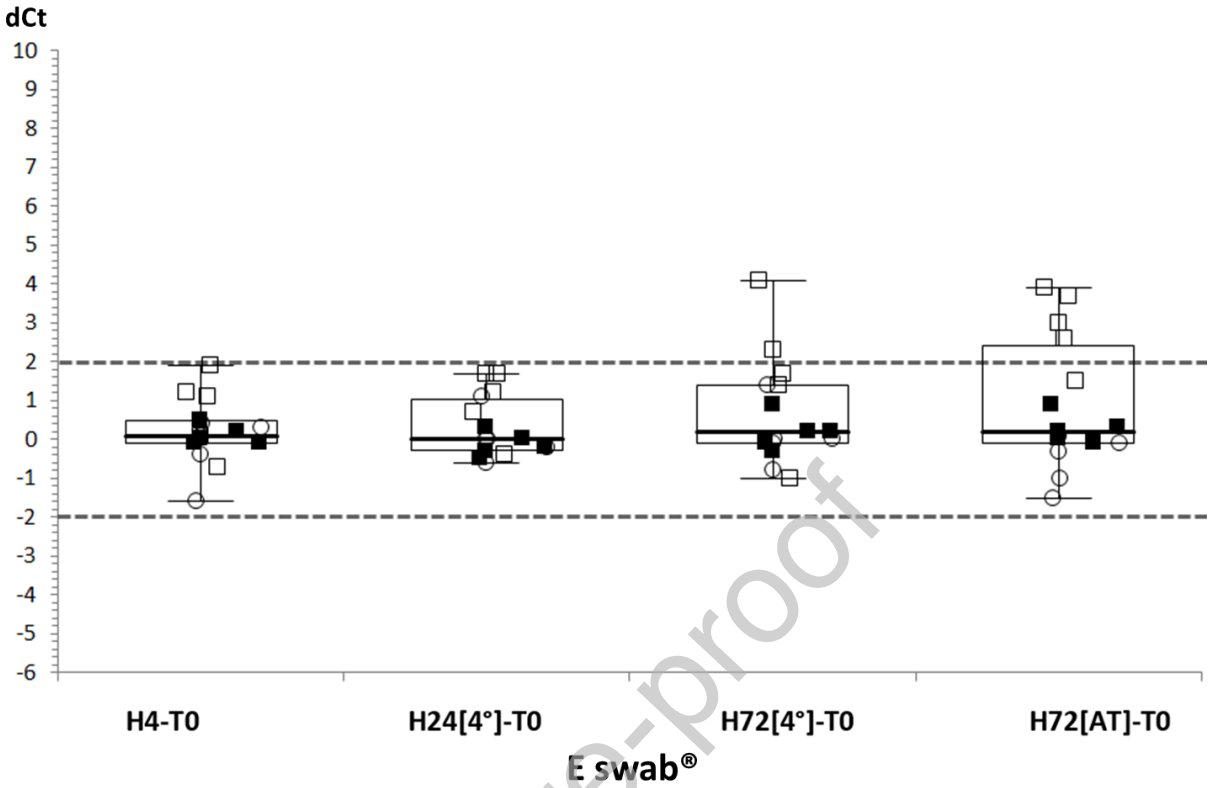

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Table 1: **Delta Ct calculated against T0 values and obtained for each virus and storage condition** *(dCt values above 2 are represented on grey background)*

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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
FluA (n=5)												
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
FluB (n=5)												
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
RSV (n=5)												
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
All viruses (n=15)												
Values over 2 dCt (n)	0	1	1	3	0	0	1	3	0	0	2	4

137

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