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A discussion of syndromic molecular testing for clinical care

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Current molecular detection methods for single or multiplex pathogens by real-time PCR generally offer great sensitivity and specificity. However, many infectious pathogens often result in very similar clinical presentations, complicating the test-order for physicians who have to narrow down the causative agent prior to in-house PCR testing. As a consequence, the intuitive response is to start empirical therapy to treat a broad spectrum of possible pathogens. Syndromic molecular testing has been increasingly integrated into routine clinical care, either to provide diagnostic, epidemiological or patient management information. These multiplex panels can be used to screen for predefined infectious disease pathogens simultaneously within a 1 h timeframe, creating opportunities for rapid diagnostics. Conversely, syndromic panels have their own challenges and must be adaptable to the evolving demands of the clinical setting. Firstly, questions have been raised regarding the clinical relevance of some of the targets included in the panels and secondly, there is the added expense of integration into the clinical laboratory. Here, we aim to discuss some of the factors that should be considered before performing syndromic testing rather than traditional low-plex in-house PCR.

Introduction

Innovations to improve rapid diagnostics for infectious diseases are essential to patient care. Traditional molecular singleplex tests involve the detection of one pathogen from a patient sample; these tests provide an affordable, rapid approach to answering a diagnostic question. However, the increasing demand for high-throughput tests that can detect a greater number of pathogens has propelled multiplex testing to the forefront of routine diagnostics.[1,2] Syndromic molecular panels are used to screen for predefined (pathogen) targets associated with a particular syndrome, most commonly for respiratory, gastrointestinal (GI), neurological or sexually transmitted disease presentation (Tables 1–4). Multiplex panels, which allow for the simultaneous detection of multiple targets (usually more than five pathogens), can provide a rapid diagnosis and inform patient management strategies.[3] Several syndromic panels are currently on the market for upper respiratory tract infections, pneumonia, gastroenteritis, meningitis/encephalitis, sepsis and sexually transmitted infections.[3–6] However, these syndromic panels have some disadvantages. They are more expensive than singleplex tests and the clinical relevance of some targets included in these broad panels has been questioned; firstly, in regard to whether broad screening should be restricted to specific patients, and secondly, making the point that the disease prognosis of many pathogens is still unknown.[7,8] As syndromic testing becomes more integrated into routine virology, it is important to acknowledge the factors that drive its application instead of traditional singleplex or low-plex (fewer than five

targets) testing. This paper discusses some of the important factors to consider before performing syndromic testing.

Factors to evaluate before performing syndromic testing

Factor 1. Quality of the syndromic panel

Quality, which is linked to diagnostic validity, is arguably the most important aspect of any diagnostic test. An inaccurate test has the potential to undermine patient safety and affect patient management, particularly if a result is believed to be a false negative or a false positive. Therefore, the accuracy and clinical utility of each molecular test assay is tested prior to its use for diagnostic purposes. For clinical purposes, this involves consideration of critical measurements such as sensitivity, specificity and limit of detection.[8] Sensitivity of a diagnostic test is defined as the proportion of positive samples that are correctly identified as positive related to disease, while specificity is the proportion of negative samples that are correctly identified as negative.

The number of syndromic panels available and the number of targets within each panel are increasing; as a result, it is important to recognize that the sensitivity and specificity of the same target within each assay may vary. For example, the FilmArray ME reportedly offers a 94.2% overall sensitivity and 99.8% specificity; however, it performs poorly in detecting *Cryptococcus neoformans* and *Cryptococcus gattii,* with 52% sensitivity and 80% specificity.[5] Such variations in sensitivity and specificity within an assay can impact

Table 1. Available syndromic respiratory panels

Characteristics	BioFire RP 2.1[a]	QIAstatDx RP V2[a]	NxTAG RPP[a]	Verigene	ePlex RP2
Virus targets					
Adenovirus	✓	✓	✓	✓	✓
CoV-NL63	✓	✓	✓		✓
CoV-2229E	✓	✓	✓		✓
CoV-HKU1	✓	✓	✓		✓
CoV-OC43	✓	✓	✓		✓
Human bocavirus		✓	✓		
HMPV	✓	✓	✓	✓	✓
Influenza A	✓	✓	✓	✓	✓
Subtype H1	✓	✓	✓	✓	✓
Subtype H3	✓	✓	✓	✓	✓
Subtype H1N1/2009	✓	✓			✓
Influenza B	✓	✓	✓	✓	✓
Parainfluenza 1	✓	✓	✓	✓	✓
Parainfluenza 2	✓	✓	✓	✓	✓
Parainfluenza 3	✓	✓	✓	✓	✓
Parainfluenza 4	✓	✓	✓	✓	✓
RSV	✓	✓			
RSV A			✓	✓	✓
RSV B			✓	✓	✓
Rhinovirus/enterovirus	✓	✓	✓	✓	✓
SARS-CoV-2	✓	✓	✓		✓
Bacteria targets					
Bordetella pertussis	✓	✓		✓	
Bordetella parapertussis	✓			✓	
Bordetella holmesii				✓	
Chlamydophila pneumoniae	✓		✓		✓
Legionella pneumophila		✓			
Mycoplasma pneumoniae	✓	✓	✓		✓
Panel information					
Platform	FilmArray system	QIAstat-Dx	Luminex Magpix	Verigen system	ePlex System
Company	BioFire/bioMérieux	QIAGEN	LUMINEX	LUMINEX	GenMark
Targets in panel	22	22	21	16	21
Throughput	Low-Medium	Low	High	Low-Medium	Low-Medium
Time to result	45 min	70 min	4 h	2 h	3.5 h

[a]The most recent panel includes SARS-CoV-2, human metapneumovirus (HMPV) and respiratory syncytial virus (RSV).

clinical accuracy. For example, in an ICU ward with 100 patients and a SARS-CoV-2 prevalence of 60%, an assay with 90% sensitivity and 95% specificity for the SARS-CoV-2 target would yield false negative results for six patients. These false negative results could lead to an increase in nosocomial transmission. Additionally, three patients would have a false positive result that could lead to unnecessary isolation.

Another important measure to consider is the limit of detection, or analytical limit, which determines the lowest pathogen load that will still be detected reliably. As syndromic panels are developed by various companies, each panel may have a different approach for each target, different subtypes for a target, or even a different selection of targets. Such variability may pose challenges when attempting to compare analytical sensitivity data, as inter-platform differences can greatly impact interpretation of

results. Additionally, given the greater number of viral targets and improvements in turnaround time, this could also lead to a reduction in sensitivity.[3,9,10] International standards and external quality assurance, such as that organized by Quality Control Medical Diagnostics (QCMD, Scotland), can help mediate inter-platform differences. Regulators can also assist with investigating and collecting information on viral load distribution of different pathogens. Investigating the limit of detection and viral load distribution of pathogens of interest can also help provide valuable information about clinical relevance and what results to expect. Figure 1 depicts the average viral load, which is represented by the cycle threshold (Ct) value, (Ct values) from a broad spectrum of respiratory viruses at the University Medical Centre Groningen from 2015 to 2020. While influenza B virus and respiratory syncytial virus (RSV) generate high viral loads, on average, parainfluenza type 2

Table 2. Available gastrointestinal panels

Targets/other details	BioFire GI	QIAstatDx GI	Luminex GPP	Verigene EP
Bacteria				
Campylobacter species	✓	✓	✓	✓
Clostridioides difficile (*tcdA/tcdB*)	✓	✓	✓	
Escherichia coli O157	✓	✓	✓	
Enterotoxigenic *E. coli*	✓	✓	✓	
Enteropathogenic *E. coli*	✓	✓		
Enteroaggregative *E. coli*	✓	✓		
Salmonella species		✓	✓	✓
Plesiomonas shigelloides	✓	✓		
Shiga toxin-producing *E. coli* (*stx1-stx2*)	✓	✓	✓	✓[a]
Shigella species/enteroinvasive *E. coli*	✓	✓	✓	✓
Vibrio species	✓			✓
Vibrio cholerae	✓	✓	✓	
Vibrio parahaemolyticus		✓		
Vibrio vulnificus		✓		
Yersinia enterocolitica	✓	✓	✓	✓
Viruses				
Norovirus	✓		✓	✓
Norovirus GI		✓		
Norovirus GII		✓		
Rotavirus A	✓	✓	✓	✓
Astrovirus	✓	✓		
Adenovirus F40, F41	✓	✓	✓	
Sapovirus	✓	✓		
Parasites				
Cryptosporidium species	✓	✓	✓	
Entamoeba histolytica	✓	✓	✓	
Giardia lamblia	✓	✓	✓	
Cyclospora cayetanensis	✓	✓		
Panel information				
Platform	FilmArray system	QIAstat-Dx	Luminex Magpix	Verigen system
Company	BioFire/bioMérieux	QIAGEN	LUMINEX	LUMINEX
Targets in panel	22	24	15	9
Throughput	Low-Medium	Low	Low-Medium	High
Time to result	<1 h	1 h		2 h

[a]*stx1-stx2* are separate targets in this assay.

virus and CoV-OC43 generate low viral loads. It should be noted that viral load does not necessarily correspond to disease severity; a relatively high viral load for one virus may be a normal viral load for another virus.

The sample matrix should also be considered when evaluating clinical relevance and determining the limit of detection. For example, the detection of an adenovirus with a high viral load in a respiratory sample may have a different clinical relevance and subsequent clinical management to the detection of an enterovirus with a low viral load in a CSF sample. Thus, viral load may be relevant depending on the pathogen and population being tested (e.g. paediatric versus haematology transplant patients). More research is needed to evaluate the accuracy of different syndromic panels in specific sample matrices and various patient populations. However, finding a sufficient number of clinical cases to test could take a substantial amount of time.

The test failure rate is another aspect of quality that should be considered. While the failure rate of a test is difficult to examine specifically, tests should ideally have low failure rates. Breakdowns of diagnostic devices can severely interfere with a laboratory's ability to maintain high throughput of patient samples.

Ensuring the quality of syndromic testing

A good method that is used routinely for evaluating the quality of syndromic testing is simply to retest—using the relevant syndromic platform—a large selection of patient samples that have already been tested with in-house methods. Testing sensitivity and specificity using patient material is a reliable way to assess potential clinical benefit; however, questions remain about the acceptable thresholds for these two factors. For instance, a short study by Rhoads *et al.*[11] in 2020 comparing the DiaSorin Simplexa (DiaSorin, Saluggia, Italy), the Abbott ID Now (Abbott, Chicago, IL, USA) and a modified CDC assay found a positive percent agreement

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Table 3. Available meningitis panels

Targets	BioFire ME
Bacteria	
Escherichia coli K1	✓
Haemophilus influenzae	✓
Listeria monocytogenes	✓
Neisseria meningitidis	✓
Streptococcus agalactiae	✓
Streptococcus pneumoniae	✓
Viruses	
Cytomegalovirus (CMV)	✓
Enterovirus	✓
Herpes simplex virus 1 (HSV-1)	✓
Herpes simplex virus 2 (HSV-2)	✓
Human herpesvirus 6 (HHV-6)	✓
Human parechovirus	✓
Varicella zoster virus (VZV)	✓
Fungi	
Yeast	
Cryptococcus neoformans/gattii	✓
Panel information	
Platform	FilmArray system
Targets in panel	14
Throughput	Low-Medium
Hands on time	<5 min
Time to result	1 h

QIAstat-Dx Meningitis/Encephalitis and the central nervous system panel (CNS) from GeneMark Dx are in development.

Table 4. Available sexually transmitted infections panels

| Targets	Xpert® CT/NG
Bacteria	
Chlamydia trachomatis	✓
Neisseria gonorrhoeae	✓
Treponema pallidum	–
Mycoplasma genitalium	–
Mycoplasma hominis	–
Ureaplasma urealyticum	–
Haemophilus ducreyi	–
Viruses	–
Herpes simplex virus 1 (HSV-1)	–
Herpes simplex virus 2 (HSV-2)	–
Human immunodeficiency virus	–
Human papillomavirus	–
Protozoa	–
Trichomonas vaginalis	–
Panel information	
Platform	Xpert
Targets in panel	2
Throughput	Low-Medium
Hands on time	<5 min
Time to result	90 min

(PPA) of 94%, 96% and 100% for the Simplexa and ID Now and CDC assay, respectively. While these assays clearly perform well, high PPA does not entirely preclude the possibility of false-negative results. For example, when treating organ transplant patients in a hospital, false negatives are unacceptable and potentially dangerous owing to the weakened immune systems of these patients.

Additional quality factors to consider before buying or applying syndromic platforms and panels in medical laboratories include laboratory requirements related to quality and employee competence, as well as the *in vitro* diagnostic medical devices (IVD) classification guidelines.[12] While it is true that IVD medical devices, such as syndromic platforms, provide analytical solutions capable of withstanding pre- and post-analytical evaluations for the detection of several pathogens, future CE-IVD decision-rules may require separation of targets or implementation of different workflows.[12] In this case, workflows would need to match the IVD's risk according to developed decision trees generated by ruled-based systems rather than prescriptive systems.[12] Thus, considering that IVDs intended for serious transmissible diseases (e.g. HIV, SARS and syphilis) and at-risk groups (e.g. pregnant women, newborns) may have different risk, the question of how to incorporate such diseases or risk groups in syndromic panels remains.

Limitations in the quality of syndromic testing

Compared with in-house real-time PCR methods, syndromic tests usually have higher limits of detection, which can potentially lead to false-negative results.[13] This could be due to the difficulty in designing commercial syndromic tests that are equally efficient for a greater number of targets, within a single PCR thermal protocol. Alternatively, it could be due to a lower input volume of extracted material. In either case, when choosing a commercial syndromic test, quality is largely outside of a laboratory's control. It is no longer possible for the end users of commercial tests to redesign the primers and probes to accommodate new strains or to re-evaluate thermal protocols due to apparent inefficiencies. Additionally, geographical variation between continents must be considered. For example, HIV-1 has a high circulation in North America and Europe, while HIV-2 has a higher circulation in (West) Africa.[14] If primer sequences were made available, they could potentially be adapted to account for such geographic variations.

In cases of novel strains, such as influenza A virus (H1N1pdm2009), the use of singleplex or low-plex tests to confirm their presence or absence might be more rapid and reliable than syndromic panels, which do not allow for much variation in the target organism.[15] Additionally, in-house tests may be better suited to larger outbreak settings, given their capacity for higher throughput. Conversely, it could be argued that syndromic panels are ideally suited for small outbreak settings, where all results can be confirmed with in-house microbiological assays to ensure no false-positive or false-negative errors occur.[16] For instance, in a hospital ward outbreak, it may be more important to have a rapid turnaround time to results than perfect testing accuracy, as long as testing accuracy does not drop below an acceptable range. The acceptable range for testing accuracy may be determined by each institution or on a per-case basis.
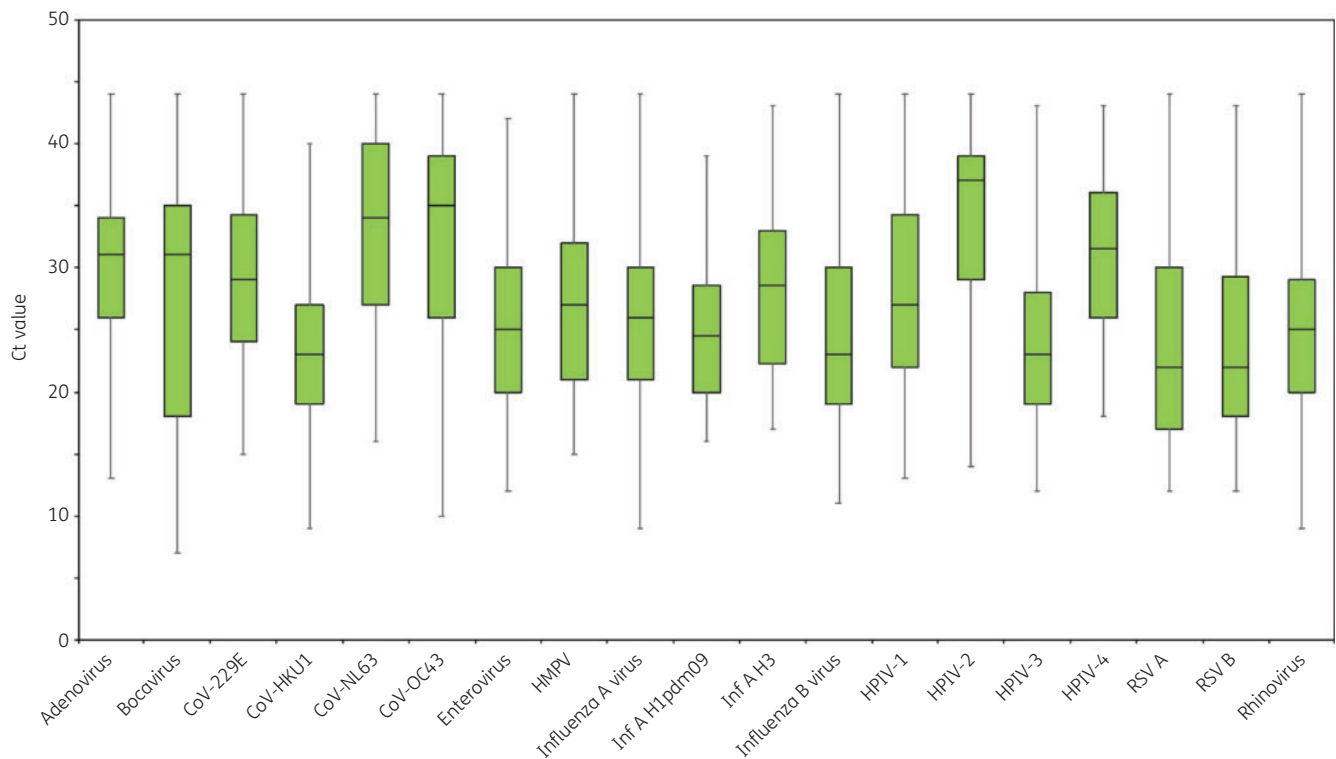
Figure 1. Distribution of cycle threshold (Ct) values of detected respiratory viruses at the University Medical Center Groningen from 2015 to 2020. Inf A, influenza A virus.

Factor 2. Turnaround time of the syndromic panel

Turnaround time (TAT) is an important factor in the selection of a syndromic test, particularly if time is in short supply. TAT is defined as the time it takes between processing the sample for testing and obtaining the result from the device. A major selling point for commercial syndromic tests is short TAT (e.g. $\leq$1 h) and low hands-on time. Decreased hands-on time reduces the potential for human error during sample processing and extraction that is inherent during in-house targeted PCR tests. Rapidly identifying the causative agent not only enables optimized therapy, but also provides information on how best to accommodate the patient, thus reducing overcrowding for unnecessary isolation and limiting the spread of infection.[16,17] Considering the time-consuming nature of in-house PCR (approximately 4–5 h, depending on the number of samples), the shorter TATs of syndromic tests are key for enabling these optimizations. A rapid TAT can also have a significant impact on hospitalization and treatment costs, both for patients and for hospitals.[18,19] For instance, one study found that the implementation of a syndromic panel (Luminex GPP) decreased yearly costs by £66 765 through a reduction of the number of isolation days for hospitalized patients from 2202 to 1447 days.[18]

TAT and throughput go hand in hand. While syndromic panels have faster turnover times, time per sample can vary. Additionally, while an in-house low-plex PCR test takes approximately 5 hours (for 48 samples) from sample processing to result interpretation, a syndromic panel can only test a limited number of samples per hour, depending on the specific syndromic panel. Therefore, it is important to consider both the patient population being tested and the diagnostic costs of implementing the syndromic panels,

extraction platforms and thermal cyclers.[20–22] For instance, Goldenberg *et al.*[18] report an initial start-up cost of £22 283 following implementation of the Luminex GPP syndromic assay.

Challenges related to turnaround time

While shorter TATs are certainly a useful feature of syndromic tests, TAT is not the only factor that determines how quickly patients receive test results. A more holistic measurement of test rapidity would be a measure of the time between sample collection and the availability of results to providers and patients. For instance, if sample collection takes 2 h due to crowded waiting rooms during influenza season, the relevance of reducing a test's TAT by 15 min could be questioned. Nevertheless, if the testing policy is adapted to accommodate these rapid syndromic panels, TAT may be highly relevant. Poelman *et al.*[16] published a new clinical pipeline that integrated sample collection into the workflow. Patients in the study were sampled as soon as they entered the hospital during the 2019 respiratory season. The subsequent results of the syndromic test were made available by the time the patient left the emergency department in 89% of cases.[16] Having this information ready when the patient leaves the emergency department is a great advantage during a respiratory season or even in an outbreak scenario, particularly when a limited number of isolation beds are available.

While the costs of syndromic testing are considered a challenge, the cost of the whole procedure, from sample preparation to result interpretation and patient management, should be considered. For example, the use of syndromic testing and the

associated reduction in TAT could lead to a reduction in unnecessary therapies, such as dispensing antibiotics or isolation. Soucek et al.[23] reported no difference in the total cost (including testing and treatment) between the recently implemented BioFire meningitis/encephalitis panel ($239.14) and in-house PCR testing ($239.63). In this case, the cost of testing per patient was compensated by the reduced antimicrobial intake.[23] Dik et al.[24] describe a cost/benefit approach, referred to as the '€hr concept', that calculates the overall cost in relation to TAT by multiplying the overall cost (€) and turnaround time (h). Analysis using the €hr concept can demonstrate the cost effectiveness of implementing a syndromic test in an emergency room during a respiratory season. The clinical and economic impact of implementing large panels has also been investigated previously.[4] In a study by Subramony et al.[25] in 2016, inpatients in a hospital were less likely to have antibiotic treatment and chest radiographs and more likely to have an additional 2 days of isolation following the introduction of a syndromic panel. TAT is also an important factor when considering diagnostic stewardship, an initiative which aims to provide a reliable and beneficial result within an optimal timeframe.

Factor 3. Number of targets of the syndromic panel

Testing for viruses based on availability of treatment options

It may be important to consider whether it is appropriate to use a syndromic panel only to test for viruses for which there are treatment options available. Although most viral infections are self-limiting, some go on to cause severe morbidity, which can result in greater burdens both in hospital settings and in communities. A limited number of viruses have some treatment options available, including influenza virus, HIV, herpes simplex virus 1 and 2, hepatitis virus and RSV. In these cases, antivirals can be administered directly to the patient as a specific treatment management option. Some studies have shown the advantage of testing only for viruses where a viable treatment option is available.[26] Other studies have suggested that laboratories test for influenza virus and RSV first then perform broader testing if samples are negative to reduce costs for the patients. This can be particularly effective if an outcome-based reimbursement system is in place.[27] Some have argued that diagnostic assays should have a clinical endpoint: i.e. that a test result should have a direct impact on patient management decisions or be epidemiologically relevant. This argument suggests that testing only for viruses such as influenza virus or RSV would be more cost- and time-effective. Thus, it could then be argued that singleplex or small targeted assays could provide attending clinicians with a more straightforward answer to therapeutic questions,[7] provided the results are given in an optimal therapeutic time frame.

While confirming the presence of therapeutically relevant viruses is advantageous, in cases where a negative result is found, it may lead to the use of unnecessary antibiotics if no other viral target is tested. Indeed, one study found that following testing with a broad syndromic panel, rhinoviruses and enteroviruses were the viruses found most frequently in patients with exacerbation of airway disease.[26] As a result, patients in this study who received broad syndromic panel testing were more likely to discontinue unnecessary antibiotics compared with those who received influenza virus and RSV testing alone. This finding was echoed by another study, which found that 43% of viruses detected in respiratory samples through an in-house multiplex PCR were either an enterovirus or rhinovirus.[28] Furthermore, it could be argued that to make the most appropriate therapeutic decision for a patient, all information relevant to the patient's clinical problem should be made available. By this rationale, a broader syndromic panel may be appropriate to help to achieve diagnostic stewardship by guiding treatment and providing resolution, both for the patient and the attending clinician.[4]

Implications of testing for multiple targets simultaneously

Respiratory infections typically have very similar clinical presentations, making them nearly impossible to distinguish without molecular testing.[29] Similarly, it is difficult to attribute gastrointestinal and neurological infections to a single specific viral pathogen. One of the most well-established advantages of syndromic testing is its capacity to detect the presence of multiple potential pathogens simultaneously using a single sample. This application of rapid diagnostics can expedite the implementation of the most appropriate patient management strategy. Whether such strategies include antibiotic management, patient isolation or discharge, the use of rapid diagnostics may lead to a reduction in the number of hospital stays. Another important benefit of testing multiple targets simultaneously with a single sample is that continuous sample collection is not required. This is especially valuable with respect to sample collection processes that are particularly uncomfortable for patients, such as the collection of CSF and nasopharyngeal swabs. Patient discomfort may lead to reluctance to undergo further testing and should thus be minimized.[4]

Despite its advantages, the ability to detect multiple targets does not necessarily translate into clinical relevance.[4] Indeed, not all viruses identified can necessarily be associated with the clinical presentation. While some viruses have a direct implication in patients, other viruses may be classified as an innocent bystander, with another pathogen determined as the causative agent. Furthermore, it could be argued that finding a rhinovirus with a low viral load is unlikely to have a high clinical relevance.[30] At the same time, a negative result or a low viral load can provide important information by informing the clinician that the patient does not have a serious viral infection, as well as providing information about the distribution of viral loads within a patient population. For example, the viral load distribution of cytomegalovirus (CMV) may be different between transplant patients and paediatric patients. Additionally, only collecting one sample type to screen for a broad spectrum of pathogens could result in sensitivity challenges. Certain sample material is more compatible with specific viral targets: for example, in lower respiratory tract infections, a bronchoalveolar lavage might be most efficient, whereas in upper respiratory tract infections, a nasopharyngeal swab might be more appropriate. Furthermore, while nasopharyngeal swabs have been found to be more sensitive for influenza viruses, a perinasal swab has been recommended for other viruses, such as parainfluenza viruses 1 to 4.[31]

Although some targets may not be directly relevant to patient management, using syndromic panels to collect data regarding background viral circulation within a hospital may provide valuable infection and control information. This was demonstrated during
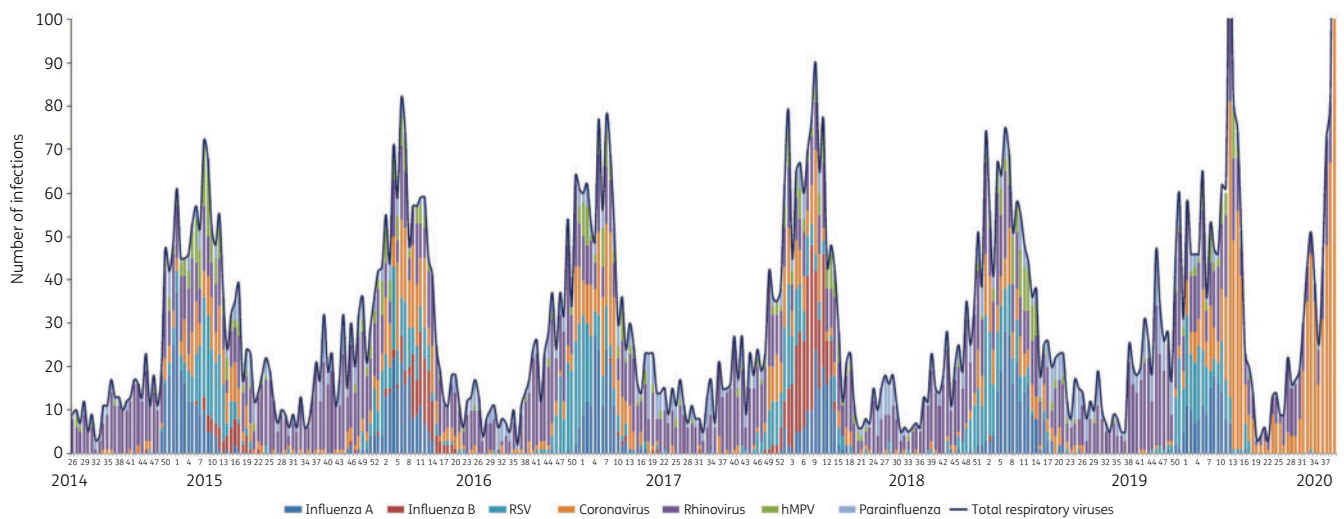
Figure 2. Viral infections during the respiratory seasons 2014 to 2020 at the University Medical Center Groningen. The *y*-axis depicts the total number of infections attributable to the viruses listed in the key. The *x*-axis shows epidemiological weeks. hMPV, human metapneumovirus.

the global enterovirus D68 (EV-D68) outbreak in 2014.[32] Following an increase of severe respiratory infections and acute flaccid myelitis, primarily in children, a link was established with an increase in enterovirus detections.

The broad nature of syndromic panels allows the wider detection of viruses that could have a new or rare pathogenicity or seasonality. Conversely, there is now a specific EV-D68 PCR test that can be used to screen for EV-D68 during the summer/autumn seasons.[33] This type of singleplex test can be crucial in rapidly targeting pathogens that may lead to significant patient morbidity.

In order to track trends and provide information on virus circulation patterns, some platforms have implemented online systems that aggregate and present the results from their syndromic panels. For example, information generated by the BioFire FilmArray is uploaded onto BioFire Syndromic Trends, a cloud-based network. Although this particular system is only available to BioFire users, the adoption of such services by more companies would greatly benefit public health.

Generating data on the viruses that have been circulating over several respiratory seasons is particularly useful for tracking trends and predicting potential prevalence. Figure 2 represents data gathered on a broad range of respiratory viruses from 2014 (week 26) to 2020 (week 36) by the University Medical Centre Groningen.

Implications of testing for all possible targets

Within a patient population, there can be significant differences between pathogen prevalence, severity and seasonality.[3] Critically ill or immunosuppressed patients are more likely to have uncommon or rare infections, which can result in more-severe clinical syndromes.[34] Furthermore, pathogens can become relevant that were not previously considered to be so. For instance, in a patient who has recently undergone a stem-cell transplant, the detection of a rhinovirus with a low viral load could provide crucial information regarding the patient's background health status e.g. potentially indicating a reduction in immune response. In this scenario, the patient would benefit from broad syndromic panel testing. It can therefore be argued that broad syndromic panels should

only be used if patients' clinical background and presentation support the use of this type of testing.[27] With the number of targets increasing in panels, the clinical background is invaluable to guide the diagnosis and aid in addressing clinical questions. By this rationale, a test should offer added value to the patient, i.e. providing information for their diagnosis and improving their outcomes.

Conversely, immunocompetent patients with self-limiting diseases may benefit from a more targeted panel.[4] Furthermore, the inclusion of rare pathogens in syndromic testing for all patients could result in an increase in false-positive results.[3] The creation of custom panels has been discussed previously and could help to account for differences within patient populations.[3] For example, paediatric or transplant patients might benefit from specifically tailored panels. Additionally, it could be argued that testing for pathogens should be guided by infection risk factors, such as cystic fibrosis and lung diseases. However, the sole use of custom panels might result in missing potential targets due to fluctuations within patient populations. Challenges might also arise in standardization, if such panels were customized by different institutes or hospitals.[3]

The costs of multiplex versus singleplex or low-plex panels should also be considered, given that the addition of multiple targets could result in insurance challenges or greater reimbursement costs.[28] One question relevant to this consideration is whether patients may be charged on the basis of their panel results (e.g. positive or negative results).[27] The increased number of potential targets could lead to a clinically 'irrelevant' positive result. In this case, syndromic testing might not be cost effective for a patient who only had incidental findings. An overview of the advantages and disadvantages of syndromic testing for each factor is provided in Table 5.

Implications of why and where the testing is performed

Performing molecular testing on clinical material is not only crucial for diagnostics, but also for public health and infection control, as demonstrated by the on-going COVID-19 pandemic. As the administration of the SARS-CoV-2 vaccine is still in its early stages,

Table 5. Syndromic testing advantages and disadvantages

Factor	Advantages	Disadvantages
Quality	Generates high volumes of data, which can be used to access background distribution in specific patient groups and to help link clinical relevance. Fewer freeze–thaw cycles impacting sample quality as multiple targets are tested simultaneously.	Challenges in designing assays that allow for a greater number of targets within a single thermal protocol may result in reduced sensitivity. Inter-platform differences can impact interpretation of results. Additional quality and employee competences. Reduced flexibility for variation in target organisms.
Turnaround time	Low time-to-result and hands-on time. Enables optimized and rapid patient management decisions. Provides fast answers to hospital ward outbreaks. Relief for the patient and unnecessary treatment. High initial implementation costs can be compensated by the reduction of unnecessary antimicrobials or isolation.	Limited number of samples per hour (lower throughput). Turnaround time is still limited by sample collection time.
High number of targets	Reduced need for continuous sampling and further testing. Provides the clinician with more clinical information to make an informed therapeutic decision. Can detect pathogens which have a rare or new pathogenicity and seasonality. Specific patient populations such as transplant recipients would benefit from a broad-spectrum panel.	Most targets will not have any specific treatment options. Challenges in clinical relevance and pathogen association with disease. A higher number of targets could increase the probability for false positive results. Addition of multiple targets could result in insurance challenges or greater reimbursement costs.

infection prevention efforts such as tracking and tracing are imperative to reduce transmission. Situations can arise where a clinician may only want to know if the patient is positive or negative for SARS-CoV-2. If the patient has a negative result, then only epidemiological information would be collected. Further testing, if warranted, would then need to be performed to confirm the causative agent for the patient's clinical syndrome. There are various reasons for clinical testing, including: (i) to answer a therapeutic question and determine whether antivirals may be given; (ii) to answer a public health question and determine whether infection control measures should be put in place; and (iii) to answer a diagnostic question, such as determining the cause of a clinical syndrome. In any case, the reasoning for a clinical test must be clearly defined.

Syndromic panels can allow the simultaneous detection of pathogens directly from patient samples to aid in answering a clinical question (most likely a therapeutic, public health or diagnostic query). In recent years, syndromic panels have been increasingly integrated into many clinical laboratories.[27,35] However, the cost of syndromic testing is an important limitation. As a result, broad syndromic panels and high throughput could be limited by laboratory capabilities, due the expense of syndromic testing, restricting their use to reference laboratories or university hospitals.[27] Conversely, smaller panels or singleplex tests may be favoured by laboratories with lower throughput capacities.

Prospects for the future of syndromic testing

Understanding the prevalence and clinical relevance of different viral targets is crucial to answering a clinical question. Similarly to traditional routine testing, diagnostic stewardship should be included in syndromic testing to ensure the most appropriate test is performed for the patient, along with timely results, to guarantee the most appropriate management decision.[14,36] Moreover, diagnostic stewardship has been shown to play a large role in addressing issues such as clinical relevance and providing guidance to help interpret results.[4] More research is needed, however, to understand the relationship between the quantity of virus and its clinical relevance in different patient populations, with more international standards being made available. Standardization of assays plays a crucial role in molecular diagnostics and in shaping future diagnostic pipelines such as syndromic testing. Efforts should be made to ensure that quantified control materials, external quality assurance (EQA) panels and data on clinical relevance are shared. Additionally, by sharing data generated by syndromic panels such as the BioFire FilmArray, we can support public health by providing updates on the prevalence and emergence of infectious disease targets in real-time. Although challenges have emerged in syndromic testing, as with all new technologies, it is crucial to continually discuss and assess new innovations for the future of clinical diagnostics.

Transparency declarations

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