Used paper tissues for pathogen identification in acute respiratory infection

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Abstract

We investigated the potential of used paper tissues as a non-invasive sampling method for the diagnosis of acute respiratory infections. The method allowed the identification and typing of respiratory pathogens in symptomatic individuals, as well as in collective samples taken at a community level. The collection of used paper tissues could therefore be useful in epidemiological surveillance for SARS-CoV-2 and other respiratory pathogens such as influenzavirus, respiratory syncytial virus, entero/rhinoviruses and *Streptococcus pneumoniae*.

Acute respiratory tract infections (ARIs), including pneumonia, constitute a major disease burden worldwide, especially in young children and the elderly [1][2]. Diagnostic testing for respiratory pathogens is usually performed on samples collected by invasive methods, such as nasopharyngeal swabs, nasopharyngeal aspirates or bronchoalveolar lavages, obtained in hospital or medical practice settings. For some respiratory viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza virus and respiratory syncytial virus (RSV), fast diagnosis using antigen rapid diagnostic test (Ag-RDTs) can be performed on self-collected nasal swabs. Since some of the most vulnerable populations for ARI outbreaks, such as residents of long-term care facilities for the elderly or mentally impaired, or infants and toddlers attending day-nurseries, are difficult to sample using these invasive methods, there is a need for less to non-invasive methods for respiratory sampling. We assessed whether paper tissues, used for nose blowing, can be used for the identification of respiratory pathogens, on an individual as well as on a community level.

Pathogen identification from used paper tissues

Used paper tissues (UPT) of eight individuals with symptoms of ARI were investigated for the presence of respiratory pathogens. Tissues were transferred with sterile tweezers into a 100 mL disposable syringe (~4 tissues). Phosphate buffered saline (PBS) was added until tissues were soaked (~25 mL). After incubation at room temperature for 5 minutes, the plunger of the syringe was pressed to recover the eluate (~ 10 mL) into a 15 mL Falcon tube. Viral nucleic acids were extracted using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit on Kingfisher Flex-96 (ThermoFisher Scientific, Europe), using 400 µL of eluate of the used paper tissues. Screening for respiratory pathogens was done using an in-house developed respiratory panel (RP) for simultaneous detection of 22 respiratory viruses (influenza A, influenza B, respiratory syncytial virus (RSV), human metapneumovirus , parainfluenzavirus -1 to -4, adenovirus , human bocavirus , enterovirus/rhinovirus (EV/RV), enterovirus D-68 , human parechovirus , human coronavirus (HCoV)-NL63, -229E, -OC43, -HKU-1, -SARS and – MERS, cytomegalovirus, herpes simplex virus -1 and -2) and 7 non-viral pathogens (*Mycoplasma pneumoniae, Coxiella burnetii, Chlamydia pneumoniae, Chlamydia psittaci, Streptococcus pneumoniae , Legionella pneumophila* and *Pneumocystis jirovecii*) [3]. An additional SARS-CoV-2 specific RT-qPCR was carried out on samples positive for HCoV-SARS using the 2019-nCoV CDC EUA kit N1 primer probe set as described earlier [4]. Tissues were stored at room temperature for up to 10 days prior to analysis.

In 2 cases, EV/RV was detected, which could be further typed as RV C in one case and as co-infection of RV B and cocksackievirus A19 in the other case (typing performed as described by Wollants et al. [5]). Three samples tested positive for RSV, all three with concomitant detection of an additional pathogen (HCoV-OC43, adenovirus and *Streptococcus pneumoniae* respectively). In one sample, HCoV-OC43 was detected in combination with *Streptococcus pneumoniae*. One sample tested positive for SARS-CoV-2 (Cq 28.1), and was further typed by complete genome Oxford Nanopore Technologies sequencing as variant BA.5.2.1, with a genome coverage of 99.3% [6].

Respiratory pathogens detected in UPT versus nasal swabs and rapid antigen tests

To further investigate the potential of UPT in comparison to standard diagnostic sampling, UPTs of 16 patients with symptoms of ARI were analysed in parallel with self-collected nasal swabs in Universal Transport Medium (UTM) (Copan). Fourteen of these patients performed an Ag-RDT on the same day. Results are listed in Table 1. In general, pathogens that were detected in the nasal swabs were also detected in the corresponding UPT. Exceptions were HSV-1 (Cq 31.9 in nasal swab) in a patient who was also influenza A positive in both nasal swab and UPT (RP008), and EV/RV (Cq 36.6 in nasal swab) in a patient who also tested positive for influenza B in nasal swab and UPT (RP015). The Cq measured in the UPT was usually higher than in the nasal swab, although in some cases it was the other way round. In all cases where the Ag-RDT tested positive, the corresponding pathogen was also detectable in the UPT.

TABLE 1. Identification of respiratory pathogens in used paper tissues versus nasal swabs and antigen tests, Belgium, winter 2022-2023 (n = 16 samples)

PATIENT ID	UPT (Cq)[a]	Nasal swab (Cq)[b]	Ag-RDT[c]	Typing result[d]
RP001	SARS-CoV-2 (19.8)	SARS-CoV-2 (15.5)	FLU A: NEG[1] FLU B: NEG[1] COVID-19: POS[1]	SARS-CoV-2: BA.4/BA.5
RP002	SARS-CoV-2 (16.8)	SARS-CoV-2 (11.7)	FLU A: NEG[2] FLU B: NEG[2] COVID-19: POS[2]	SARS-CoV-2: BA.4/BA.5
RP003	HSV-1 (34.1) SARS-CoV-2 (21.5)	HSV-1 (30.5) SARS-CoV-2 (19.6)	SARS-CoV-2: POS[3]	SARS-CoV-2: BA.4/BA.5
RP004	NEG	NEG	ND	NA
RP005	SARS-CoV-2 (31.9)	SARS-CoV-2 (25.6)	FLU A: NEG[2] FLU B: NEG[2] COVID-19: NEG[2]	SARS-CoV-2: BA.4/BA.5
RP006	EV/RV (23.9)	EV/RV (19.5)	SARS-CoV-2: NEG[4]	EV/RV: RV-C
RP007	NEG	NEG	SARS-CoV-2: NEG[4]	NA
RP008	Influenza A (30.8)	HSV-1 (31.9) Influenza A (31.1)	FLU A: POS[1] FLU B: NEG[1] COVID-19: NEG[1]	Influenza A: untypeable*
RP009	EV/RV (37.4) HSV-1 (33.8) SARS-CoV-2 (30.0)	EV/RV (35.4) SARS-CoV-2 (38.6)	FLU A: NEG[1] FLU B: NEG[1] COVID-19: NEG[1]	EV/RV: untypeable* SARS-CoV-2: BA.4/BA.5
RP010	Influenza B (27.6)	Influenza B (22.0)	FLU A: NEG[5] FLU B: NEG[5] COVID-19: NEG[5] RSV: NEG[5]	Influenza B: Victoria lineage
RP011	EV/RV (20.74)	EV/RV (22.64)	FLU A: NEG[5] FLU B: NEG[5] COVID-19: NEG[5] RSV: NEG[5]	EV/RV: Rhinovirus A
RP012	Influenza A (21.5)	Influenza A (33.8)	FLU A: POS[1] FLU B: NEG[1] COVID-19: NEG[1]	Influenza A: H1N1
RP013	Influenza B (30.4) *S. pneumoniae* (28.2)	Influenza B (22.2) *S. pneumoniae* (19.5)	FLU A: NEG[5] FLU B: POS[5] COVID-19: NEG[5] RSV: NEG[5]	Influenza B: Victoria lineage
RP014	NEG	NEG	FLU A: NEG[2] FLU B: NEG[2] COVID-19: NEG[2]	NA
RP015	Influenza B (26.6)	EV/RV (36.6) Influenza B (19.3)	FLU A: NEG[1] FLU B: POS[1] COVID-19: NEG[1]	Influenza B: Victoria lineage
RP016	NEG	NEG	ND	NA

Ag-RDT: antigen rapid diagnostic test; COVID-19: coronavirus disease; EV/RV: enterovirus/rhinovirus; HSV: herpes simplex virus; FLU: influenza; NA: not applicable; ND: not done; NEG: negative; POS: positive; RV-C: rhinovirus C; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; UPT: used paper tissue.

[a] Respiratory pathogens detected in UPT by RP and SARS-CoV-2 N1 RT-qPCR, with Cq values.

[b] Respiratory pathogens detected in nasal swab by RP and SARS-CoV-2 N1 RT-qPCR, with Cq values.

[c] Lateral flow Ag-RDT test result per respiratory pathogen, with pathogen names as indicated on the test. Ag-RDT brands that were used are indicated with numbers behind the test results.

[d] Typing performed on UPT based on partial sequencing as described previously for SARS-CoV-2 [7], EV/RV [5] and influenza[8][9].

[1] Influenza & COVID-19 Ag Combo Rapid Test (Orientgene)

[2] SARS CoV-2 and Influenza A+B Antigen Combo Rapid Test (All test)

[3] CerTest SARS-CoV-2 Ag-Nasal Sample-Self Test (Certest Biotech)

[4] SARS-CoV-2 Antigen Rapid Test (Flowflex)

[5] SARS-CoV-2 & Influenza A/B & RSV Antigen Combo Test Kit (Fluorecare)

* viral load too low for typing

SARS-CoV-2 load in UPT versus nasal swab and Ag-RDT over the course of infection

One patient was followed over the course of COVID infection, from the time of first symptom up to complete symptom resolution, with UPTs, nasal swabs in UTM and Ag-RDTs being collected daily (Fig). SARS-CoV-2 was detectable in UPT as of the start of symptoms, whereas the Ag-RDT only turned positive on Day 4. For as long as the Ag-RDT remained positive, SARS-CoV-2 was also detectable in UPT. In the nasal swab, SARS-CoV-2 was still detectable after Ag-RDTs turned negative, and remained detectable up to the last symptomatic day.

FIGURE. Evolution of SARS-CoV-2 load over the course of an infection, as captured by Cq values in UPT and nasal swab, and Ag-RDT test result, Belgium, February 2023.
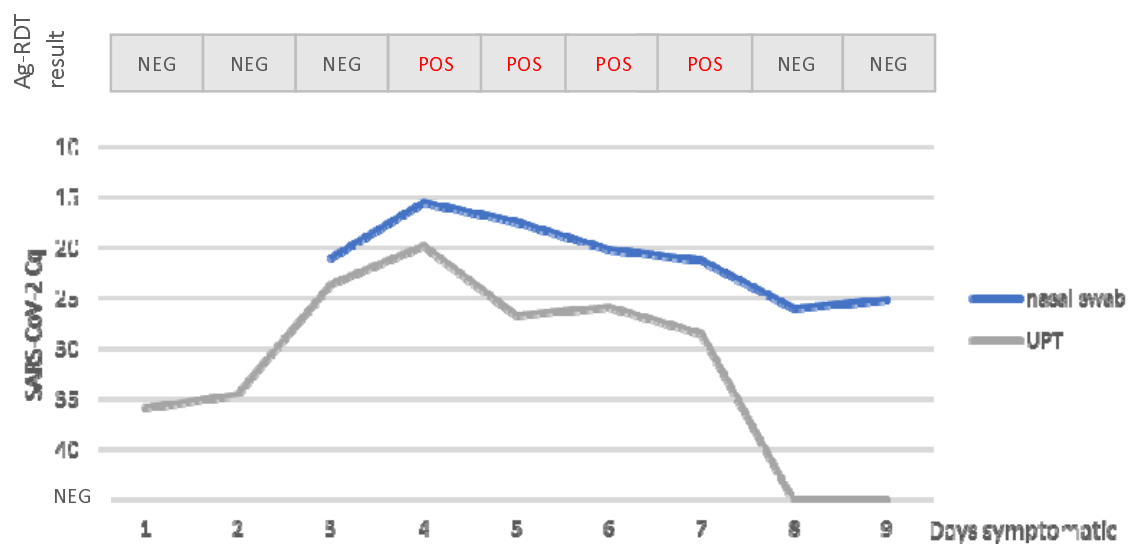
Respiratory pathogens in combined UPTs of collectivities

Combined UPTs from 6 collectivities (one childcare centre, 3 kindergartens and 2 primary schools) were collected by anonymously gathering tissues used in a classroom or childcare group over the course of one day. The maximal amount of tissues was used for pathogen elution, and further investigated as described above. The presence of multiple respiratory pathogens was detected in these combined samples (Table 2).

TABLE 2. Identification of respiratory pathogens in combined used paper tissues from collectivities, Belgium, November 2022 (n = 6 samples)

Collectivity	Respiratory pathogen	Cq
Childcare	Bocavirus	33.4
	Enterovirus / rhinovirus	28.2
	Respiratory syncytial virus	27.9
	Streptococcus pneumoniae	34.5
Kindergarten A	Enterovirus / rhinovirus	26.6
	Streptococcus pneumonia	23.6
Kindergarten B	Adenovirus	33.0
	Enterovirus / rhinovirus	25.0
	Cytomegalovirus	34.7
	Streptococcus pneumoniae	23.2
Kindergarten C	Adenovirus	29.3
	Enterovirus / rhinovirus	31.3
	Cytomegalovirus	33.5
	Streptococcus pneumoniae	24.4
Primary school A	Respiratory syncytial virus	33.0
	Enterovirus / rhinovirus	26.7
	Enterovirus D68	30.4
Primary school B	Human coronavirus OC43	32.2
	Streptococcus pneumoniae	26.2

Discussion

Our method for detection of respiratory pathogens from UPT samples allowed the identification of respiratory pathogens responsible for ARI, with adequate sample quality to allow further genetic characterization. We were able to detect respiratory viruses as well as bacteria, with most pathogens that were identified in nasal swabs also being detected in concurrent UPTs from the same patient. Pathogens that were not detected in the UPT samples were co-infecting pathogens which were only present in low amounts in the corresponding nasal swab (Cq ≥ 32).

Bacterial pathogens have been shown to be reliably detectable from paper tissues of patients with upper respiratory tract infections [10]. In a recent study, Lagathu et al. were able to identify multiple

respiratory viruses in pooled facial tissues obtained in communities of children. They also compared
SARS-CoV-2 Cq values between nasopharyngeal swabs and facial tissues of individual COVID-19
patients and found a higher signal from the tissues in 11 out of in 15 cases [11]. In our study, we
compared Cq values for SARS-CoV-2 but also for other common respiratory viruses such as
enterovirus/rhinovirus and influenzavirus A and B, and *S. pneumoniae*, obtained from 16 UPT and
nasal swabs, and found a high variety in Cq difference between both samples. We also were able to
detect the presence of multiple respiratory pathogens in pooled UPT samples of collectivities,
confirming its applicability for community testing. This would especially be useful in schools and
preschool daycare centers, since taking nasal samples from (young) children is an invasive method
and requires training, or in elderly homes and homes for disabled people, in whom taking nasal
samples is less well tolerated. Because sequencing a complete genome is possible from UPT this
method can also be applied for epidemiological surveillance. Furthermore, UPT samples can easily be
transported to diagnostic laboratories, even by regular mail.

Since our sample contains eluted material from entire paper tissues, the pathogen load in the sample
is not only dependent on the amount of virus shedding but also on the amount of nasal discharge
collected in the tissue. This makes the method less suited for (semi-) quantitative analyses. It also
implies that the method cannot be used when there is very little to no nasal discharge, or when nasal
discharge is difficult to collect by nose blowing or wiping with a tissue.

We were able to detect the corresponding virus in UPT of all Ag-RDT positive cases, indicating that
UPTs are sufficiently sensitive to detect individuals with high virus shedding, who are most likely to
be infectious. As such, UPT could provide an interesting non-invasive sampling method for screening
of individuals. In the patient that was followed over the course of a COVID infection, UPTs tested
positive as of the start of symptoms, whereas Ag-RDTs turned positive only on day 4. This is in
accordance with the notion that SARS-CoV-2 viral loads in persons with pre-existing immunity (by
previous infection or by vaccination) may only rise to Ag-RDT detectable levels after several days of
symptoms. Although only based on a single observation, UPT testing seems to be sensitive enough to
allow detection as of the start of infection, reducing the amount of false negative test results.

Since pathogen detection was possible from combined UPTs obtained in collectivities, it can also
provide a good alternative to sampling of sewage water of buildings or aircraft wastewater to obtain
a community sample for pathogen screening. This would be very useful to complement the current
strategy of wastewater testing of incoming aircraft for SARS-CoV-2 variant screening [12], [13].

Ethical statement

Ethics committee of UZ/KU Leuven gave ethical approval for this work.

Informed consent, approved by the UZ Leuven Ethics Committee, was obtained from all individuals providing
self-collected samples.

Conflict of interest

None declared.

Authors' contributions

Annabel Rector: Conception and design of the study, data analysis and interpretation, writing of the
manuscript, final approval of the version to be published.

Mandy Bloemen: Conception and design of the study, data collection, data analysis and interpretation, writing
of the manuscript, final approval of the version to be published.

Marc Van Ranst: Conception and design of the study, final approval of the version to be published.

Elke Wollants: Conception and design of the study, data collection, data analysis and interpretation, writing of
the manuscript, final approval of the version to be published.

References

[1]   WHO, "The World health report : 2004 : Changing history," 2004.
        https://apps.who.int/iris/handle/10665/42891

[2]   H. Nair *et al.*, "Global burden of respiratory infections due to seasonal influenza in young
        children: A systematic review and meta-analysis," *Lancet*, vol. 378, no. 9807, pp. 1917–1930,
        2011, doi: 10.1016/S0140-6736(11)61051-9.

[3]   J. Raymenants *et al.*, "Indoor air surveillance and factors associated with respiratory pathogen
        detection in community settings in Belgium," pp. 1–12, 2023, doi: 10.1038/s41467-023-
        36986-z.

[4]   E. Wollants *et al.*, "Monitoring of SARS-CoV-2 concentration and circulation of variants of
        concern in wastewater of Leuven, Belgium," *J. Med. Virol.*, 2023, doi: 10.1002/jmv.28587.

[5]   E. Wollants *et al.*, "A decade of enterovirus genetic diversity in Belgium," *J. Clin. Virol.*, vol.
        121, Dec. 2019, doi: 10.1016/J.JCV.2019.104205.

[6]   T. Wawina-Bokalanga *et al.*, "Genetic diversity and evolution of SARS-CoV-2 in Belgium during
        the first wave outbreak," *bioRxiv*, p. 2021.06.29.450330, 2021.

[7]   M. Bloemen *et al.*, "Fast detection of SARS-CoV-2 variants including Omicron using one-step
        RT-PCR and Sanger sequencing," *J. Virol. Methods*, vol. 304, no. March, p. 114512, 2022, doi:

10.1016/j.jviromet.2022.114512.

[8]   B. Schweiger, I. Zadow, R. Heckler, H. Timm, and G. Pauli, "Application of a fluorogenic PCR assay for typing and subtyping of influenza viruses in respiratory samples," *J. Clin. Microbiol.*, vol. 38, no. 4, pp. 1552–1558, 2000, doi: 10.1128/jcm.38.4.1552-1558.2000.

[9]   A. Rector, M. Bloemen, G. Schiettekatte, P. Maes, M. Van Ranst, and E. Wollants, "Sequencing directly from antigen-detection rapid diagnostic tests in Belgium, 2022: a gamechanger in genomic surveillance?," *Euro Surveill.*, vol. 28, no. 9, 2023, doi: 10.2807/1560-7917.ES.2023.28.9.2200618.

[10]   M. R. Van Den Bergh *et al.*, "Alternative sampling methods for detecting bacterial pathogens in children with upper respiratory tract infections," *J. Clin. Microbiol.*, vol. 50, no. 12, pp. 4134–4137, 2012, doi: 10.1128/JCM.02376-12.

[11]   G. Lagathu *et al.*, "Using Discarded Facial Tissues to Monitor and Diagnose Viral Respiratory Infections," *Emerg. Infect. Dis.*, vol. 29, no. 3, pp. 511–518, 2023, doi: 10.3201/eid2903.221416.

[12]   European Commission, "European Commission. Adhoc guidance: Wastewater sampling of aircrafts and airports for SARS-CoV-2 surveillance. 10 January 2023. European Commission Brussels 2023.," 2023.

[13]   European Centre for Disease Prevention and Control/European Union Aviation Safety Agency, "Guidelines in response to the worsening of the epidemiological situation: addendum to the Aviation Health Safety Protocol." 2023.