Surveillance of the respiratory syncytial virus outside infancy: impact of testing methods, a retrospective observational study

Nicolas Yin [1], Sigi Van den Wijngaert [1], Magali Wautier [1], Delphine Martiny [1,2], Marie Hallin [3,6] and Olivier Vandenberg [3,4,5,6]

[1]Department of Microbiology, Laboratoire Hospitalier Universitaire de Bruxelles–Universitair Laboratorium Brussel (LHUB-ULB), Université Libre de Bruxelles (ULB), Brussels, Belgium. [2]Faculté de Médecine et Pharmacie, Université Libre de Mons, Mons, Belgium. [3]Centre for Environmental Health and Occupational Health, School of Public Health, Université Libre de Bruxelles (ULB), Brussels, Belgium. [4]Clinical Research and Innovation Unit, Laboratoire Hospitalier Universitaire de Bruxelles–Universitair Laboratorium Brussel (LHUB-ULB), ULB, Brussels, Belgium. [5]Division of Infection and Immunity, Faculty of Medical Sciences, University College London, London, UK. [6]These authors have contributed equally to this work and share senior authorship.

Corresponding author: Nicolas Yin (nicolas.yin@lhub-ulb.be)

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RSV surveillance may be affected not only by the type of assay used for its detection (molecular or antigenic), but also by the use of multiplex assays that detect other viruses in a single test https://bit.ly/3H4YWWA

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Abstract

Background The European Medicines Agency has approved several vaccines to protect the elderly against respiratory syncytial virus (RSV) infections. However, differences in performance between antigen and PCR tests, especially in adults, can make monitoring RSV difficult. This study aims to assess the impact of the chosen diagnostic methods on the surveillance of RSV.

Methods RSV and influenza test results obtained from July 2022 to June 2023 in a consolidated clinical laboratory in Brussels, Belgium, were collected. These results included antigen tests, quadruplex PCR tests and viral cultures on respiratory samples. Epidemiological trends related to the age of patients and the diagnostic methods were analysed.

Results Among 14 761 RSV tests, the overall number of positive tests for infants until 1 year of age peaked on 5 November 2022 (67 per 7 days) whereas it peaked on 22 December 2022 for adults (33 per 7 days). Positive antigen tests peaked on 7 November 2022 (56 per 7 days) whereas positive PCRs peaked on 19 December 2022 (36 per 7 days). Nevertheless, the positivity rate of RSV PCRs had peaked 1 month previously. Infants were mainly diagnosed through antigen testing, contrary to older patients. The influenza epidemic was probably the cause of the increased use of a quadruplex PCR, leading to a delayed increase in the absolute number of PCRs positive for RSV.

Conclusion This study shows that the use of different diagnostic methods could lead to an erroneous representation of RSV epidemiology in adults due to the lack of sensitivity of antigen detection. RSV surveillance in the elderly should rely rather on molecular methods.

Introduction

The respiratory syncytial virus (RSV) is now recognised as an important cause of serious illness in the elderly [1] and at the time of writing, two vaccines recently approved by the European Union's health regulator have been made available to older adults for the coming winter [2]. Therefore, monitoring the impact of the vaccination on this specific population requires efficient surveillance. The use of laboratory data to assess the occurrence of specific microorganisms in a population represents one of the most common established public health surveillance approaches for infectious diseases [3]. Since 1983, the Belgian authorities have implemented such a strategy through the set-up of the Belgian Sentinel Network of Laboratories, which collect data on the epidemiology of 43 microorganisms [4].

However, monitoring the number of RSV cases can be tricky because of 1) the difference in sensitivity of the affordable antigen testing between adults and infants [5]; 2) a probable lack of access to PCR tests; as well as 3) the disregard by clinicians of the impact of this pathogen in adults. Furthermore, 4) the molecular detection of RSV is frequently paired with the detection of other respiratory viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and/or influenza viruses [6], potentially adding another confusion bias in its surveillance. The aim of this study was to examine the testing data coming from a single large clinical microbiology laboratory for five hospitals in Brussels and to assess the impact of diagnostic methods on the surveillance of RSV during the winter of 2022–2023.

Material and methods

Data were coming from a single consolidated clinical laboratory, the LHUB-ULB (Laboratoire Hospitalier Universitaire de Bruxelles–Universitair Laboratorium Brussel, Brussels, Belgium). This is a clinical laboratory serving five university hospitals (with a capacity of ∼3000 beds) as well as a network of general practitioners in Brussels, covering a service area of 700 000 inhabitants [7]. RSV positive test results as well as influenza-positive PCR results were collected from July 2022 to June 2023. These results included RSV antigen detection tests (RSV K-set; Coris Bioconcept, Belgium), quadruplex PCR tests (Alinity m RESP-4-PLEX assay; Abbott Molecular, USA) as well as viral cultures coming from respiratory samples which were routinely performed in addition of the antigen detection test. The quadruplex PCR test allowed the simultaneous detection of RSV, SARS-CoV-2 and influenza A and B viruses. In the routine surveillance perspective, all patients diagnosed with RSV or influenza infection by rapid antigen detection tests, molecular diagnostic tests or by viral culture are considered as notifiable cases of RSV or influenza infection in the frame of the Belgian Sentinel Network of Laboratories. Multiple positive results for the same patient were deduplicated to keep only the first positive result per patient. The daily positivity rate for RSV and influenza PCR was calculated by using the number of non-deduplicated tests performed in the previous 7 days. The age of the patients at sampling date was also collected. Patients aged <1 year were considered as infants, patients aged 2–14 years were considered as children and patients aged ⩾15 years were considered as adults. Epidemiological trends were analysed by cumulating daily positive tests per 7 days to minimise day-to-day and holiday-related fluctuations from 1 July 2022 to 30 June 2023.

Results

From 25 June 2022 to 30 June 2023, 14 761 RSV diagnostic tests (7280 PCR and 7581 antigen detection tests followed by viral culture) and 7282 influenza PCR tests were performed (table 1). Viral cultures yielded 123 RSV cases not detected by antigen detection test. 162 RSV PCR tests were performed on the same day as a negative antigen test, of which 19 yielded a positive result. 944 patients had a positive RSV test during this period, including 608 (64.4%) infants. During the same period, 901 patients had a positive influenza PCR tests. The overall number of positive tests for RSV in infants peaked twice: on 5 November 2022 and on 26

TABLE 1 Antigen detection and nucleic acid amplification (PCR) tests performed for the detection of therespiratory syncytial virus (RSV) and influenza virus and patients' ages from 25 June 2023 to 30 June 2023

	Antigen RSV	PCR RSV	PCR influenza
Overall			
Age years	3 (0–52)	61 (37–75)	61 (37–75)
Number of tests	7581	7280	7282
Age <2 years	3278	336	334
Age 2–14 years	1408	227	228
Age ⩾15 years	2895	6717	6720
Positive			
Age years	4 (0–55)	50 (2–73)	40 (26–63)
Number of tests	528	340	934
Age <2 years	458	76	34
Age 2–14 years	44	35	66
Age ⩾15 years	26	229	834
Negative			
Age years	0 (0–0)	61 (38–75)	63 (41–76)
Number of tests	7053	6940	6348
Age <2 years	2820	260	300
Age 2–14 years	1364	192	162
Age ⩾15 years	2869	6488	5886

Data are presented as median (interquartile range) or n.

November 2022, with 67 and 61 positive tests per 7 days, respectively (figure 1). Conversely, the overall number of RSV positive tests for adults peaked on 22 December 2022, with 33 positive tests per 7 days. For children, the overall number of positive tests for RSV was lower and reached its maximum at 12 positive tests per 7 days on 23 November 2022. When analysing the nature of the positive tests, the number of positive antigen tests peaked twice, on 7 November 2022 and on 24 November 2022, with 56 and 55 positive tests per 7 days, respectively. In contrast, the number of positive RSV PCRs peaked on 19 December 2022, with 36 positive tests per 7 days. However, the positivity rate of RSV PCRs peaked 1 month earlier, on 17 November 2022, with 21.5% of PCR tests. This can be explained by the fact that the median (interquartile range) age for positive antigen tests was 0 (0–0) years, whereas it was 50 (2–73) years for the positive PCR tests. The influenza epidemic, for which the number of positive PCRs peaked on 30 December 2022 with 181 positive tests per 7 days, probably indirectly drove the increasing number of positive PCRs for RSV after the epidemic peak observed for antigen testing and infants. The positivity rate of influenza PCR tests peaked on 29 December 2022 at 43.8% and the positivity rate of RSV antigen detection tests peaked on 7 November 2022 at 46.0%.

Discussion

The multiplication of rapid detection methods for respiratory viruses, ranging from antigen tests [5] to rapid point-of-care multiplex PCRs [6] has allowed for a broader detection of RSV. However, this can also complicate its surveillance. For passive surveillance using the RSV laboratory surveillance database, recommendations in Europe are to gather positive test results as well as the overall number of RSV tests and the type of test (*e.g.* antigen, PCR, culture) [8]. This study highlights the importance of gathering these data as the number of cases on its own was not reflecting the actual epidemiological situation. There appeared to be a delay between the number of positive cases identified by antigen tests and those identified by PCR. This delay was artificially created by two factors: the lack of sensitivity of the antigen tests in adults and the fact that the molecular diagnostic used a quadruplex PCR which was also used for influenza and SARS-CoV-2 testing. The number of quadruplex PCR tests performed increased dramatically because of the spread of the influenza increasing the number of diagnosed RSV cases, while its actual prevalence was decreasing.

In a previous study [9], we showed that for the PCR detection of SARS-CoV-2, the average cycle threshold values (Ct) of the positive PCRs varied in advance of the absolute number of positive results. Thus, when the number of positive PCRs peaked, the proportion of recently infected (hence contagious) patients had already been decreasing for a few weeks. Indeed, due to the high sensitivity of the PCR, testing positive for a respiratory virus by PCR does not necessarily indicate illness. Antigen tests detect patients with higher viral loads [10] and are less affected by this effect. In our setting, antigen testing was the favoured RSV diagnostic method for infants aged <1 year. This can be easily explained by the good performance of this test in this population [5], combined with its rapidity and ease of use, making it available in on-site laboratories. Conversely, point-of-care PCRs are usually expensive, while larger molecular diagnostic platforms, which allow a lower cost per test, are performed in our central laboratory during business hours, taking more time (a few hours), thus decreasing its interest for the rapid management of patients. Therefore, for the five partner hospitals of our clinical laboratory (LHUB-ULB), the diagnostic algorithm for the management of patients with influenza-like illness frequently includes the performance of a rapid antigenic diagnostic test as a first step. If the latter is negative and if the patient requires hospitalisation, a molecular technique is performed. Such a strategy allows for rapid and sensitive diagnosis at the best cost, by avoiding unnecessary molecular testing. In addition, viral cultures are performed on samples with a negative antigen test. These cultures are reimbursed by the Belgian national health insurance system as an alternative to molecular tests, which, in contrast, are not reimbursed. They allow confirmatory diagnosis in mildly ill patients. However, beyond their epidemiological interest, the time-to-result of viral cultures hampers their clinical interest. In the context of epidemiological surveillance, it would also be interesting to focus on the positivity rate in each municipality located in the direct service area of the LHUB-ULB. This would show which populations are the most at risk of developing influenza, but would also help hospital managers to target vulnerable populations or to rapidly identify clusters. As originally demonstrated by John Snow, such mapping proved their usefulness in showing differences in rates of disease between communities and in identifying clusters of disease [11].

The findings in this report are subject to at least two limitations. First, sentinel surveillance based on LHUB-ULB data only may not provide a fully representative sample of the epidemiological situation in Belgium, as influenza testing is mainly performed on inpatients and patients attending emergency departments with respiratory symptoms. This was underlined by Jester *et al*. [12], who highlighted that influenza surveillance relies on specimens collected from symptomatic patients during medical encounters, where the purpose of testing is primarily patient diagnosis rather than surveillance. Furthermore, additional

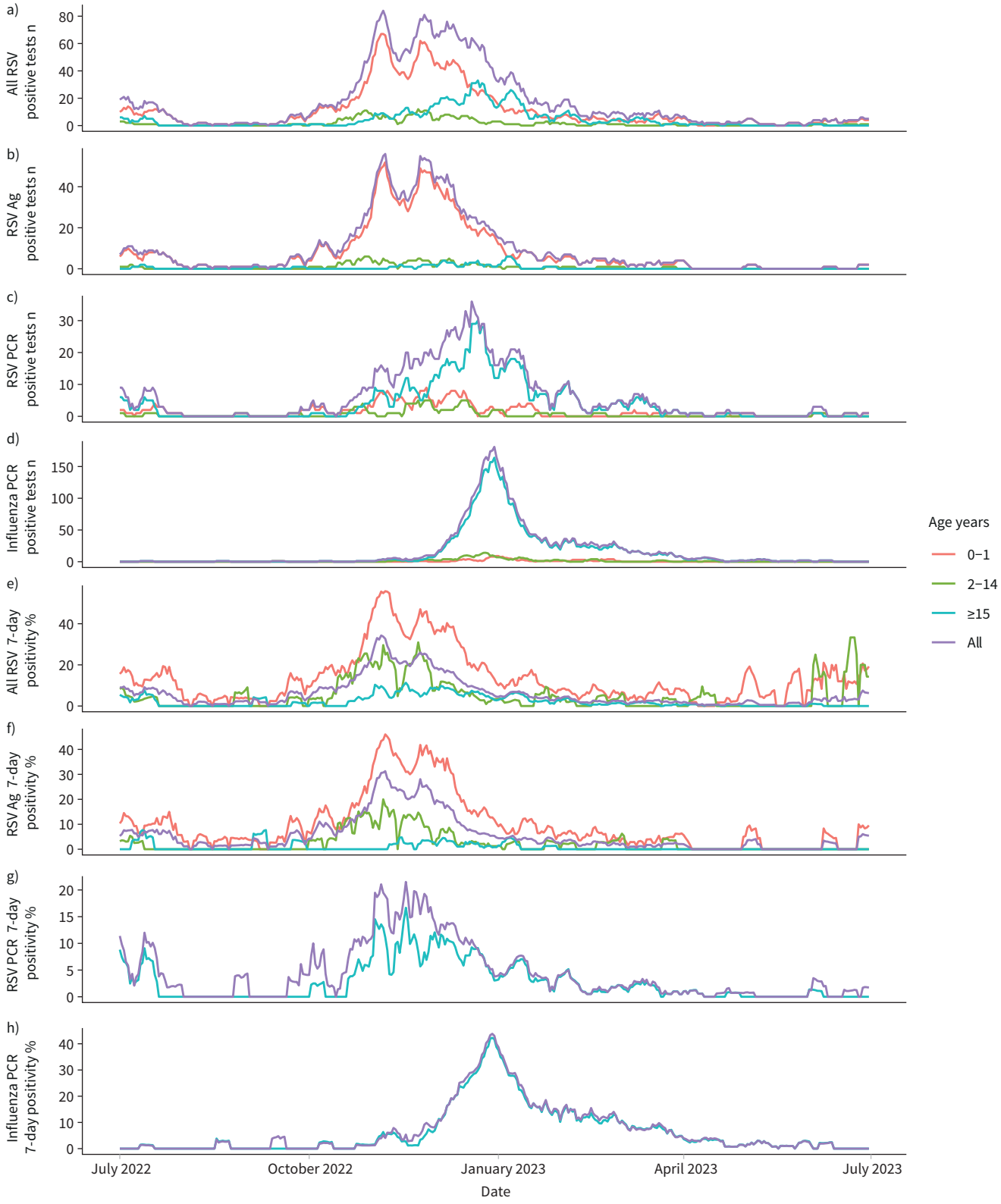

FIGURE 1 Compared evolution of the 7-day non-deduplicated number of a) overall respiratory syncitial virus (RSV) positive tests, b) RSV antigen (Ag) detection tests, c) RSV PCR tests, d) influenza PCR tests, as well as e–h) their respective 7-day positivity rate (%) from July 2022 to June 2023.

studies must be carried out to judge whether data unification from large consolidated laboratories located, for instance, in the three different regions of Belgium (Brussels, Wallonia and Flanders), could be sufficient to describe the infectious events in Belgium, as we did previously for influenza [7]. At the European level, the interconnection of consolidated clinical microbiology laboratories (where each laboratory could be seen as a real-time sensor in its area) would move laboratory surveillance from public health structures to clinical laboratories [13]. Such a network, more directly linked to the field, demonstrated their abilities to adequately support public health responses during the coronavirus disease 2019 (COVID-19) pandemic. Second, because of their reimbursement by the national health insurance system in Belgium, antigen testing and viral cultures are the main diagnostic methods used before molecular tests for RSV and influenza. At the time of writing, only the SARS-CoV-2 PCR is reimbursed in a limited number of indications (mainly symptomatic or fragile patients requiring admission), therefore clinical laboratories may perform a quadruplex PCR during influenza and RSV season instead of a single SARS-CoV-2 PCR. Routine use of a quadruplex PCR increases the overall number of detected cases compared to targeted PCR.

In the frame of future RSV surveillance, especially with the distribution of vaccines and the development of therapeutic interventions, it seems important that decision-makers favour tools that allow efficient detection and thus better surveillance of RSV. Indeed, with an ageing population, RSV may become a growing burden and surveillance of RSV will be of interest to promote vaccination [14]. Although antigen testing can be used in infants due to its relative cost-effectiveness, this method is inaccurate in older patients [5]. Patients requiring hospitalisation should benefit from more expensive rapid multiplex PCRs to allow better clinical management and relevant hygiene precautions [6]. As shown in this study, the intertwinement of influenza, RSV and probably SARS-CoV-2 could clinically justify the systematic use of such multiplex PCRs during the epidemic season. Likewise, the surveillance of epidemic trends would benefit from data more accurate than the sole number of positive cases, such as the positivity rate and perhaps semi-quantitative approaches based on Ct values [15]. Indeed, as described for COVID-19, the use of the Ct value of RT-PCR could help a better prediction of influenza and RSV trends [9]. Furthermore, the use of molecular diagnostic methods applied in the frame of syndromic approaches would also allow the detection of multiple respiratory pathogens and, for some, the testing of influenza virus subtypes [16]. The overlap of the epidemics of RSV, influenza and COVID-19 during winter as well as the difficulties, especially for elderly, to clinically distinguish these infections [17], makes it more convenient for both laboratories and clinicians to use one multiplex assay. However, the use of such an assay may lead to an increase in the detection of RSV, as a side-effect of the spread of the other viruses it detects at a given time. This should be taken into consideration for the passive surveillance of respiratory viruses.

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Ethics statement: This study is an epidemiological retrospective observational study using aggregated anonymous data; therefore, no ethical approval was required.

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