



Comprehensive real-time epidemiological data from respiratory infections in Finland between 2010 and 2014 obtained from an automated and multianalyte mariPOC® respiratory pathogen test

M. Gunell^{1,2} · P. Antikainen³ · N. Porjo³ · K. Irjala⁴ · J. Vakkila⁴ · K. Hotakainen^{4,5} · S. S. Kaukoranta⁶ · J. J. Hirvonen⁶ · K. Saha⁷ · R. Manninen⁸ · B. Forsblom⁹ · K. Rantakokko-Jalava² · V. Peltola¹⁰ · J. O. Koskinen³ · P. Huovinen¹

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Abstract Respiratory viruses cause seasonal epidemics every year. Several respiratory pathogens are circulating simultaneously and typical symptoms of different respiratory infections are alike, meaning it is challenging to identify and diagnose different respiratory pathogens based on symptoms alone. mariPOC® is an automated, multianalyte antigen test which allows the rapid detection of nine respiratory infection pathogens [influenza A and B viruses, respiratory syncytial virus (RSV), human metapneumovirus, adenovirus, parainfluenza 1–3 viruses and pneumococci] from a single nasopharyngeal swab or aspirate samples, and, in addition, can be linked to laboratory information systems. During the

study period from November 2010 to June 2014, a total of 22,485 multianalyte respi tests were performed in the 14 participating laboratories in Finland and, in total, 6897 positive analyte results were recorded. Of the tested samples, 25 % were positive for one respiratory pathogen, with RSV (9.8 %) and influenza A virus (7.2 %) being the most common findings, and 0.65 % of the samples were multivirus-positive. Only small geographical variations in seasonal epidemics occurred. Our results show that the mariPOC® multianalyte respi test allows simultaneous detection of several respiratory pathogens in real time. The results are reliable and give the clinician a picture of the current epidemiological situation, thus minimising guesswork.

✉ M. Gunell
marianne.gunell@utu.fi

¹ Medical Microbiology and Immunology Unit, University of Turku, Kiinamylynkatu 13, 20520 Turku, Finland

² TYKS-Sapa, Microbiology and Genetics Service Area, Microbiology Branch, Turku University Hospital, Turku, Finland

³ ArcDia International Oy Ltd, Turku, Finland

⁴ Mehiläinen Private Health Care Center, Espoo, Helsinki, Oulu, Tampere, and Turku, Finland

⁵ Department of Clinical Chemistry, University of Helsinki, Helsinki, Finland

⁶ Vaasa Central Hospital, Vaasa, Finland

⁷ Seinäjoki Central Hospital, Seinäjoki, Finland

⁸ SataDiag, Satakunta Central Hospital, Pori, Finland

⁹ Carea, Kymenlaakso Social and Health Services, Kotka, Finland

¹⁰ Department of Pediatrics and Adolescent Medicine, Turku University Hospital and University of Turku, Turku, Finland

Introduction

Every year, respiratory viruses cause seasonal epidemics. In Northern Europe, the epidemic season starts in September–October and lasts until April. Since several respiratory pathogens are circulating at the same time and typical symptoms of different respiratory infections (cough, fever, rhinitis, headache etc.) are quite similar, it can be difficult to identify and diagnose different pathogens based on symptoms alone. To be able to avoid unnecessary antimicrobial treatment and to focus antiviral treatment properly, rapid and reliable detection of respiratory pathogens is needed. At the moment, verified viral results in Finland are reported to the Register for Infectious Diseases of the National Institute for Health and Welfare (NIHW, <http://www.thl.fi/ttr/gen/rpt/tilastot.html>, [1]). Data from this national register go to The European Surveillance System (TESSy), a data bank organised by the European Centre for Disease Prevention and Control (ECDC, [2]), and

these European-wide data are further combined with data collected all over the world by the World Health Organization (WHO, [3]). After the data are collected, they need to be analysed and further processed into reports, and this can take 1–2 weeks. Thus, although these reports are extensive and usually rapidly and widely available in electronic format, the data themselves have already expired. In addition, at least in Finland, the data do not represent the outpatient setting, but hospitalised patients and military recruits. New rapid and automated multianalyte tests could potentially help to provide more accurate and real-time data to help health care professionals in infection management and containment, especially in outpatient settings.

mariPOC[®] (ArcDia International Oy Ltd, Turku, Finland) is an automated, multianalyte antigen test for the rapid detection of respiratory infection pathogens from a single nasopharyngeal swab or aspirate sample [4, 5]. The mariPOC[®] respi test allows the detection of nine clinically relevant pathogens from the upper and lower respiratory tracts. The test panel includes: influenza A and B viruses (IAV and IBV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), adenovirus (AdV), parainfluenza 1–3 viruses (PIV 1, 2, 3) and *Streptococcus pneumoniae*. The automated mariPOC[®] analyser has been designed for point-of-care use and it can be connected to laboratory information systems (LIS). The majority (75 %) of positive results are reported within 20 min, while final results are reported after 2 h for low positive and negative samples.

The aim of our study was to investigate and analyse the epidemiological data of respiratory pathogens in Finland recorded by the mariPOC[®] respiratory tests during the seasonal epidemics between November 2010 and June 2014. In addition, the benefits of using the mariPOC[®] multianalyte test in rapid diagnostics and in collecting epidemiological data, for doctors and for patients, are discussed.

Materials and methods

Specimen collection

The nasopharyngeal swab samples and aspirates for mariPOC[®] analyses were collected in 14 hospitals and private health care centres in different parts of Finland: Oulu, Vaasa, Seinäjoki, Pori, Turku, Tampere, Espoo, Helsinki, Vantaa, Kotka and Kouvola (Fig. 1). mariPOC[®] tests performed between week 48 in 2010, i.e. at the beginning of the seasonal epidemic, and week 24 in 2014 were included in this study. During the first respiratory virus season, the only participant was Satakunta Central Hospital (Pori). The majority of laboratories started to analyse samples at the beginning of the epidemic season 2011–2012, and continued until the end of season 2014 (Fig. 2). Samples were taken from patients

suffering from typical symptoms of respiratory tract infection, whenever aetiological diagnostics was considered necessary by the attending physician. Nasopharyngeal swab samples were collected and handled as previously described [4, 5]. This study was performed retrospectively, based on epidemiological data collected as part of routine diagnostic procedures and, therefore did not require any ethical committee approval. Patient data were not handled in any way.

mariPOC[®] rapid antigen detection

The mariPOC[®] platform (ArcDia International Oy Ltd, Turku, Finland) is based on a two-photon fluorescence excitation detection technique, where micro-volume immunometric antigen detection is performed in a separation-free assay format [6, 7]. mariPOC[®] is a fully automated system, allowing random-access analysis. Preliminary results are automatically reported after 20 min. Samples were analysed with the mariPOC[®] respi test according to the instructions for use. It was used in various different types of clinical units, including point-of-care use by nurses where tests were done 24/7, private health care clinics, and clinical microbiology and clinical chemistry emergency laboratories, where tests were performed by biomedical lab nurses.

mariPOC[®] data collection

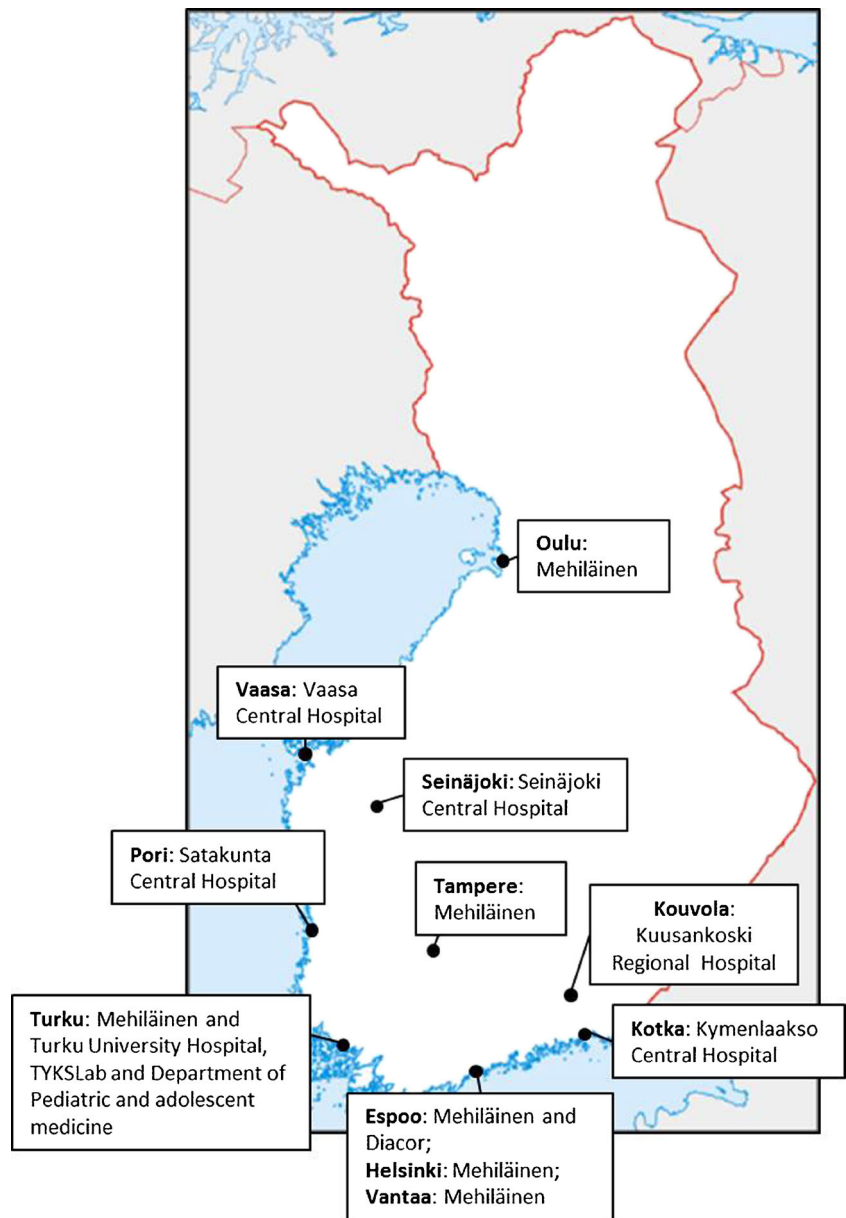
Epidemiological data were collected by the remote access service of the test system. This allows simultaneous and retrospective analysis of the data recorded by all mariPOC[®] users. Both site-specific data and various combinations were analysed. The incidence of viruses recorded by mariPOC[®] was compared to the surveillance data collected by the virology unit of the NIHW. The total number of performed mariPOC[®] tests and the number of positive results for each tested respiratory pathogen were downloaded from participating laboratories on a weekly basis by ArcDia's personnel.

Results

Respiratory pathogen results

During the study period from November 2010 to June 2014, a total of 22,485 multianalyte respi tests were performed in the 14 participating laboratories and a total of 6897 positive analyte results were recorded. A summary of the positive samples recorded by the mariPOC[®] respi test is shown in Fig. 3. Three clear epidemiological peaks were detected in the second (2011–2012) to fourth (2013–2014) seasonal respiratory pathogen epidemics. During the first annual epidemic, 2010–2011, only one unit participated in this study and 475 tests were performed. The peak in testing was detected at week 10/

Fig. 1 Finnish health care centres and hospitals participating in this study



2011, when 68 samples were tested and nine of these were influenza B virus positive. Also, a small RSV peak (eight positive samples) was detected at week 15/2011.

RSV and IAV were the most commonly detected respiratory viruses included in the test panel in Finland during the study

period. Ten percent of the tested samples were positive for RSV and 7 % for IAV (Table 1). During the annual epidemics, equal amounts of positive IAV and RSV findings were detected. The proportion and the number of positive IAV and RSV findings were highest during the 2011–2012 epidemic

Fig. 2 The time span for mariPOC® respi test data collection from different units in Finland

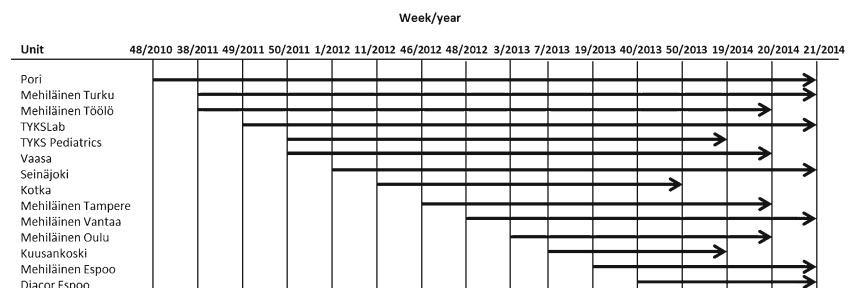
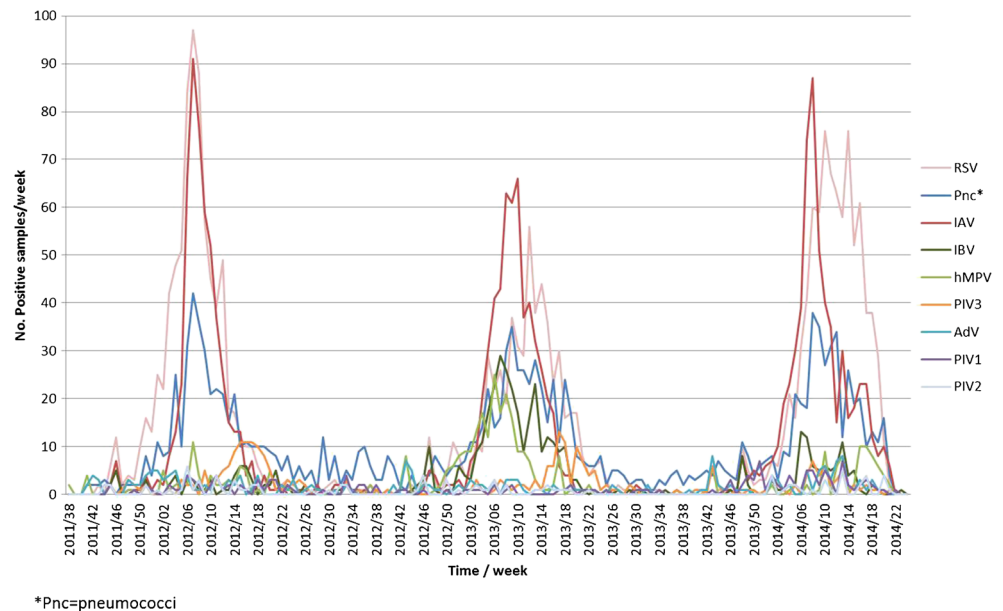


Fig. 3 The numbers of positive respiratory pathogen results in Finland between 2011 and 2014, detected with the mariPOC[®] respi test



(Table 2). The annual epidemiological peaks of RSV and IAV also occurred almost simultaneously, RSV a few weeks later than IAV (Table 2 and Fig. 3).

Other respiratory pathogens included in the mariPOC[®] respi test panel were found less frequently, except IBV and hMPV, of which larger epidemics were seen during the winter of 2013 (Table 2 and Fig. 3). Pneumococci were the second most common respiratory pathogen detected (Table 1). Three distinct peaks for pneumococci were detected during the annual epidemics (Table 2). However, samples positive for pneumococci were also detected outside the seasonal epidemics, especially in late summer to early fall (weeks 29–41) in 2012, when 14 % (78/577) of the samples were positive for pneumococci (Fig. 3).

A total of 6150 (27 %) patient samples were positive for the respiratory pathogens included in the mariPOC[®] respi test panel. During the seasonal epidemics, up to 44–58 % of the

samples were positive for at least one respiratory pathogen (Fig. 4). Excluding pneumococci (due to the substantially high carriage rate [8]), viral co-infection was detected among 0.65 % of the tested samples (3 % of the positive samples, $n = 146$). Of these, 67 % ($n = 98$) were positive for two viruses. RSV was the most common virus present in co-infections: it was found in 70 % of two-virus infections, in 61 % of infections with three viruses and in 100 % of the findings positive for four viruses. RSV/IAV co-infection was the most common combination ($n = 26$), followed by RSV/PIV1 ($n = 12$), RSV/PIV3 ($n = 7$) and RSV/hMPV ($n = 6$) combinations.

Epidemiological peaks of different respiratory pathogens varied annually. During the 2011–2012 epidemic, the highest incidences of IAV, RSV, pneumococci and hMPV were detected simultaneously at week 7, whereas during the 2012–2013 epidemic, peaks in the positive findings of different respiratory pathogens varied from week 6 (hMPV) to week 12

Table 1 The number and proportions of positive results, and the proportions of different respiratory pathogens among all positive findings recorded by the mariPOC[®] respi test during the study period

| Respiratory pathogen | No. of positive findings (% of tested) | No. of tested samples | Proportion of all positive findings |
|-----------------------------|--|-----------------------|-------------------------------------|
| Respiratory syncytial virus | 2197 (9.8) | 22,422 | 32 % |
| Pneumococci | 1456 (9.4) | 15,559 | 21 % |
| Influenza A virus | 1625 (7.2) | 22,420 | 24 % |
| Influenza B virus | 489 (2.2) | 22,324 | 7 % |
| Human metapneumovirus | 386 (1.7) | 22,354 | 6 % |
| Parainfluenza virus 3 | 280 (1.3) | 22,298 | 4 % |
| Adenovirus | 219 (1.0) | 22,309 | 3 % |
| Parainfluenza virus 1 | 151 (0.7) | 22,259 | 2 % |
| Parainfluenza virus 2 | 94 (0.4) | 22,263 | 1 % |
| Total | 6897 (30.7) | 22,485 | 100 % |

Table 2 Annual epidemiological peaks, and the number and proportion of positive respiratory syncytial virus (RSV), pneumococcal, influenza A and B viruses (IAV and IBV) and human metapneumovirus (hMPV) findings during the epidemiological peaks

| Respiratory pathogen | Epidemiological peak (week) | No. of positive findings (%) |
|----------------------|-----------------------------|------------------------------|
| RSV | 7/2012 | 97 (21) |
| | 12/2013 | 56 (16) |
| | 10/2014 | 76 (16) |
| | 14/2014 | 76 (24) |
| Pneumococci | 7/2012 | 42 (16) |
| | 9/2013 | 35 (11) |
| | 8/2014 | 38 (9) |
| IAV | 7/2012 | 91 (20) |
| | 10/2013 | 66 (16) |
| | 8/2014 | 87 (15) |
| IBV | 15/2012 | 6 (3) |
| | 16/2012 | 6 (3) |
| | 7/2013 | 29 (8) |
| | 6/2014 | 13 (3) |
| hMPV | 7/2012 | 11 (2) |
| | 6/2013 | 25 (8) |
| | 16/2014 | 10 (4) |
| | 17/2014 | 10 (4) |

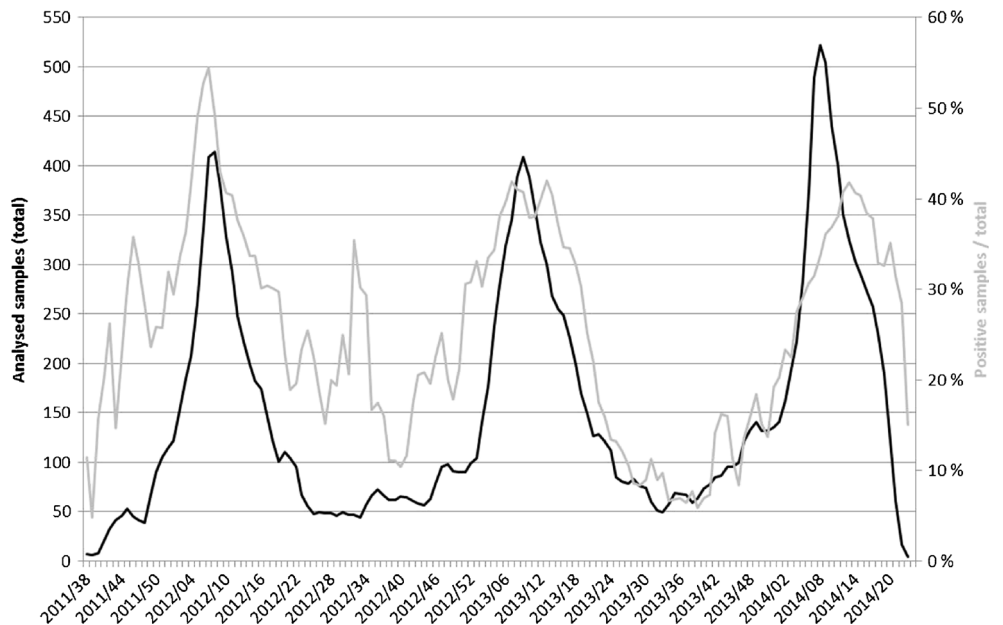
(RSV). A wide variation in the incidence of respiratory pathogens was also seen during the 2013–2014 annual epidemic (Table 2 and Fig. 3).

Geographical variation

mariPOC[®] respi test results from different laboratories participating in this study were also analysed by dividing units into geographical areas: Western Finland (Satakunta Central Hospital, Vaasa Central Hospital and Southern Ostrobothnia

Central Hospital), South-West Finland (Mehiläinen Turku, TYKSLab and Department of Pediatrics/Turku University Hospital), Southern Finland (Mehiläinen Espoo, Vantaa and Töölö; Diacor Espoo) and South-East Finland (Kuusankoski Regional Hospital and Kymenlaakso Central Hospital). Small variations in prevalence and epidemiological peaks of different respiratory viruses were seen. In our study, RSV was the most commonly detected respiratory virus, and IAV was more prevalent only in South-East Finland during the 2012–2013 and 2013–2014 epidemics (Fig. 5a, b). During the 2011–2012

Fig. 4 The number of tested samples compared with the percentage of positive results, detected with the mariPOC[®] respi test



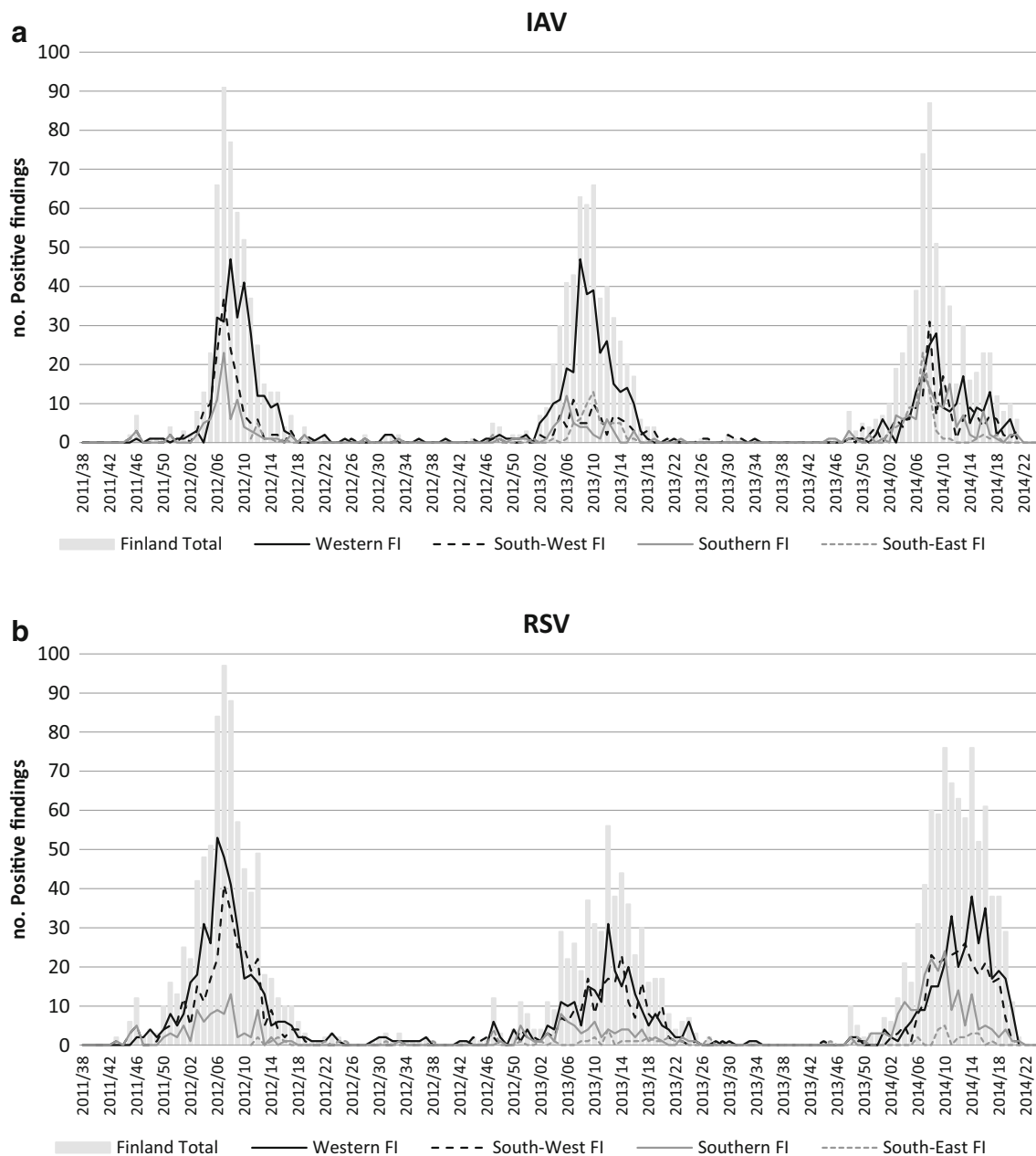


Fig. 5 Influenza A virus (IAV, **a**) and respiratory syncytial virus (RSV, **b**) epidemics in different parts of Finland

annual epidemic, RSV and IAV peaks were detected almost simultaneously at week 7/2012. During the 2012–2013 annual epidemic, the RSV peak in Southern Finland had occurred already in week 5/2013, 7–9 weeks earlier than in other areas, whereas the IAV epidemic occurred almost simultaneously, between weeks 6–10/2013. During the 2013–2014 annual epidemic, IAV and RSV peaks arrived in adjacent weeks (7 and 8/2014) in Southern Finland, whereas in other parts of Finland, the IAV peak came 3 to 5 weeks earlier than the RSV peak (Fig. 5a, b). Geographically, a wider variation was seen in the RSV annual epidemiological peak compared to the IAV peak. RSV epidemics also lasted longer than IAV epidemics, and broader peaks were detected especially on the west coast

of Finland (Fig. 5b). In early 2013, there was also a small IBV epidemic in Western Finland, which was detected in week 8/2013, simultaneously with the IAV peak (Fig. 3).

Frequency of testing versus positive findings

During the seasonal epidemics, the number of performed respiratory tests was eight times higher compared to tests performed outside the epidemic season. In Fig. 5, the three-week running average of the number of analysed samples and the proportion of positive samples are presented. Despite the fluctuation in the proportion of positive findings, especially outside the epidemics, the highest numbers of positive findings

were detected during the epidemiological peaks, when respiratory pathogens are circulating abundantly. It also shows that a peak in testing appears at the same time (weeks 8 and 9) in different years (2012–2014), whereas the peak in the proportion of positive samples varied between different years: in 2012, the epidemiological peak was 1 week ahead of the testing peak; in 2013, there were two distinct epidemiological peaks and the testing peak hit in the middle of these peaks; and in 2014, the testing peak was actually 5 weeks ahead of the epidemiological peak (Fig. 4).

Respiratory pathogen epidemics occurred simultaneously all over Finland during the 2011–2012 and 2013–2014 annual epidemics. However, during the 2012–2013 epidemic, a variation between different geographic areas was seen, when a peak in testing was detected at week 6 in Southern Finland, week 9 in Western Finland, week 10 in South-East Finland and as late as week 16 in South-West Finland. A comparison of the positive results with the number of tested samples showed that the relative proportion of positive IAV and RSV samples correlated with the number of positive findings per week. During the seasonal epidemics, the percentage of IAV- and RSV-positive samples were up to 20 and 25 % of the tested samples, respectively, and outside the epidemics, the percentage of positive samples was less than 5 %. The percentage of findings positive for pneumococci remained the same, approximately 10 %, during the whole study period.

Discussion

The mariPOC[®] respi test is a rapid and validated test methodology for respiratory pathogens: more than half of the positive results are obtained within 20 min and the final results within 2 h, with sensitivities for influenza viruses and RSV of 80–90 % and analytical specificities ≥ 99 %, on average [4, 5]. The mariPOC[®] respi test preliminary results at 20 min showed 99.9 % analytical specificity, and, on average, a maximum 1 out of 100 tested samples gives a false-positive result. A positive preliminary result is true in 97 % of cases, on average. For IAV, the positive predictive value is up to 97.8 %, and for RSV 99.1 % (data not shown). Negative fluorescence readouts (results) are not reported in the preliminary phase, since some may turn positive in the 2 h that it takes for the assay to reach equilibrium. Nevertheless, the negative predictive value of the preliminary results (no results shown) is 98 %, indicating that most of the preliminary negatives remain negative for that particular analyte also after 2 h. Typically, POC tests are rapid, but less sensitive than polymerase chain reaction (PCR)-based methods. However, mariPOC[®] antigen detection has shown good sensitivity, especially in the detection of influenza viruses and RSV and group A *Streptococcus*, and the specificities of all methods have been excellent [4, 5, 9]. Ivaska et al. reported that mariPOC[®] had, in their study, only a moderate sensitivity for AdV and hMPV

compared to PCR, but also noted that, sometimes, DNA/RNA findings by PCR may be clinically non-relevant [4]. Thus, PCR methods can be sometimes even too sensitive.

The LIS feature allows a rapid diagnosis, accurate reporting of results and data storage, and use of the results in real-time. This real-time epidemiological data can be a beneficial tool for health care professionals; based on the local epidemiology of circulating respiratory pathogens, testing procedures can be optimised based on real knowledge on what and when certain respiratory pathogens should be tested and, therefore, diagnoses can be made more accurately. Sharing this information with patients might make it easier for doctors to justify not prescribing antibiotics. Mehiläinen, a country-wide private health care provider in Finland, already has such a tool, where positive virus results and the numbers of tests performed in the laboratory are updated on a daily basis, and the epidemiological trend is presented in real time to their clinicians and laboratories all over the country.

The introduction of mariPOC[®] respi testing in Finland has significantly increased the number of tests performed, and has enabled pathogen-specific diagnosis, especially for viruses for which no other rapid multianalyte tests are available. Individual users may run 1000–2000 tests a year, a magnitude similar to that reported by official national epidemiological follow-up systems (e.g. the NIHW). Thus, potentially available epidemiological data have reached completely new levels, and this should be put into use more efficiently for real-time epidemiological surveillance. In contrast to conventional rapid assays, the mariPOC[®] technique is characterised by an automated results readout. Thus, the analysers could be connected not only to LIS but also via the internet to a national or global database and surveillance system, allowing health care authorities to monitor ongoing infections online. Such a surveillance system would support the control of infectious diseases and epidemics, and provide an ‘early warning’ system for changes in pathogen activities [10–12]. Influenza virus, for example, causes milder disease before the epidemic peak arrives. Once the number of positive cases increases, the incidence and proportion of more severe diseases also increase [13]. This would have a high impact both for the individual and for society.

Our results show that, within the limits of the test’s sensitivities, all the respiratory pathogens included in the mariPOC[®] respi test panel were detected, although a wide variation in frequencies was seen. Our study shows that RSV (10 %) and IAV (7 %) were the most commonly detected viruses in Finland during the whole study period, whereas other tested viruses (AdV, IBV, PIV 1–3 and hMPV) were found only in small proportions (<2 %). Although the test’s slightly variable sensitivity against different viruses might have some impact on the results, the epidemiological picture produced by the mariPOC[®] respi test was well in line with virus results reported from the virology unit at the NIHW. Proportions of AdV and IBV findings detected with

mariPOC[®] were the same size as those reported in the Register for Infectious Diseases of the NIHW (<http://www.thl.fi/ttr/gen/rpt/tilastot.html>). When IAV and RSV results are compared, RSV is detected more frequently with mariPOC[®], whereas higher numbers of IAV are reported in the NIHW register compared to RSV (data not shown). However, the NIHW register is currently updated only monthly, although the data is collected on a weekly basis; thus, it is retrospective and does not provide the current clinical situation. In contrast, mariPOC[®] provides real-time data also for end-point users, i.e. clinicians. Moreover, the data for the NIHW register are collected from hospitals and garrisons and, therefore, do not reflect the epidemiological situation in the population visiting health care centres in Finland. Our data include probably more samples from child patients compared to the NIHW register.

The number of participating laboratories increased during the study, and the number of tested samples varied between the annual epidemics. It is notable that the highest numbers of positive findings were detected already in 2012 at week 7, when only seven out of 14 units participated in this study (Fig. 2); thus, the 2011–2012 seasonal epidemic was actually more severe than the following epidemics. Our results show that the seasonal epidemics of respiratory pathogens start almost simultaneously all over Finland, and only slight variations in epidemiological peaks were detected geographically. Our geographically divided data also showed that the prevalence of different pathogens varied between different areas. For example, no clear RSV peak was detected in South-Eastern Finland, and the IBV epidemic in 2013 was concentrated in Southern Finland. In Northern Europe, IAV epidemics are concentrated in the winter months, and the peak is usually in February [14]. RSV epidemics occur simultaneously with IAV, and the peak is in February, but with a wider time span [14, 15]. Our results were in concordance with these reports: the IAV peak was commonly detected between weeks 7 and 9, whereas the annual RSV peak had a wider range (from week 7 in 2012 to week 14 in 2014). Minor geographical differences were also detected: in South-Eastern Finland, RSV epidemics were smaller, and in Southern Finland, the 2013 epidemiological peak was detected already at week 5.

We have shown in this study that 27 % of all the tested nasopharyngeal swabs were positive for at least one of the respiratory pathogens included in the mariPOC[®] respi test and, during the epidemics, the percentage was even higher. Samples were taken from patients suffering from symptoms of respiratory infection; thus, the patients with a sample negative for the tested viruses probably had some other respiratory virus. For example, rhinovirus, which is the predominant cause of common cold, coronaviruses, which are a significant cause of common cold in adults, human bocavirus, which might cause lower respiratory tract infections and non-polio enteroviruses, which can cause mild respiratory symptoms,

circulate at the same time as the pathogens currently included in the mariPOC[®] respi test panel [16–18]. Since the sensitivity of any rapid POC immunoassay test can hardly be 100 %, some positive cases have supposedly gone undetected. Based on the previous studies, this fraction may be expected to be 10–20 % with mariPOC[®] [4, 9].

Our results show that the percentage of positive findings followed the seasonal epidemiology. During the epidemics, the number of positive findings was highest, but also the proportion of positive findings increased, indicating a real epidemiological event. This also indicates that the testing for respiratory viruses, especially IAV and RSV, is correctly scheduled. During off-season, between May and October, only sporadic infections are encountered, and only 1.4 % of tested samples are positive. Findings positive for pneumococci did not follow the seasonal epidemics, and the percentage of positive findings, 10 %, can signify carriage: 5–10 % of adult and >30 % of child patients have been reported to be carriers of pneumococci [8, 19].

It is commonly known that the annual circulation of different respiratory pathogens follow a certain pattern. For example, IAV and RSV circulation shows a wave-like pattern, whereas adenovirus is present throughout the year. It is also commonly known that RSV causes larger epidemics biannually [15, 20], which could also be seen in our material: RSV was detected more frequently in samples in 2012 and 2014 compared to 2013 (Table 2 and Fig. 3). Based on these commonly known patterns for respiratory virus circulation, clinicians probably use this ‘educated guess’ when they order laboratory tests for their patients. However, this educated guess can also cause bias: if RSV is tested more often every other year, it can be expected to be found more frequently. If the mariPOC[®] multianalyte test is used, this forethought has no impact on results. With multianalyte testing, it is possible to get results on several pathogens simultaneously, and although the swab might have been taken to assure RSV positivity, the result might show that the patient actually has an adenovirus or an RSV–pneumococcal co-infection. Our results show that viral co-infection was detected among 0.65 % of the tested samples (3 % of the positive samples), and 67 % of these were double infections, with RSV being the most common virus present in co-infections. Thus, mariPOC[®] detects and reports the actual epidemiological situation of respiratory pathogens.

In conclusion, this is the first comprehensive study of respiratory virus epidemiology in Finland. We have shown that, with the mariPOC[®] multianalyte respi test, several respiratory pathogens could be detected in real time, and the results were reliable and reflected the true epidemiological situation, not only common expectations. Seasonal epidemics of respiratory pathogens occurred simultaneously all over Finland, with small geographical variations, and RSV and IAV were the most common viruses in Finland, as detected with the mariPOC[®] respi test.

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Compliance with ethical standards

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Conflict of interest None.

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