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COVID-19 – KCE CONTRIBUTIONS

CLINICAL UTILITY OF MULTIPLEX PCR ASSAYS FOR THE DIAGNOSIS OF ACUTE RESPIRATORY INFECTIONS DURING THE SARS-COV-2 PANDEMIC

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This document is based on a rapid review of the scientific literature and guidelines from national and international agencies, and on an ad-hoc consultation of Belgian experts. KCE compiled the evidence in short time frames to respond to urgent questions and could therefore not follow its regular methodological procedures. This work is used to inform guidance of other governmental agencies (like RIZIV/INAMI, Sciensano, CSS/HGR, AFMPS/FAGG and SPF/FOD).

TABLE OF CONTENTS

Contents

LIST OF TABLES **1**
LIST OF ABBREVIATIONS **2**
1 BACKGROUND **4**
2 SCOPE **5**
2.1 RESEARCH QUESTION 5
2.2 QUESTIONS OUT-OF-SCOPE 5
3 METHODS **6**
4 RESULTS **8**
4.1 MULTIPLEX TESTS COMBINING AT LEAST SARS-COV-2 AND INFLUENZA 8
4.2 MULTIPLEX TESTS INCLUDING INFLUENZA (NO SARS-COV-2 STUDIES) 8
4.2.1 Clinical trials 9
4.2.2 Systematic reviews 18
4.3 INTERNATIONAL GUIDELINES 20
4.4 CONSULTATION OF STAKEHOLDERS 25
5 DISCUSSION **26**
5.1 LITERATURE REVIEW 26
5.2 STAKEHOLDER CONSULTATION 27
5.3 RECOMMENDATIONS 27
6 APPENDIXES **28**
7 REFERENCES **34**

LIST OF TABLES

Table 1 – PICO terms 6
Table 2 – Description and quality evaluation of included studies 11
Table 3 – Results of included studies 15
Table 4 – International guidelines 20
Table 5 – Number of clinical trials per outcome 26
Table 6 – Studies excluded 28
Table 7 – Belgian experts consulted 29
Table 8 – Summary of results from included clinical trials 30

LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AB	Antibiotic
Adv	Adenovirus
AOR	Adjusted odds ratio
ARI	Acute respiratory infection
BAL	Broncho-alveolar lavage
COPD	Chronic obstructive pulmonary disease
COVID-19	Corona virus disease- 2019
CRP	C-reactive protein
CT	Computed tomography
ED	Emergency department
EWS	Early Warning Score
ICU	Intensive care unit
IFA	Immunofluorescence assay
ITT	Intention to treat
LRTI	Low respiratory tract infection
mPCR	Multiplex polymerase chain reactions
PCR	Polymerase chain reaction
PIV	Parainfluenza virus
POCT	Point of care test
RAG	Risk assessment group
RR	Risk ratio
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcriptase polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
TAT	Turn-around time
UK	United Kingdom
US	United states of America
WCC	Peripheral white cell count

ABSTRACT

Multiplex polymerase chain reactions (mPCR) tests allow for the rapid diagnosis (point of care) of several pathogens in patients suffering low respiratory tract infections (LRTI). Some of them combine the detection of SARS-CoV-2 and influenza viruses (duplex), possibly combined with the detection of the Respiratory Syncitial Virus (RSV; triplex). Other mPCR tests target a much broader panel of respiratory pathogens (maxipanels). The mPCR tests are currently not reimbursed by the National Institute for Health and Disability Insurance (NIHDI) This report aimed at assessing the clinical utility of (mPCR) tests for the diagnosis of acute respiratory infections during the SARS-CoV-2 pandemic in terms of differential patient's management, including infection control policies, avoidance of investigations or unnecessary treatment.

A rapid review of the scientific literature and national and international guidelines was carried out. We retrieved very limited evidence on the clinical benefit of using mPCR tests combining SARS-CoV-2 and influenza detection (duplex), or mPCR tests including at least influenza detection in patients diagnosed with COVID-19. We extended our literature review to studies assessing the benefit of mPCR tests for influenza in non-COVID-19 patients. Results from the 6 clinical trials included were inconsistent, except for a reduction of turn-around time of test results.

Several national and international health agencies have published recommendations or guidelines for the use of mPCR testing when influenza viruses are circulating. Recommendations are consistent across guidelines:

- For individuals with a severe LRTI requiring hospitalization, guidelines advocate for PCR testing of SARS-CoV-2 and influenza. Likewise, simultaneous testing of both viruses is recommended in hospitalized severely-ill children (combined with RSV testing if appropriate). Some guidelines also recommend a duplex test in out-patients (emergency department) at high-risk of developing a severe LRTI (e.g. immunocompromised persons).
- For non-severely ill patients who do not require hospitalization, including people living in congregate settings such as nursing homes, testing of influenza (combined with SARS-CoV-2) is supported only if the result might influence the clinical management or an infection control decision.

The recommendations of the consulted Belgian experts are in line with those of the international guidelines:

- During the COVID-19 pandemic and influenza season, duplex tests are recommended (or triplex tests, mainly in children) in patients presenting at the emergency room (or hospitalized) with signs and symptoms of LRTI, essentially to guide isolation measures. Outside the season of high transmission of respiratory viruses, only a rapid SARS-CoV-2 PCR test is indicated.
- The price of a duplex/triplex PCR would not be much higher than a single SARS-CoV-2 PCR. The nomenclature code currently used for the reimbursement of SARS-CoV-2 PCR test by NIHDI could apply. Price is expected to decrease with increasing volumes of utilization.
- The use of duplex/triplex PCR assays is not recommended in the ambulatory sector (nursing homes, primary care).
- Maxipanels are not recommended, except for immunocompromised patients and patients with a severe community-acquired pneumonia with unfavourable evolution after empiric therapy.

Despite the lack of high-quality scientific evidence on the clinical utility of mPCR tests, KCE recommends in line with recommendations of Belgian experts and other national and international agencies the possible use of duplex/triplex PCR assays for patients presenting at the emergency room (or hospitalized) with signs and symptoms of severe LRTI during the season of high virus transmission, and their reimbursement by NIHDI (INAMI/RIZIV).

INTRODUCTION

1 BACKGROUND

Rapid rule-in or rule-out of viral infections caused by respiratory viruses such as severe acute respiratory distress coronavirus type 2 (SARS-CoV-2), influenza virus or respiratory syncytial virus (RSV), is key to take adequate infection control measures in hospital in-patients presenting with an acute lower respiratory tract infections (LRTI), and to avoid unnecessary additional diagnostic procedures.[1] Furthermore, early detection of eventual viral co-infection in COVID-19 patients, and in particular influenza virus, could allow starting antiviral treatment earlier when appropriate (severe pneumonia). This concern is acute as the season of high influenza incidence will start soon.

COVID-19 patients can also present with a bacterial co-infection. For example, the incidence of bacterial coinfection was reported to be between 15% based on conventional cultures[2, 3] and 33% based on polymerase chain reaction (PCR) tests.[4] Since the signs and symptoms in case of a respiratory infections are generally non-specific, the diagnosis uncertainty can lead to inappropriate antibiotic prescribing or resources utilization. Because antibiotic overuse drives antimicrobial resistance, it is essential to quickly document the pathogen responsible for lower respiratory tract infections and decide whether antibiotic treatment is required.[3, 5, 6]

Multiplex polymerase chain reactions (mPCR) tests allow for the rapid diagnosis (point of care) of several pathogens in patients suffering low respiratory tract infections (LRTI). Some of them combine the detection of SARS-CoV-2 and influenza viruses (duplex), possibly combined with the detection of the RSV (triplex). Other mPCR tests target a much broader panel of respiratory pathogens (maxipanels). For example, the Biofire® FilmArray® Respiratory 2.1 plus Panel simultaneously tests for 23 of the most common pathogens which cause respiratory tract infections, including the three viruses mentioned above and four bacteria.[a] These automated molecular diagnostic assays display good diagnostic performance, e.g. according to the manufacturer the Biofire® FilmArray® Respiratory 2.1 plus Panel has a sensitivity and specificity of 97.4% and 99.4%, respectively, and the Xpert Xpress SARS-CoV-2 reaches an agreement of 100 % with results from in-house RT-PCR.[7] Several of these tests were granted emergency-use authorization by the FDA.[b] mPCR tests can allow point-of-care testing and possibly more rapid clinical management decisions. Immunoassays tests that are based on antigenic detection are also being routinely used for the diagnosis of viral or bacterial infections. However, they provide a lower sensitivity than molecular-based techniques.[c8] The evaluation of antigenic tests is not the purpose of the present report.

In Belgium, the Risk Assessment Group (RAG) very recently (September 2021) published recommendations regarding the use of multiplex PCR tests to differentiate infections by SARS-CoV-2 and influenza.[d] These recommendations, which were endorsed by the Risk Management Group, are as follows:

- INAMI/RIZIV should urgently make a decision on the reimbursement of PCR tests for respiratory infections other than COVID-19 (including multiplex PCR tests)
- Multiplex tests that co-detect SARS-CoV-2 and other respiratory pathogens should be used in priority in the following categories of patients:
    - patients at the hospital with or at increased risk for severe COVID-19 and/or severe disease by other respiratory infections. These include patients presenting with severe acute respiratory infection (ARI) and patients at risk for severe ARI, such as immunocompromised patients, patients with severe comorbidities (pulmonary, cardiac…) and the elderly. Both

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a https://www.biomerieux-diagnostics.com/filmarrayr-respiratory-panel

b https://www.cdc.gov/flu/professionals/diagnosis/table-flu-covid19-detection.html

c Viral culture is not performed for clinical management since it is time consuming (3-10 days) and requires skilled laboratories. It is mainly used for the genetic characterization of Influenza https://www.cdc.gov/flu/professionals/diagnosis/overview-testing-methods.htm

d https://covid-19.sciensano.be/sites/default/files/Covid19/20210906_Advice_RAG_Update%20use%20of%20multiplex%20tests_FR.pdf

hospitalized in-patients and out-patients at the emergency department could also be considered;

- nursing home residents with ARI, in particular in a context of a cluster;

- SARS-CoV-2/other respiratory pathogen multiplex test should be used only in periods during which both SARS-CoV-2 and other respiratory pathogens are simultaneously and strongly circulating, such as during the influenza season.
- Multiplex tests should only be used if the differential diagnosis effectively results in a different management of the patient, such as different isolation measures, avoidance of further investigations and unnecessary therapies, or a different clinical management.

Determining if multiplex PCR tests of respiratory infections result truly in a different management of patients (including in-hospital infection control strategies), the avoidance of investigations (e.g. unnecessary chest computed tomography (CT) or bronchoscopy with broncho-alveolar lavage (BAL)) or unnecessary therapies is crucial to make an evidence-informed decision on the reimbursement of such tests. This is the focus of the present report.

Multiplex PCR tests are currently in use in Belgium. But the indications and volumes are unclear, as no utilization data is available in the absence of reimbursement by INAMI/RIZIV. It is likely that the cost of the test is charged to the patient (between €15 and €90; personal communication Eva Van Braeckel).

2 SCOPE

2.1 Research question

What is the clinical utility of multiplex tests for the diagnosis of acute respiratory infections during the SARS-CoV-2 pandemic in terms of differential patient management, including infection control policies, avoidance of investigations and unnecessary treatments?

2.2 Questions out-of-scope

Given the short timeframe allowed for this research project, the following research questions are considered out-of-scope:

1. Reviewing the evidence on the usefulness of multiplex tests which do not include the detection of SARS-CoV-2
2. Assessing the performance (sensitivity, specificity, likelihood) of the various multiplex tests, including those allowing the detection of SARS-CoV-2
3. Assessing the cost-effectiveness of the various multiplex tests in the Belgium settings (data are lacking)

If these questions are deemed relevant by NIHDI (INAMI/RIZIV) to decide, they could be addressed by a standard KCE research project at a later stage.[e]

[e] The research question was introduced by Dirk Ramaekers (Head of the Vaccination Taskforce) and Pieter Geentjens (RIZIV/INAMI) on September 30th. The scope of the research was approved by all parties during a tele-conference on October 5th. The final recommendations for INAMI/RIZIV based on this assessment were expected within 4 weeks.

3 METHODS

Given the short timeframe allowed for this research project, the proposed recommendations for INAMI/RIZIV responding to the research question was be based on:

1. A rapid literature review. Published peer-reviewed scientific studies were searched for in Medline. Published or on-going HTA reports were searched for on websites of HTA agencies (EUnetHTA (including the POP database), INAHTA and NHS HTA). The grey literature was searched for reports and guidelines from prominent healthcare institutions (e.g. WHO, CDC, NHS, HAS) or expert societies (e.g. European Respiratory Society, American Thoracic Society). The search was limited to evidence on multiplex PCR including at least the combined detection of SARS-CoV-2 and influenza viruses. The PICO elements can be found in Table 1. For randomized controlled studies, the risk of bias and quality of included studies was assessed using the revised Cochrane risk-of-bias tool for randomized trials (RoB 2).
2. Consultation of a panel of Belgian specialist in emergency care, geriatrics, pediatrics, pulmonology, general medicine, and microbiology on their practice regarding the use of multiplex tests. This panel was constituted of hospital and primary line practitioners with experience in the use of multiplex tests.

Table 1 – PICO terms

	Inclusion	Exclusion
Patients	Adults and children with respiratory infection (any setting)	Remnant laboratory samples
Intervention	Multiplex PCR tests including at least the combined detection of SARS-CoV-2 and influenza viruses[f]	Singleplex PCR test
Comparator	Conventional management	Comparison of various multiplex tests
Outcomes	• Antibiotic prescribing • Antiviral prescribing • Length of stay at ED • Hospital admission • Isolation measures • Recurrent health care visits • Cost consequences, cost-effectiveness	Diagnosis performance
Study types	Systematic literature review RCT, quasi-randomized trials, prospective studies with control group	Before-after studies* Retrospective studies Editorial, comments

** Studies are excluded because of confounding by concomitant changes in other dimensions of care*

We applied the following search string in MEDLINE on 11/10/2021:

1. SARS-CoV-2 AND ("Xpert Xpress" OR "GeneXpert Dx" OR "GeneXpert Infinity" OR "GeneXpert Xpress System" OR Powerchek OR "BD MAX" OR "Flu SC2" OR “Applied Biosystems 7500 Fast Dx” OR "Sofia FIA" OR “Cobas 6800/8800” OR “Cobas Liat Systems” OR “Roche cobas SARS-CoV-2” OR QIAstat OR “ePlex System” OR FilmArray OR "BioFire Respiratory Panel") (n=130)
2. (SARS-CoV-2 AND influenza) AND (multiplex OR point-of-care) (n=143)
3. 1 OR 2 (n=262)
4. Antibiotic OR antimicrobial OR antiviral OR ((turn-around OR turnaround) AND time) OR length of stay OR hospitalization OR isolation OR mortality OR survival OR cost (n=10,445,278)

[f] Concomitant singleplex tests, e.g. SARS-CoV-2 detection together with influenza, were also considered

5. 3 AND 4 (n=180)

The brand name of the multiplex assays for simultaneous detection of SARS-CoV-2 and influenza viruses used in the search where those which were granted an emergency-use authorization by the FDA.[g] Reference lists of included studies were also searched. We did not apply any language restrictions. For the search of HTA reports a very sensitive strategy was applied using the key word "COVID" (7/10/2021).

Given the paucity of publications on multiplex tests including SARS-CoV-2 detection, and to draw possible lessons from the evaluation of other multiplex respiratory PCR tests, we extended our search to mPCR including at least influenza detection. The following search strategy limited to systematic literature reviews and clinical trials was applied in Medline (15/10/2021):

1. Influenza AND (multiplex OR point-of-care) *Filter: systematic review* (n=20)
2. (Influenza OR respiratory) AND (multiplex OR point-of-care) AND PCR *Filter: clinical trial* (n=53)
3. 1 AND 3 (n=73)

[g] https://www.cdc.gov/flu/professionals/diagnosis/table-flu-covid19-detection.html

4 RESULTS

4.1 Multiplex tests combining at least SARS-CoV-2 and influenza

The search for HTA in EUnetHTA (including the POP database), INAHTA and NHS HTA database (CRD) returned no reference pertaining to multiplex PCR tests.

The search for peer-reviewed papers in MEDLINE returned 180 references. Seventeen publications were potentially relevant based on title and abstract, among which two were systematic reviews.[4, 9] One additional study was identified by screening the references list of the two systematic reviews (Verroken et al. 2020, no abstract[10]). Thus, in total, 18 publications were screened. None fulfilled our inclusion criteria (for excluded studies see Appendixes, Table 6). The systematic review by Dinnes et al. 2020 focused on diagnostic performances of rapid SARS-CoV-2 tests, most of which were rapid singleplex test for SARS-CoV-2 detection only, and was thus discarded.[9] The second systematic review assessed the impact of the BioFire® FilmArray® Pneumonia plus Panel[h] in detecting bacteria and clinical management among critically ill COVID-19 patients admitted to the intensive care unit (ICU).[4] Only 2 of the 7 studies included in that review reported on a relevant outcome (i.e. antibiotic prescribing) but no comparison groups were included.[10, 11]

Given the absence of relevant high-quality studies, we decided to describe these 2 studies, although they assessed a mPCR with no virus and had no comparison group.[10, 11] Their characteristics are presented in Table 2.

In Maataoui 2021, a mPCR (BioFire® FilmArray® Pneumonia plus Panel) was performed at the physician's request in COVID-19 patients suspected of bacterial co/superinfections in two ICUs (67 patients).[11] The Pneumonia plus Panel was associated with early therapy changes in 34% (38 of 112) episodes.[i] Positive tests led to antibiotic initiation or adaptation in 15% of episodes and de-escalation in 4%. When negative, 28% of episodes remained antibiotic-free (14% no initiation, 14% withdrawal). Limitations of this study include: 1. no comparative design, i.e. it was impossible to assess the added value of mPCR against conventional care; 2. mPCR could be applied several times in some patients, potentially limiting the external applicability; 3. selection of patients was performed by the physician, also potentially limiting the external applicability; 4. antibiotic use before admission was observed in 79% of patients.

Verroken et al. 2020 published a preliminary analysis of a study conducted over a 1-month period at the ICU of the Cliniques universitaires Saint-Luc and included all COVID-19 adult patients from whom a lower respiratory tract sample could be obtained.[10] Here, again the BioFire® FilmArray® Pneumonia plus Panel was used. Direct communication of mPCR results led to speeded-up antibiotic modifications in 15/32 (46.9%) patients.[j]

4.2 Multiplex tests including influenza (no SARS-CoV-2 studies)

The search for peer-reviewed articles in MEDLINE yielded 73 hits. An additional paper was retrieved from another source,[12] as a result 74 papers were screened. Among them, two systematic reviews[13, 14] published in 2018 and five more recent primary studies[6, 12, 15-17] met our inclusion criteria. An additional randomized controlled study was identified in the reference list of one of the systematic reviews.[18] As a result, eight articles were included in our review (six primary studies and two systematic reviews). A meta-analysis was available in one of the systematic reviews.[14]

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h BioFire® FilmArray® Pneumonia plus Panel does not include SARS-CoV-2 but directly detects 15 bacteria with a semiquantitative value, 3 atypical bacteria, 9 viruses, and 7 antimicrobial resistance genes within 1:15 h

i Patients had ≥ 1 mPCR

j Antibiotic therapy was stopped in 26% (5/19) of patients with a negative bacteria test. AB therapy was started in 69% (9/13) patients with a positive bacteria test (the others were already receiving AB)

4.2.1 Clinical trials

Among primary studies, five were randomized controlled trials, and one a quasi-randomized trial conducted in one center.[6] Studies were conducted in the UK[6, 18], Finland[15], US[12], Argentina[16] and China.[17] Five studies were conducted on patients admitted to the emergency department[6, 12, 15, 16, 18]; one of them also included out-patients observed at the daycare medical assessment center [6, 18] while both others were conducted in ward-hospitalized patients.[17,19] Four studies comprised adults[6, 15, 17, 18] while the two others either focused on children[12], or mixed both populations[16]. The study by Andrew et al.[6] was also included in the systematic review by Egilmezer[13]. The evaluation of the quality of evidence is presented in Table 2 Studies were overall of moderate quality and were rated as 'some concerns' by using the RoB 2 tool. Concerns about bias were mainly detected in the outcome assessment domain.[k] Two studies were rated at high-risk of bias: one was a quasi-randomized trial[6] and the other one had an unbalanced randomisation[16].

- UK: The quasi-randomized trial from Andrews et al. did not identify an association between mPCR (Film Array) and the length of hospital stay in comparison to a routine, laboratory-based respiratory PCR and serology testing.[6] Turn-around time (TAT) for multiplex was one day faster (19 hours) than routine testing. Moreover, mPCR was significantly associated with the antibiotic prescribing decision within 24 hours after the result.[l] Nevertheless, similar percentages of patients received antibiotics in both arms and the time to antibiotic within the first 72 hours did not differ between groups.
- US: In a randomized controlled trial, Rao et al. assessed the impact of a mPCR (BioFire FilmArray RP2 Panel) for respiratory pathogens, including four bacteria (*Bordetella pertussis* and *B. parapertussis*; *Chlamydophila pneumoniae*; and *Mycoplasma pneumoniae*), in a population of children with influenza-like symptoms admitted to the emergency department.[12] Children who underwent a multiplex test were more likely to receive antibiotics (and to have a diagnosis in which antibiotics would be indicated), with no significant differences in antiviral prescribing, medical visits, and hospitalization. Authors also reported that in an adjusted ITT analysis[m], children were more likely to receive antivirals, had longer emergency department (ED) and hospital stays when compared to children whose physicians did not know the testing result.[n] However, these results are difficult to interpret given the statistical modelling without clear-cut underlying assumptions.
- Finland: In a monocentric randomized controlled trial, Saarela et al. showed that knowing the results of multiplex respiratory virus testing (Anyplex™II RV16 Detection; detects 16 respiratory viruses and no bacteria) did not reduce antimicrobial consumption, nor the hospital length of stay in adults admitted in the emergency department.[15] About two thirds of the patients received antibiotics in both arms.

  Of importance, the authors mentioned that the clinicians received the results of the mPCR after 24 hours and that this could have accounted for the absence of strategy care modifications. Besides, it should be noted that the cohort had a low rate of virus positivity reaching only 17.5%.

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[k] It should be noted that all trials were unblinded. However, an open-labelled approach was unavoidable given the nature of the research question.

[l] Antibiotic therapy was continued in 60% of cases in the control group versus 49% in the Intervention group

[m] Adjustment was performed for a priori variables of high-risk comorbidity, age, and diagnoses classified by appropriate antibiotic therapy

[n] In the protocol, clinicians could order complementary diagnostic tests. Therefore, authors conducted a modified ITT analyses whereby the intervention group included children whose clinicians knew the multiplex test results (children in the intervention group with results given to clinicians and children in the control group whose clinician ordered clinical testing). The control group comprised children whose clinicians did not know the multiplex test results. According to this modified ITT analysis, children whose physician knew the test result were more likely to have a diagnosis in which antibiotics would be indicated than those without test results (risk difference, 6.0%; 95% CI, 0.4% to 11.6%). Children with a viral pathogen detected were significantly less likely to receive antibiotics than those with a negative test result (20% vs 30%, p=0.01).

The population studied included many patients with comorbidities and one third of the cohort were immunocompromised patients.

- Argentina: In a sample of adults and children admitted to the emergency department, Echevarria et al. reported in a monocentric unblinded randomized trial that significant changes in clinical management occurred when the result of a mPCR for respiratory pathogens was rapidly available when compared to antigenic testing (immunofluorescence assay (IFA)).[16] By using FilmArray RP®, respiratory pathogens were detected in 93% of the children (99% viruses, 1% *Mycoplasma pneumoniae*) and 74% of adults (100% viruses). In contrast, testing through IFA allowed to identify a respiratory virus in 49% of children and only in 23% of adults.

  The main changes consisted in a decrease of the proportion of patients being prescribed antibiotics (children and adults) and less complementary exams (children) in the intervention group. Prescription of antivirals was little affected. There was no evidence of an effect on hospitalization and length of stay but few patients required to be hospitalized in the cohort. Interestingly, the percentage of viral co-infections with mPCR was 31% in children and 7% in adults. IFA did not detect any co-infections. Results from this trial should be considered with caution as the risk of selection bias was high (unbalanced randomization).

- China: In a monocentric randomized controlled trial of patients hospitalized for lower respiratory tract infection, the duration of intravenous antibiotic therapy was significantly shortened by 1 day by the use of mPCR (FilmArray RP®), when compared with routine real-time PCR assays.[17] However, it should be noted that the median antibiotic therapy duration was still 7 days in the intervention group. An early antibiotic de-escalation (defined as a reduction of antibiotic types, a change from intravenous antibiotics to oral antibiotics, or from broad-spectrum antibiotics to narrower spectrum) was more frequent in the intervention group (de-escalation within 72 hours : 7.9% vs 3.2% (p=0.005)). In addition, the length of hospital stay and expenditures associated to the antibiotherapy and hospitalization were significantly reduced (see Table 3 for details).

- UK: Brendish et al. conducted a randomized controlled trial on adults patients who presented to the emergency department or acute medical unit for acute respiratory illness.[18] The use of mPCR (FilmArray RP®) did not reduce the proportion of patients treated by antibiotics, nor the duration of the treatment, when compared to a clinical routine management (single PCR at the discretion of clinicians). However, they demonstrated that the hospital length of stay was reduded in the intervention arm by 1.1 day, and that a greater proportion of influenza-positive patients were treated with antiviral drugs when mPCR was used. Interestingly, they showed that a higher proportion of patients were treated with only a single dose or brief duration (less than 48 hours) in the mPCR group. The subgroup analysis revealed that this reduction mostly occurred in patients with exacerbation of airway diseases. Chronic pulmonary disease and asthma were associated with shorter antibiotic durations, mainly in patients testing positive for respiratory viruses.

Table 2 – Description and quality evaluation of included studies

Authors	Index Test	Comparator	Population	Setting	Patient sampling	Study type	Quality
Maataoui 2021[11]	BioFire® FilmArray® Pneumonia plus Panel (bioMérieux, France)	-	67 COVID-19 patients with ≥ 1 mPCR (112 mPCR) Median age: 57 Males: 82% ≥1 comorbidity: 87% AB use before admission: 79% Mortality rate: 57%	2 ICUs	mPCR performed at physician request in patients suspected of bacterial co/superinfections (n=67/191)	Single arm cohort	Unclear patient selection
Verroken 2020[10]	BioFire® FilmArray® Pneumonia plus Panel (bioMérieux, France)	-	32 COVID-19 patients	1 ICU	all COVID-19 adult patients from whom a lower respiratory tract sample could be obtained	Single arm cohort	Unclear patient selection
Andrews 2017[6]	FilmArray® Respiratory Panel (BioFire Diagnostics, Salt Lake City, UT, BioMerieux)	Routine, laboratory-based respiratory pathogen PCR testing ± atypical serology	In- and out- adult patients (n=545) Median age: 61 Males: 52% Median Charlson score[o]: 0 30-days post enrolment mortality: 4%	Acute medical wards for patients admitted from the emergency department, and medical assessment centre for out-patients	mPCR performed on even days in the month and routine testing on odd days (during 6 months)	Single centre quasi-randomized unblinded trial	High-risk

[o] Comorbidity score based on age and the presence of cardiovascular and cerebrovascular diseases, dementia, connective tissue diseases, peptic ulcer disease, liver disease, chronic kidney disease, diabetes, hemiplegia, solid or liquid tumor, AIDS.

Authors	Index Test	Comparator	Population	Setting	Patient sampling	Study type	Quality
Rao 2021[12]	BioFire FilmArray RP2 Panel (BioFire Diagnostics)	Results of the swab test not given, routine clinical care	Children (1 month- 18 years) (n=908) Median age: 2.1 years Males: 56% High-risk medical condition: 35% Hospitalized: 16%	Emergency department	Nasopharyngeal swabs performed on all included participants	Single center, randomized unblinded trial	Some concern
Saarela 2020[15]	Anyplex™II RV16 Detection (Seegene, Seoul, Korea)	Delayed viral diagnostics group: results received 7 days after sampling (method for viral detection not clearly mentioned)	Adults (n=998) Males: 53% (Interv);52% (Ctrl) Comorbidities: 75% (Interv); 71% (Ctrl) Immunosuppression: 31% (Interv); 28% (Ctrl) Discharged home from emergency department: 23% (Interv); 21% (Ctrl)	Emergency department	Nasal swabs for viral detection on all included participants	Single center, randomized unblinded trial	Some concern
Echavarria 2018[16]	FilmArray® Respiratory Panel (FilmArray-RP) (BioFire/bioMérieux, Salt Lake City, UT)	Immunofluorescence assay with specific monoclonal antibodies for RSV, FluA, FluB, PIV 1–3 and AdV (Millipore/Chemicon, Temecula, CA)	Adult (n=276) and children between 2 months-6 years (n=156) Median age: • Children: 9 • Adults: 46 Males: 64% (children); 41.5% (adults) Presence of pneumonia: 4.5%	Emergency department	• Nasopharyngeal swabs performed on all included participants. The physician called the laboratory for randomization between FilmArray or IFA • Physicians have to document a presumptive medical	Single center randomized unblinded trial	High-risk

Authors	Index Test	Comparator	Population	Setting	Patient sampling	Study type	Quality
			(children); 22.5% (adults) Hospitalization: 18.6% (children); 12.3% (adults)		management plan (planned antibiotic and antiviral prescriptions and complementary studies) on a standardized form		
Shengchen 2019[17]	FilmArray Respiratory Panel (BioFire; Salt Lake City, UT, USA)	RT- PCR assays for the detection of viral pathogens (influenza A (H1N1, H7N9) and B, respiratory syncytial virus, parainfluenza virus, adenovirus, Epstein Barr virus, herpes simplex virus, human cytomegalovirus)	Hospitalized adult patients (n=800) Age: 60.8 (Interv), 60.9 (Ctrl) Males: 58.3% (Interv), 55.7% (Ctrl) Acute exacerbation of COPD : 24.6% (Interv), 26.9% (Ctrl) Chronic respiratory disease: 42.2% (Interv), 44.8% (Ctrl) Cardiovascular disease: 44.2% (Interv), 45.3% (Ctrl) Diabetes: 20.1% (Interv), 25.1% (Ctrl) Antibiotic use before admission: 1.5% (Interv), 1.7% (Ctrl)	Hospitalized (medical ward)	Nasopharyngeal swabs were taken within 4 hours in the intervention group and immediately analyzed. The results were reported to physicians (with mandatory feedback) on the day of admission. In the control group, the results were reported and explained to physicians once obtained.	Single center randomized unblinded trial	Some concern
Brendish[18] 2017	FilmArray Respiratory PanelBioFire; Salt Lake City, UT, USA)	Conventional PCR at the discretion of the physician	Age: 63 (Interv), 62 (Ctrl) Males: 49% (Interv), 48% (Ctrl)	Emergency department and acute medical unit	Nose and throat swab in intervention group. Management of control group according to	Single center unblinded randomized controlled trial	Some concern

Authors	Index Test	Comparator	Population	Setting	Patient sampling	Study type	Quality
			Antibiotics within 14 days: 25% (Interv), 26% (Ctrl) Chronic respiratory disease: 59% (Interv), 58% (Ctrl) Cardiovascular disease: 37% (Interv), 38% (Ctrl) Renal disease: 6% (Interv), 6% (Ctrl) Diabetes: 13% (Interv), 18% (Ctrl) Immunocompromised: 5% (Interv), 6% (Ctrl) Cancer: 6% (Interv), 7% (Ctrl)		routine clinical care (testing at discretion of physician)		

Legend: AdV: adenovirus; COPD: chronic obstructive pulmonary disease; Ctrl: control group; IFA: immunofluorescence assay; Interv: Intervention group; mPCR: Multiplex Polymerase Chain Reaction; PIV: Parainfluenza virus

Table 3 – Results of included studies

Authors	Antibiotics	Antivirals	TAT	Hospitalisation	Length of hospital stay	Others
Maataoui 2021[11]	• Antibiotic changes[p] in 34% (38/112) of episodes[q] • In positive tests: antibiotic initiation/ adaptation in 15% of episodes; de-escalation in 4%. • In negative tests, 28% of episodes remained antibiotic-freer	-	-	-	-	-
Verroken 2020[10]	• antibiotic modifications in 15/32 (46.9%) of patients.[s]					
Andrews 2017[6]	• Antibiotic use during hospital stays: 77% (Ctrl) vs 75% (Interv); p=0.99, aOR[t] (95%CI): 1.0 (0.6-1.5) • Duration of antibiotics: 6 days (Ctrl) vs 6 days (Interv); p=0.23 • Time to antibiotic within the first 72h: difference in days (95% CI): 2.2 (-1.4-5.8) • Significant association between POCT and antibiotic decision within 24 hours after the result[u] (p<0.001)	Among patients who received antiviral, the time to the first dose (from time of admission) was reduced in the intervention group (24 hours vs 60.4 hours)	Reduction from 39.5h to 19h (p<0.001) (Defined as time between hospital admission and the time of the result)	-	No effect Median (hours) *: 79.6 (control) vs 98.6 (intervention) In a linear model adjusted on age, sex, Charlson score, EWS, WCC, CRP: increase of length of stay of 11% (95%CI: 9-36%)	• Appropriate treatment of *Mycoplasma pneumoniae* in mPCR group (0 detection in controls) • Readmission within the first 30 days: no effect (20% control vs 19% intervention, p=0.70); aOR (95%CI): 0.9 (0.6-1.4) • Mortality post enrolment: 4% (Ctrl) vs 4% (Interv), p=0.79; aOR (95%CI): 0.9 (0.3-2.2)

p Antibiotics were recorded at D−1, D0, D+1, and D+2 following mPCR

q 16 withdrawals, 13 initiations, 3 adaptations (introduction of an effective antibiotic), 5 de-escalations (appropriate use of a narrower spectrum antibiotic for beta-lactam antibiotics), and one change resulting in inadequacy (details in table 3 of the paper)[11]

r 14% no initiation, 14% withdrawal

s AB therapy was stopped in 26% of 19 patients with a negative test. AB therapy was started in 69% of 13 patients with negative test (the others were already receiving AB)

t adjustement for age, sex, Charlson and Potts (Physiological Observation Track and Trigger System) scores, day of week, and admission values of WCC (peripheral white cell count) and CRP (C-reactive protein)

u Antibiotherapy were continued in 60% of cases in the Control group versus 49% in the Intervention group

Authors	Antibiotics	Antivirals	TAT	Hospitalisation	Length of hospital stay	Others
Rao 2021[12]	Antibiotherapy more likely in intervention group*: 25% vs 19% (Ctrl); RR (95%CI) 1.3 (1.0-1.7)	Prescription: 7% (Interv) vs 5% (Ctrl); RR (95%CI): 1.3 (0.8-2.1) Prescription and influenza test positive : 6% (Interv) vs 5% (Ctrl); RR (95%CI) : 1.1 (0.6-1.9)	-	17% (Interv) vs 15% (Ctrl); RR (95%CI) : 1.1 (0.8-1.5)	Median 3.1 days (Interv) vs 2.8 days (Ctrl); RR (95%CI): 1.0 (0.9-1.1)	• Additional hospitalization within 10 days: 3% (Interv) vs 3% (Ctrl); RR (95%CI):1.3 (0.6-2.7) • Additional emergency department visit within 10 days: 6% (Interv) vs 6% (Ctrl); RR (95%CI):1.0 (0.6-1.7) • Additional medical visit within 10 days: 27% (Interv) vs 25% (Ctrl); RR (95%CI):1.1 (0.8-1.4)
Saarela 2020[15]	• Days on antibiotics within 30 days*: 11.3 (Interv) vs 10.4 (Ctrl); p= 0.23 • Patients with antibiotic treatment in 30 days: 64% (Interv) vs 64% (Ctrl); p= 0.89	-	-	-	Mean duration (days)*: 4.2 (Interv) vs 4.1 (Ctrl); p= 0.81	• Radiological examination: 2.5 (Interv) vs 2.2 (Ctrl); p= 0.41 • ICU admission: 5.4% (Interv) vs 6.6% (Ctrl); p= 0.43 • Mortality:3% (Interv) vs 3% (Ctrl); p= 0.99
Echavarria 2018[16]	Decrease in antibiotic prescriptions • Children: 23% vs 2% (p=0.001) • Adults: 14% vs 1% (p=0.001)	• Decrease in oseltamivir prescription in adults with negative influenza: 11.9% vs 9% (p=0.042) • Increase in oseltamivir prescription in adults with positive influenza: 6.8% vs 3% (p=0.091)	FilmArray: 1h52 min vs IFA 26h40 min (p<0.001)	Hospitalization rate: • children: 17.7% vs 20.9% (p=0.643) • adults: 13.6% vs 10% (p=0.38)	Median length of stay: • Children: 3 days vs 5 days (p=0.218) • Adults:4 days vs 10 days (p=0.382)	Decrease of additional diagnostic tests: • Children[£]: 25.7% vs 4.7% (p=0.001) • Adults:0.6% vs 0% (p=0.594)
Shengchen 2019[17]	• Duration of IV antibiotic treatment*: 7 days vs 8 days (p<0.001) • Proportion of participants who received IV antibiotics: 92.1% vs 93.8% (p=0.38)	-	Reduction from 29 hours to 1.6 hour (p<0.001)	-	Length of stay decrease: 8 days vs 9 days (p<0.001)	• Median cost of IV antibiotics: $189.9 vs $245.8 (p<0.001) • Median cost of hospitalization: $1804.7 vs $2042.5 (p=0.002)

Authors	Antibiotics	Antivirals	TAT	Hospitalisation	Length of hospital stay	Others
	• Antibiotic de-escalation within 72 hours[v] :7.9% vs 3.2% (p=0.005) • De-escalation between 72 h and 7 days: 29.7% vs 22% (p=0.024)					• ICU admission: 1% vs 1.5% ; OR (95%CI) : 0.67 (0.19-2.39) • Hospital death : 0.5% vs 0.8% ; OR (95%CI): 0.67 (0.11-4.04) • Readmission within 30 days: 5.9% vs 6.9%; OR (95%CI): 0.85 (0.48- 1.51) • Death within 30 days: 0.8% vs 1%; OR (95%CI): 0.75 (0.17-3.38)
Brendish[18] 2017	• Antibiotic prescription: 84% vs 83% (p=0.96) • Intravenous antibiotic prescription: 54% vs 52% (p=0.46) • Antibiotic duration: 3.1 days vs 2.9 days (p=0.48)	Neuraminidase inhibitors (hospitalized Influenza positive patients): 91% vs 65% (p=0.0026)	Reduction from 37.1 hours to 2.3 hours (p<0.0001)	Hospital admission: 92% vs 92% (p=0.94)	Length of stay: 5.7 days vs 6.8 days (p=0.0443)	• Time to isolation: 0.5 days vs 1 day (p=0.0071) • Time to de-isolation: 1 day vs 3.1 days (p=0.0057) • Proportion of patients isolated with diagnosis of Influenza or RSV: 17% vs 9% (p=0.0217) • ICU admission: 3% vs 2% (p=0.36) • Died within 30 days: 3% vs 5% (p=0.15) • Re-presented within 30 days: 14% vs 14% (p=1.00) • Readmission within 30 days: 13% vs 16% (p=0.28)

**: primary outcome; £: Reduction in ordering blood cell count and chest-X ray*
aOR: adjusted Odds ratio; CRP: C-reactive protein; EWS: Early warning score; ICU: Intensive Care Unit; IV: intravenous; IFA: Immunofluorescence assay; min: minutes; TAT: turn-around time; RR: risk ratio; WCC: peripheral white cell count

[v] defined as reduction of antibiotic types, change from intravenous antibiotics to oral antibiotics, or from broad-spectrum antibiotics to narrower spectrum antibiotics

4.2.2 Systematic reviews

4.2.2.1 Descriptions of studies and populations

The systematic review from Egilmezer et al. included randomized trials and cohort studies that compared POCT to standard care, and studies with before-after designs (and comparisons of pre-implementation and post-implementation of POCTs as well).[13] Of note, most of the POCTs were singleplex antigenic tests. It comprised 5 randomized controlled trials, 2 quasi-randomized trials, 1 cross-sectional study and 22 observational prospective or retrospective studies. Studies were conducted in emergency department, clinical and primary care settings. Populations included adults and children (15 studies).

The systematic review/ meta-analysis from Lee et al. retrieved 7 randomized studies and 6 non-randomized studies that compared POCT to standard testing (including also before-after designs).[14] All POCTs were singleplex antigenic tests. The included studies were exclusively performed in primary care settings (3 studies) and emergency departments (10 studies). Studies conducted with hospitalized patients were excluded. They mainly comprised children (8/13 studies). Eleven of the thirteen studies were common with the review from Egilmezer.[13]

4.2.2.2 Impact on anti-viral prescription

In the systematic review from Egilmezer, 12 studies among the 14 retrieved studies reported on a significant increase of anti-viral drug prescriptions associated with a positive diagnosis by POCT.[13] Six studies were conducted on children, 5 on adults and 3 on mixed samples. Three of them were randomized control trials. It is worthy to point out that most of the studies used a POCT based on viral antigen detection[w] and that only two studies used multiplex PCR (mPCR) techniques[x]. We describe these two studies here.

The first study was a retrospective study assessing a triplex test (Influenza A,B, Respiratory Syncitial Virus) in 350 US adults participants. It identified a trend of antiviral prescription: two time periods were compared (before implementation of mPCR and after). The use of antivirals went up from 45 to 55% (p= 0.05) whereas the duration of antivirals (oseltamivir) in influenza-negative patients significantly decreased.[19]

The second study was a RCT conducted in the UK between 2015 and 2016 that made use of a wider detection of several respiratory viruses. Adults with acute respiratory illness (n= 720) were randomly assigned to routine testing versus mPCR. Antiviral treatment among influenza-positive patients was more common in the mPCR group than in the control group.[18]

Lee et al identified in their review 6 studies reporting on antiviral use (3 randomized and 3 non-randomized).[14] It should be noted that antigen detection tests were exclusively used, and no study used mPCR techniques. An association between POCT and antiviral prescribing was shown in 3 randomized studies (RR: 2.65; 95%CI: 1.95-3.60; I^2 = 0%), while 3 non-randomized studies showed no significant difference in antiviral prescribing, along with great imprecision and heterogeneity across studies (RR: 11.36; 95% CI, 0.82 to 157.12; I^2 = 88%).

4.2.2.3 Impact on antibiotherapy prescription

Egilmezer et al. retrieved 26 studies assessing the impact of POCT on antibiotic use. Results were contrasted. While in the 20 observational studies a decrease of antibiotic use was reported, such effect was not observed in the 6 studies of higher methodological quality (3 were randomized controlled trials, 2 were quasi-randomized trials and 1 was a prospective observational study). Only two studies were about mPCR (and not antigenic POCTs) and both were part of these 6 high quality studies.[6, 18]

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[w] Simplexa Flu a/B & RSV (Focus Diagnostics®)

[x] Film Array Respiratory Panel (BioFire; Salt Lake City, UT, USA)

Also, in Lee et al. this split between RCTs and observational was observed. There was no effect of POCT on antibiotic prescribing in 7 RCTs (RR: 0.97; 95%CI: 0.82-1.15; I^2 = 70%).[14] In a sensitivity analysis using data from 3 randomized trials, antibiotic prescribing was compared among patients who tested positive for influenza with patients who tested negative: no significant effect on prescribing antibiotics in patients with influenza was demonstrated (RR: 0.63; 95% CI: 0.32-1.22; I^2 = 64%), nor an increase in antibiotic prescribing in patients without Influenza (RR: 1.02; 95%CI: 0.85 to 1.23; I^2 = 0%). In contrast, among the 5 non-randomized studies that reported on antibiotic prescribing, 4 studies reported significant reductions of antibiotic prescribing. Meta-analysis showed a strong association between POCTs and reduced antibiotic prescribing but with great imprecision and statistical heterogeneity (RR, 0.64; 95% CI, 0.48 to 0.86; I^2 = 81%).

4.2.2.4 Decision on care pathway management

Length of stay in the emergency department

Egilmezer et al. retrieved 9 non-randomized studies assessing the impact of POCT on the length of stay in the emergency department.[13] Only five of them reported a significant decrease of the time spent in ED. Times were highly variable and differences were generally modest.[y]

In the review and metanalysis by Lee et al., the time that patients spent in emergency departments was unchanged by POCTs.[14]

Admission to hospital

Only Lee et al. assessed the impact of POCT on the admission to hospital.[14] The latter was not lessened in the meta-analysis of randomized (RR: 0.93; 95%CI: 0.61-1.42; I^2 = 34%) or non-randomized studies (RR: 0.73; 95%CI: 0.49-1.09; I^2 = 0%).

4.2.2.5 Use of other diagnostic tests

Lee et al. identified studies reporting the impact of POCT on the use of additional diagnostic tests.[14] Among randomized studies, POCT was associated with a decrease of routine blood tests (RR 0.80; 95%CI: 0.69-0.92; I^2 = 0%), blood culture analysis (RR 0.82; 95%CI: 0.68-0.99; I^2 = 0%) and chest-X ray (RR 0.81; 95%CI: 0.68-0.96; I^2 = 32%), while no effect was shown on lumbar puncture (RR 1.07; 95%CI: 0.45-2.54; I^2 = 0%) and urinalysis (RR 0.91; 95%CI: 0.78-1.07; I^2 = 20%). A study reported a decrease of respiratory syncytial virus testing (RR 0.40; 95%CI: 0.26-0.63).[20] Only 3 non-randomized trials reported the impact of influenza POCT on additional tests. Meta-analysis did not provide statistically significant impact on routine blood tests (RR 0.85; 95%CI: 0.18-4.1; I^2 = 92%), nor for chest radiography (RR 0.77; 95%CI: 0.57-1.05; I^2 = 65%). The single non-randomized study assessing the use of urinalysis showed a significant decrease (RR 0.47; 95%CI: 0.37-0.61).

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[y] The RCT by Brendish was not considered for the assessment of the length of stay in the review by Egilmezer although it reported on that parameter. In that study, mPCR was associated with a shorter hospital length of stay and decreased time-to-isolation.[18]

4.3 International guidelines

Several national and international health agencies have published recommendations or guidelines for the use of mPCR testing when SARS-CoV-2 and influenza viruses are circulating.[z] All are consistent and address rather similar diagnostic strategies:

- For individuals with LRTI who need to be hospitalized, guidelines advocate for the PCR testing of SARS-CoV-2 and influenza. Likewise, a simultaneous testing for both viruses is recommended in hospitalized severely ill children (combined with RSV testing if appropriate). Some guidelines also recommend testing high-risk out-patients.
- For non-severely ill patients who do not require hospitalization, including people living in congregate settings such as nursing homes, the testing of influenza (combined with SARS-CoV-2) is supported only if the result might influence the clinical management or for infection control decisions.

Multiple PCR testing (combining SARS-CoV-2 with duplex or triplex PCR for influenza A-B and RSV) is generally prioritized rather than single target PCR. The guidelines from the American Thoracic Society support the testing of respiratory pathogens other than influenza through multiplex PCR only in hospitalized patients with severe pneumonia, or for those who are immunocompromised.

Table 4 – International guidelines

Institution	Objective	Methods	Recommendations
Haute Autorité de Santé (HAS)[aa] November 2020	Assess the relevance of multiplex tests combining influenza and SARS-CoV-2 (and other viruses) among patients with symptoms	• Rapid non-systematic evaluation of literature Medline, Embase, Covid-19 Research, WHO COVID-19 database, Medxriv until 29th September 2020 • Search among HTA agencies until October 2020 (Agence Santé Publique Canada, Australian Clinical Practice Guidelines, CADTH, CDC, KCE, Centres for reviews and Dissemination, department for Health, ECDC, FDA, GIN, HCSP, INESSS, Ministère de la Santé, NHS, NICE, Tripdatabase, WHO)	1) For hospitalized patients (or ED) with respiratory infection: PCR SARS-CoV-2 and influenza A-B (nasopharyngeal swab) to be performed through: • Multiplex for SARS-CoV-2 and influenza; • Or Multiplex PCR for respiratory viruses (including influenza) combined with PCR SARS-CoV-2 • Or a minimum of both single target PCR for SARS-CoV-2 and influenza A-B (2) In nursing homes for patients with symptoms but who do not require hospitalization: • If SARS-CoV-2 rapid antigen test is positive, it can be followed by a RT-PCR for influenza A-B • If SARS-CoV-2 rapid antigen test is negative: double RT-PCR testing (single target or duplex) for both viruses (SARS-CoV-2: influenza)

[z] ERS guidelines on severe community acquired pneumonia are currently under revision

[aa] https://www.has-sante.fr/jcms/p_3216748/fr/tests-diagnostiques-pour-differencier-la-covid-19-des-infections-respiratoires-hivernales-en-periode-de-co-circulation-des-virus

Institution	Objective	Methods	Recommendations
		• Expert's consultation (2 virologists, 6 clinicians: general practitioners, pediatrician, infectiologist, geriatricians, 1 patient)	• RT-PCR for SARS-CoV-2 and Influenza can directly be performed (without performing rapid antigen test) (3) For children: • Hospitalized: idem than for adults + RSV testing • Not hospitalized children: no PCR testing (antigenic testing)
Haut Conseil de la Santé Publique France (HCSP)[bb] September 2020	Experts consulting about the use of diagnostic tests during seasonal influenza (and other respiratory viruses) that may occur simultaneously with SARS-CoV-2 pandemic	Experts consulted in September 2020 (9 experts, unanimity agreement)	• Molecular testing should be prioritized and clearly state when the testing is recommended • Indication of simultaneous testing for SARS-CoV-2/influenza A-B: prioritizing patients at high-risk for severe LRTI when influenza viruses are circulating (combined testing or two separate tests) • Molecular based testing should be used for the elderly living in the community (with the aim to use preventive/curative antiviral drugs) • Testing for SARS-CoV-2 with Multiplex-PCR is indicated for newborns and children with severe symptoms or hospitalized • Indications of Multiplex-PCR: singleplexPCR testing is possible but HCSP advocates for Multiplex-PCR if available at a reasonable cost.

Institut national d'excellence en santé et services sociaux (INESSS)[cc], Québec
 	
October 2020
	Use of PCR tests during SARS-CoV-2 pandemic and influenza season	• Non-systematic review of literature from July to September 2020 (PubMed, Live COVID-19, BioRxiv2 and grey-literature)
		• Consultation of governmental scientific health agencies (WHO, Global influenza Surveillance and Response System, CDC, Canadian Public Health Laboratory Network, BC Renal –Provincial Health Services Autority, Alberta Health Services, Public health Ontario, Australian Government-Department of Health, FDA, IDS, John Hopkins Medicine)
			During SARS-CoV-2 pandemic and Influenza season:
			
			• PCR SARS-CoV-2 test for everyone with symptoms
			• PCR influenza and/or RSV if respiratory symptoms for:
			  ○ hospitalized patients
			  ○ people living in a collectivity
			  ○ high-risk patients (and people living in the same place)
			  ○ children < 2 years
			
			• Duplex PCR (influenza A-B) and triplex PCR (influenza A-B + RSV) has to be prioritized rather than Multiplex PCR (8 viruses + SARS-CoV-2)
			
			• Multiplex combining SARS-CoV-2 with Duplex PCR (Influenza A-B) and triplex PCR (influenza A-B + RSV detection) may be used according to their availability

[bb] https://www.hcsp.fr/Explore.cgi/Groupe?clef=3281

[cc] https://www.inesss.qc.ca/covid-19/investigation-procedures-diagnostiques/utilisation-pertinente-des-analyses-pour-la-detection-des-virus-respiratoires-durant-la-saison-grippale-en-contexte-de-pandemie.html

Institution	Objective	Methods	Recommendations
		• Consultation of 15 experts	
Center for Disease Control and Prevention (CDC), US[dd] October 2020	Information for clinicians on SARS-CoV-2 and influenza viruses testing	Based upon local public health surveillance data and testing at local healthcare facilities	1) In patients who require hospitalization for acute respiratory illness symptoms: • Testing of SARS-CoV-2 and influenza through multiplex PCR (if not available, both separate PCR tests) 2) In patients who do not require hospitalization: • Testing of SARS-CoV-2 by nucleic acid detection; OR if not available, by SARS-CoV-2 antigen detection assay • Testing of influenza only if the management will change, or for infection control decisions (living in congregate setting): influenza nucleic acid detection assay; if rapid influenza nucleic acid detection assay is not available on-site, order rapid influenza antigen assay 3) Nursing home residents: testing for both SARS-CoV-2 and influenza: if available, multiplex nucleic acid detection assay for both viruses can be performed. Two different specimens may need to be collected if a multiplex nucleic acid detection assay including both influenza viruses and SARS-CoV-2 is unavailable.
National Institute of health (NIH)[ee], US October 2020	To provide regularly updated guidelines	Related references available on the NIH website; group of associated experts (no mention of systematic approach)	When SARS-CoV-2 and influenza viruses are co-circulating: • For hospitalized patients with acute respiratory illness: testing for both viruses • For out-patients with acute respiratory illness: SARS-CoV-2 plus influenza testing if the results will change clinical management of the patient • Testing for other pathogens should be considered depending on clinical circumstances, especially in patients with influenza in whom bacterial superinfection is a well-recognized complication.

dd https://www.cdc.gov/coronavirus/2019-ncov/lab/multiplex.html; https://www.cdc.gov/flu/professionals/diagnosis/index.htm

ee https://www.covid19treatmentguidelines.nih.gov/special-populations/influenza/

Institution	Objective	Methods	Recommendations
Academy of Medical Science (AMS)[ff], UK July 2021	Prioritize the measures for preparing the upcoming months (winter season) during COVID-19 pandemic	National expert advisory group (many medical areas)	Multiplex strongly supported for surveillance, treatment decisions (use of antivirals for influenza), minimizing isolation times, and avoiding and reducing rates of transmission*. * If this is not feasible, well-evaluated and accurate point-of-care testing (POCT) for influenza should be deployed in hospitals, primary care settings, care homes and community pharmacies
World Health Organization (WHO) & Global Influenza Surveillance and Response System (GISRS)[gg] November 2020	Organize the monitoring of SARS-CoV-2 and influenza for public health professionals and laboratories	Redacted by international experts (epidemiology and laboratory)	Specimens from sentinel sites should be tested for both influenza and SARS-CoV-2 viruses. If possible, multiplex PCR assays for the simultaneous detection of influenza and SARS-CoV-2 viruses should be selected for efficient use of reagents, consumables, and hands-on time.
World Health Organization (WHO) Policy Brief[hh] November 2020	Address to policymakers the question of organizing influenza reaction during SARS-CoV-2 pandemic	Narrative summary of the guidance from WHO-GISRS	Prioritize sampling and testing and use multiplex testing for influenza and COVID-19 in routine sentinel surveillance systems.
Infectious Disease Society of America (IDSA)[8] 2019	Clinical practice guidelines on the diagnosis, treatment, chemoprophylaxis, and management of seasonal influenza	Systematic review Panel members of 16 experts in infectious diseases	• During influenza activity, recommendations on the patients to be tested are: ○ High-risk out-patients or ED (including immunocompromised persons) ○ Out-patients with acute symptoms and either an exacerbation of a chronic medical condition or a known complication of influenza, and if the testing result will influence the management ○ Out-patients discharged home (from ED), and if the result will influence the management ○ Patients requiring hospitalization

ff https://www.gov.uk/government/publications/ams-covid-19-preparing-for-the-future-looking-ahead-to-winter-2021-2022-and-beyond-15-july-2021

gg https://www.who.int/publications/i/item/maintaining-surveillance-of-influenza-and-monitoring-sars-cov-2-adapting-global-influenza-surveillance-and-response-system-(gisrs)-and-sentinel-systems-during-the-covid-19-pandemic

hh Readiness for influenza during the COVID-19 pandemic: policy brief, 6 November 2020 (who.int)

Institution	Objective	Methods	Recommendations
			○ Hospitalized patients with exacerbation of a chronic medical condition ○ High-risk hospitalized patients (including immunocompromised persons) • Other recommendation for testing: ○ Clinicians should use rapid molecular assays (nucleic acid amplification tests) over rapid influenza diagnostic tests ○ Clinicians should use RT-PCR over other tests in hospitalized patients ○ Clinicians should use Multiplex-PCR targeting a panel of respiratory pathogens including influenza in hospitalized immunocompromised patients. Multiplex-PCR may be used in non-immunocompromised persons if it might influence care (cohorting decision, reduce testing, decrease antibiotic use)
American Thoracic society (ATS)[21] 2021	Clinical practice guidelines on nucleic-acid-based testing for Noninfluenza Viral Pathogens in Adults with suspected community-acquired pneumonia	Systematic review International panel of experts in respiratory infections	In adults with suspected community-acquired pneumonia: In out-patients, the use of routine nucleic acid-based testing of respiratory samples for viral pathogens other than influenza is not suggested. In hospitalized patients, routine nucleic acid-based testing of respiratory samples for viral pathogens other than influenza is suggested only in patients who meet one of the following conditions: ○ Severe pneumonia (≥ 1 major criterion or ≥ 3 minor criteria[ii]) OR ○ Immunocompromised patients (neutropenia, ongoing active cancer therapy, solid-organ or blood component transplantation, chronic use of immunosuppressive medications including corticotherapy)

[ii] Major criteria: septic shock, mechanical ventilation. Minor criteria: respiratory rate > 30/min; hypoxemia (PaO_2/FiO_2 <250), multilobar infiltrates, confusions/disorientation; uremia; leukopenia; thrombocytemia; hypothermia; hypotension requiring aggressive liquid infusion

4.4 Consultation of stakeholders

A panel of experts was consulted (see invitees and participants in Appendixes, Table 7). The objective of this consultation was to discuss the key findings of the literature review and collect information on current real-life clinical practice. We present hereunder the main discussion points of the meeting (videoconference) held on 25/10/2021.

1. The main interest of duplex/triplex PCR assays (mini panel) is to assess the presence of viral co-infections to implement infection control policies and avoid unnecessary investigations (chest CT, bronchoscopy, repeat SARS-CoV-2 testing). Moreover, in-hospital acquired influenza and RSV increase mortality (also Human Metapneumovirus (hMPV)).
2. During the COVID-19 pandemic and influenza season, a rapid duplex/triplex PCR for SARS-CoV-2 + Influenza A/B + RSV is recommended in patients either presenting at the emergency room or when hospitalized with signs and symptoms of a LRTI. Outside the season of high transmission of respiratory viruses, only a rapid SARS-CoV-2 PCR test is indicated.
3. The price of a duplex/triplex PCR would not be much higher than a single SARS-CoV-2 PCR. The nomenclature code currently used for the reimbursement of SARS-CoV-2 PCR test by NIHDI could apply. The current price of a commercial mPCR assay for 23 pathogens is reported to be around €55. Price is expected to decrease with increasing volumes of utilization. Tests with short turn-around time (commercial tests) are more expensive than in-house PCR tests. Maxi panels are also more expensive.
4. The utilization of mPCR is currently not recommended in nursing homes. Stakeholders put forward the following arguments: the proportion of people vaccinated against SARS-CoV-2 and influenza is high; isolation measures may reduce the quality of life whereas life expectancy is reduced; it is uncertain if the results of the mPCR would result in a modified prescribing behaviour. More research is needed.
5. The utilization of mPCR is currently not recommended in primary care, as it is unlikely that the results of the test would modify patient management.
6. The utilization of broader multiple mPCR tests (maxi panels) also detecting a wide range of viruses and bacteria is not recommended in patients either presenting at the emergency room or when hospitalized with signs and symptoms of a LRTI. Their utilization might augment (unnecessary) antibiotic prescribing for commonly residing micro-organisms in the respiratory tract. Moreover, at the first hospital presentation, bacterial co-infection in COVID-19 admitted patients is low and antibiotics are not routinely recommended in absence of clinical suspicion of bacterial sur-infection. However, these tests might be useful:
    a. in immunocompromised patients presenting with LRTI: detection of important viral pathogens such as rhinovirus or hMPV can avoid unnecessary antibiotic escalation or prolonged duration of antibiotic therapy;
    b. in (severe) CAP patients with unfavourable evolution after empiric therapy (either on nasopharyngeal swab or BAL) to avoid unnecessary broad-spectrum antibiotics and to enable pathogen-directed antimicrobial therapy.

5 DISCUSSION

5.1 Literature review

We retrieved limited evidence on the clinical utility of multiplex tests for the diagnosis of acute respiratory infections during the SARS-CoV-2 pandemic in terms of differential patient's management, including infection control policies, avoidance of investigations and unnecessary therapies. The main findings are:

- There is a great paucity of scientific evidence on the clinical benefit of using mPCR tests combining SARS-CoV-2 and influenza detection, or mPCR tests including at least influenza detection in patients diagnosed with COVID-19
- We extended our literature review to studies assessing the benefit of mPCR tests for influenza in non COVID-19 patients. Two systematic reviews (with a majority of antigenic studies) and 6 more recent clinical trials on mPCR indicate:
    - There is little high quality evidence that influenza mPCR or antigenic point-of-care testing has a substantial impact on antibiotic prescribing.
    - The evidence indicates an effect of influenza antigenic point-of-care testing on the prescription of antivirals. The effect size is unclear and the evidence for mPCR tests is limited.
    - Influenza antigenic point-of-care testing may decrease the utilization of additional diagnostic tests such as blood tests or chest-X ray, mainly in children. The evidence for mPCR tests is scarce.
    - There is no high quality evidence of a substantial reduction of the length of stay at ED or hospital, or for the admission rate.
    - We retrieved very little evidence on the cost consequences or cost-effectiveness of using mPCR (1 study).
    - The evidence from clinical trials were inconsistent, except for a reduction in turn-around time (see Table 5, and Table 8 in the appendixes).

Table 5 – Number of clinical trials per outcome

Outcomes	Increased	Decreased	No effect
Antibiotherapy	2	2	3
Antivirals	1	1	1
Turn-around time	-	4	-
Hospitalisation	-	-	3
Length of stay	-	-	5

- Several national and international health agencies have published recommendations or guidelines for the use of mPCR testing when influenza viruses are circulating. Recommendations are quite consistent across guidelines:
    - For individuals with LRTI who need to be hospitalized, guidelines advocate for the PCR testing of SARS-CoV-2 and influenza. Likewise, a simultaneous testing for both viruses is recommended in hospitalized severely-ill children (combined with RSV testing if

appropriate). Some guidelines also recommend a duplex test in out-patients (emergency department) at high-risk of developing a severe LRTI (e.g. immunocompromised persons).[jj]

- For non-severely ill patients who do not require hospitalization, including people living in congregate settings such as nursing homes, the testing of influenza (combined with SARS-CoV-2) is supported only if the result might influence the clinical management or for infection control decisions.
- For severe community-acquired pneumonia (CAP) and pneumonia in immunocompromised patients, respiratory multiplex PCR testing for viral pathogens other than SARS-CoV-2 and influenza is recommended.

Several limitations were observed:

- No hard outcomes were reported, but rather the prescription of antibiotics and/or antivirals. Moreover, main outcomes (antibiotics and/or antivirals) were reported in a heterogeneous way (e.g. time to treatment start, proportion of patients receiving a prescription, change in therapeutic plan, etc…). It was therefore difficult to assess the effect size, if any, and the clinical significance of the effect.
- The two systematic reviews on influenza testing included mostly antigenic studies; and this was not the focus of the report. However, we summarized these reviews as the benefits of antigenic POCT for influenza (rapid tests are likely to lead to more rapid adaptation of treatment) could be reasonably extrapolated to mPCR tests.
- Available evidence concerns a limited number of assays, in particular the FilmArray®. Moreover, studies poorly described the algorithm that was used in relation to subsequent treatment or prescriptions and the external applicability of their results is thus unknown.

5.2 Stakeholder consultation

A panel of Belgian experts was consulted (see section 4.4). Their recommendations are in line with those of international guidelines (see section 4.3). Their recommendations can be summarized as follows:

- During the COVID-19 pandemic and influenza season, a rapid duplex/triplex PCR assay (SARS-CoV-2, influenza A/B, RSV) is recommended in patients presenting at the emergency room (or hospitalized) with signs and symptoms of LRTI. Outside the season of high transmission of respiratory viruses, only a rapid SARS-CoV-2 PCR test is indicated.
- The utilization of duplex/triplex PCR assays is not recommended in the ambulatory sector (nursing homes, primary care).
- The utilization of broader multiple mPCR tests (maxi panels) is not recommended, except in immunocompromised patients and in patients with a severe community-acquired pneumonia with unfavourable evolution after empiric therapy.

5.3 Recommendations

Despite the lack of high-quality scientific evidence on the clinical utility of mPCR tests, KCE recommends in line with recommendations of Belgian experts and other national and international agencies the possible use of duplex/triplex PCR assays for patients presenting at the emergency room (or hospitalized) with signs and symptoms of severe LRTI during the season of high virus transmission, and their reimbursement by NIHDI (INAMI/RIZIV).

[jj] Patients at high risk of severe LRTI include elderly, people living in nursing home or chronic care facilities, young children, pregnancy (or postpartum), people with extreme obesity, comorbidities or who are immunocompromised[8]

6 APPENDIXES

Table 6 – Studies excluded

Authors	Reason for exclusion	Additional information
Alosaimi 2021[22]	Wrong outcomes *"we investigated the implications of viral and bacterial co-infection in COVID-19 clinical outcomes*" (no comparison multiplex with other approach)	We found evidence of co-infection in 34 COVID-19 patients (71%). Influenza A H1N1 (n = 17), Chlamydia pneumoniae (n = 13) and human adenovirus (n = 10) were the most commonly detected pathogens
Bouzid 2021[5]	Wrong comparison "*to measure the impact of using the same rapid syndromic test as a POCT versus using it in the central laboratory*"	
Brendish 2020[23]	Wrong comparison and design (*open-labelled trial of QIAstat-Dx Respiratory SARS-CoV-2 Panel for rapid detection of SARS-CoV-2 compared with laboratory PCR*)	
Camelena 2021[24]	Wrong design "retrospective analysis on a consecutive cohort"	At least one pathogen was detected in 46% (68/147) of samples by FA-PP and 39% (57/147) of samples by culture. The overall percentage agreement between FA-PP and culture results was 98% (93-100%).
Dinnes 2020[9]	Wrong outcome (diagnosis performance, mainly from rapid singleplex test for SARS-CoV-2 detection)	
Foschi 2021[25]	Wrong outcome and design "*In this retrospective study, we assessed respiratory bacterial coinfections in lower respiratory tract samples taken from intensive care unit-hospitalized COVID-19 patients, by comparing the conventional culture approach*"	
Gupta 2021[26]	Wrong design "*evaluate 120 archived respiratory clinical specimens for SARS-CoV-2 on the SARS-CoV-2 panel*."	QIAstat-Dx SARS-CoV-2 panel sensitivity and specificity for SARS-CoV-2 on SARS panel was found to be 90.00 % and 100 %
Hansen 2021[27]	Wrong comparison "*clinical performance of the first U.S. Food and Drug Administration (FDA)-authorized POC RT-PCR for detection of SARS-CoV-2 in 20 min, the cobas Liat SARS-CoV-2 and influenza A/B nucleic acid test, to the most widely used RT-PCR laboratory test, the cobas 68/8800 SARS-CoV-2 test*"	
Kolenda 2020[3]	Wrong design and outcome (no comparator; assessment of co-infections)	
Mancini 2021[28]	Wrong design and outcome (no comparator; assessment of co-infections)	
Marshal 2021[29]	Wrong design and outcome (assessment of co-infections)	
Perlitz 2021[30]	Wrong intervention (singleplex influenza in non-covid patients)	

Authors	Reason for exclusion	Additional information
Perng 2021[31]	Wrong comparison “BD MAX platform compared to in-house real-time RT-PCR methods”	
Timbrook 2021[4]	Systematic review with 2 relevant papers already included as primary studies	
Wolters 2020[7]	Wrong comparison “Cepheids GeneXpert Xpert Xpress SARS-CoV-2 compared to in-house real-time RT-PCR methods”	
Zhen 2020[32]	Wrong design (comparison of 3 platforms)	

Table 7 – Belgian experts consulted

Name	Specialty	Affiliation	E-mail
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Geentjens Pieter*		RIZIV/INAMI	Pieter.Geentjens@riziv-inami.fgov.be
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*Participated to expert meeting on 25/10/2021; # Contributed written comments

Table 8 – Summary of results from included clinical trials

Outcomes	Increased	Decreased	No effect
Antibiotherapy	• **Rao; n= 908, children, ED** Antibiotic received: 25% vs 19%; RR (95%CI): 1.3 (1.0-1.7) • **Andrews; n=545, adults, ED/ward** Antibiotic continued: 60% vs 49% (p<0.001)	• **Echeverria; n=432, adults/children, ED** ○ Children: 2% vs 23% (p=0.001) ○ Adults: 1% vs 14% (p=0.001) • **Shengchen; n=880; adults hospitalized** ○ Duration: 7 days vs 8 days (p<0.001) ○ De-escalation within 72 hours: 7.9% vs 3.2% (p=0.005) ○ De-escalation between 72 h and 7 days: 29.7% vs 22% (p=0.024)	• **Andrews; n=545, adults, ED/ward** ○ Antibiotic use during hospital stays[kk]: 75% (Ctrl) vs 77% (p=0.99) ○ Duration of antibiotics: 6 days vs 6 days (p=0.23) ○ Time to antibiotic within the first 72h: difference in days (95% CI): 2.2 (-1.4-5.8) (p=0.28) • **Saarela; n= 998, adults, ED** ○ Days on antibiotics within 30 days*: 11.3 vs 10.4 (p=0.23) ○ Patients with antibiotic treatment in 30 days: 64% vs 64% (p=0.89) • **Brendish n= 720, adults, ED/ward** ○ Antibiotic prescription: 84% vs 83% (p=0.96) ○ Antibiotic duration: 3.1 days vs 2.9 days (p=0.48)
Antivirals	• **Brendish n= 720, adults, ED/ward** In Influenza-positive patients: 91% vs 65% (p=0.0026)	• **Echevarria; n=432, adults/children, ED** In Influenza-negative patients: 11.9% vs 9% (p=0.042)	• **Rao; n= 908, children, ED** ○ Prescription: 7% vs 5% ; RR (95%CI): 1.3 (0.8- 2.1) ○ Prescription and influenza test positive : 6% vs 5%
TAT	-	• **Andrews; n=545, adults, ED/ward** TAT: 19 hours vs 39.5 hours • **Echeverria; n=432, adults/children, ED** 1hour 52 min vs 26 hours 40 min (p<0.001)	

[kk] Andrews et al. also described that antibiotherapy was continued in 60% of cases in the Control group versus 49% in the Intervention group (p<0.001)

Outcomes	Increased	Decreased	No effect
		• **Shengchen; n=880; adults hospitalized** 1.6 hours vs 29 hours (p<0.001) • **Brendish n= 720, adults, ED/ward** 2.3 hours vs 37.1 hours (p<0.0001)	
Hospitalization	-		• **Rao; n= 908, children, ED** 17% vs 15% ; RR (95%CI): 1.0 (0.8- 1.5) • **Echevarria; n=432, adults/children, ED** ○ Children: 17.7% vs 20.9% (p=0.643) ○ Adults: 13.6% vs 10% (p=0.382) • **Brendish n= 720, adults, ED/ward** Hospital admission: 92% vs 92% (p=0.94)
Length of stay	-	• **Shengchen; n=880; adults hospitalized** 8 vs 9 days (p<0.001)	• **Andrews; n=545, adults, ED/ward** 98.6 hours vs 79.6 hours • **Rao; n= 908, children, ED** Median 3.1 vs 2.8 days; RR (95%CI): 1.0 (0.9- 1.1) • **Saarela; n= 998, adults, ED** 4.2 vs 4.1 days (p=0.81) • **Echevarria; n=432, adults/children, ED** ○ Children: 3 days vs 5 days (p=0.218) ○ Adults: 4 days vs 10 days (p=0.382) • **Brendish; 720, adults, ED/ward** 5.7 days vs 6.8 days (p=0.0443)

Outcomes	Increased	Decreased	No effect
Others	• **Andrews; n=545, adults, ED/ward** Identification of Mycoplasma pneumoniae (n=5 vs n=0)	• **Shengchen; n=880; adults hospitalized** ○ Median cost of intravenous antibiotics: $189.9 vs $245.8 (p<0.001) ○ Median cost of hospitalization: $1804.7 vs $2042.5 (p=0.002) • **Echevarria; n=432, adults/children, ED** Decrease of additional diagnostic tests in children (blood cell count and chest-X ray): 25.7% vs 4.7% (p=0.001)	• **Andrews; n=545, adults, ED/ward** ○ Readmission within the first 30 days: 19 vs 20% (p=0.70) ○ Mortality post enrolment: 4% vs 4% (p=0.79) • **Rao; n= 908, children, ED** ○ Additional hospitalization within 10 days: 3% vs 3%; RR (95%CI): 1.3 (0.6-2.7) ○ Additional emergency department visit within 10 days: 6% vs 6%; RR (95%CI): 1.0 (0.6- 1.7) ○ Additional medical visit within 10 days: 27% vs 25%; RR (95%CI): 1.1 (0.8-1.4) • **Saarela; n= 998, adults, ED** ○ Radiological examination: 2.5 vs 2.2 (p=0.41) ○ ICU admission: 5.4% vs 6.6 (p=0.43) ○ Mortality: 3% vs 3% (p=0.99) • **Echevarria; n=432, adults/children, ED** ○ Decrease of additional diagnostic tests in adults: 06% vs 0% (p=0.594) • **Brendish; = 720, adults, ED/ward** ○ ICU admission: 3% vs 2% (p=0.36) ○ Died within 30 days: 3% vs 5% (p=0.15) ○ Re-presented within 30 days: 14% vs 14% (p=1.00)

Outcomes	Increased	Decreased	No effect
			○ Readmission within 30 days: 13% vs 16% (p=0.28) ○ Time to isolation: 0.5 days vs 1 day (p=0.0071) ○ Time to de-isolation: 1 day vs 3.1 days (p=0.0057) ○ Proportion of patients isolated with diagnosis of Influenza or RSV: 17% vs 9% (p=0.0217) • **Shengchen; n=880; adults hospitalized** ○ ICU admission: 1% vs 1.5% ○ Hospital death: 0.5% vs 0.8% ○ Readmission within 30 days: 5.9% vs 6.9% ○ Death within 30 days: 0.8% vs 1%

The first presented result is always the intervention group and the second the control group.

7 REFERENCES

1. Poelman R, der Meer JV, der Spek CV, Riezebos-Brilman A, Knoester M, Leer-Buter CV, et al. Improved diagnostic policy for respiratory tract infections essential for patient management in the emergency department. Future Microbiol. 2020;15:623-32.
2. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect. 2020;81(2):266-75.
3. Kolenda C, Ranc AG, Boisset S, Caspar Y, Carricajo A, Souche A, et al. Assessment of Respiratory Bacterial Coinfections Among Severe Acute Respiratory Syndrome Coronavirus 2-Positive Patients Hospitalized in Intensive Care Units Using Conventional Culture and BioFire, FilmArray Pneumonia Panel Plus Assay. Open Forum Infect Dis. 2020;7(11):ofaa484.
4. Timbrook TT, Hueth KD, Ginocchio CC. Identification of bacterial co-detections in COVID-19 critically Ill patients by BioFire(R) FilmArray(R) pneumonia panel: a systematic review and meta-analysis. Diagn Microbiol Infect Dis. 2021;101(3):115476.
5. Bouzid D, Casalino E, Mullaert J, Laurent O, Duval X, Lescure FX, et al. Added value of rapid respiratory syndromic testing at point of care versus central laboratory testing: a controlled clinical trial. J Antimicrob Chemother. 2021;76(Supplement_3):iii20-iii7.
6. Andrews D, Chetty Y, Cooper BS, Virk M, Glass SK, Letters A, et al. Multiplex PCR point of care testing versus routine, laboratory-based testing in the treatment of adults with respiratory tract infections: a quasi-randomised study assessing impact on length of stay and antimicrobial use. BMC Infect Dis. 2017;17(1):671.
7. Wolters F, van de Bovenkamp J, van den Bosch B, van den Brink S, Broeders M, Chung NH, et al. Multi-center evaluation of cepheid xpert(R) xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic. J Clin Virol. 2020;128:104426.
8. Uyeki TM, Bernstein HH, Bradley JS, Englund JA, File TM, Fry AM, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenzaa. Clin Infect Dis. 2019;68(6):895-902.
9. Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 2020;8:CD013705.
10. Verroken A, Scohy A, Gerard L, Wittebole X, Collienne C, Laterre PF. Co-infections in COVID-19 critically ill and antibiotic management: a prospective cohort analysis. Crit Care. 2020;24(1):410.
11. Maataoui N, Chemali L, Patrier J, Tran Dinh A, Le Fevre L, Lortat-Jacob B, et al. Impact of rapid multiplex PCR on management of antibiotic therapy in COVID-19-positive patients hospitalized in intensive care unit. Eur J Clin Microbiol Infect Dis. 2021;40(10):2227-34.
12. Rao S, Lamb MM, Moss A, Mistry RD, Grice K, Ahmed W, et al. Effect of Rapid Respiratory Virus Testing on Antibiotic Prescribing Among Children Presenting to the Emergency Department With Acute Respiratory Illness: A Randomized Clinical Trial. JAMA Netw Open. 2021;4(6):e2111836.
13. Egilmezer E, Walker GJ, Bakthavathsalam P, Peterson JR, Gooding JJ, Rawlinson W, et al. Systematic review of the impact of point-of-care testing for influenza on the outcomes of patients with acute respiratory tract infection. Rev Med Virol. 2018;28(5):e1995.
14. Lee JJ, Verbakel JY, Goyder CR, Ananthakumar T, Tan PS, Turner PJ, et al. The Clinical Utility of Point-of-Care Tests for Influenza in Ambulatory Care: A Systematic Review and Meta-analysis. Clin Infect Dis. 2019;69(1):24-33.
15. Saarela E, Tapiainen T, Kauppila J, Pokka T, Uhari M, Kauma H, et al. Impact of multiplex respiratory virus testing on antimicrobial consumption in adults in acute care: a randomized clinical trial. Clin Microbiol Infect. 2020;26(4):506-11.

16. Echavarria M, Marcone DN, Querci M, Seoane A, Ypas M, Videla C, et al. Clinical impact of rapid molecular detection of respiratory pathogens in patients with acute respiratory infection. J Clin Virol. 2018;108:90-5.

17. Shengchen D, Gu X, Fan G, Sun R, Wang Y, Yu D, et al. Evaluation of a molecular point-of-care testing for viral and atypical pathogens on intravenous antibiotic duration in hospitalized adults with lower respiratory tract infection: a randomized clinical trial. Clin Microbiol Infect. 2019;25(11):1415-21.

18. Brendish NJ, Malachira AK, Armstrong L, Houghton R, Aitken S, Nyimbili E, et al. Routine molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial. Lancet Respir Med. 2017;5(5):401-11.

19. Chu HY, Englund JA, Huang D, Scott E, Chan JD, Jain R, et al. Impact of rapid influenza PCR testing on hospitalization and antiviral use: A retrospective cohort study. J Med Virol. 2015;87(12):2021-6.

20. Abanses JC, Dowd MD, Simon SD, Sharma V. Impact of rapid influenza testing at triage on management of febrile infants and young children. Pediatr Emerg Care. 2006;22(3):145-9.

21. Evans SE, Jennerich AL, Azar MM, Cao B, Crothers K, Dickson RP, et al. Nucleic Acid-based Testing for Noninfluenza Viral Pathogens in Adults with Suspected Community-acquired Pneumonia. An Official American Thoracic Society Clinical Practice Guideline. Am J Respir Crit Care Med. 2021;203(9):1070-87.

22. Alosaimi B, Naeem A, Hamed ME, Alkadi HS, Alanazi T, Al Rehily SS, et al. Influenza co-infection associated with severity and mortality in COVID-19 patients. Virol J. 2021;18(1):127.

23. Brendish NJ, Poole S, Naidu VV, Mansbridge CT, Norton NJ, Wheeler H, et al. Clinical impact of molecular point-of-care testing for suspected COVID-19 in hospital (COV-19POC): a prospective, interventional, non-randomised, controlled study. Lancet Respir Med. 2020;8(12):1192-200.

24. Camelena F, Poncin T, Dudoignon E, Salmona M, Le Goff J, Donay JL, et al. Rapid identification of bacteria from respiratory samples of patients hospitalized in intensive care units, with FilmArray Pneumonia Panel Plus. Int J Infect Dis. 2021;108:568-73.

25. Foschi C, Zignoli A, Gaibani P, Vocale C, Rossini G, Lafratta S, et al. Respiratory bacterial co-infections in intensive care unit-hospitalized COVID-19 patients: Conventional culture vs BioFire FilmArray pneumonia Plus panel. J Microbiol Methods. 2021;186:106259.

26. Gupta A, Soni A, Rooge S, Paul D, Agarwal R, Tarai B, et al. Syndromic approach to SARS-CoV-2 detection using QIAstat-Dx SARS-CoV-2 panel from clinical samples. J Virol Methods. 2021;298:114300.

27. Hansen G, Marino J, Wang ZX, Beavis KG, Rodrigo J, Labog K, et al. Clinical Performance of the Point-of-Care cobas Liat for Detection of SARS-CoV-2 in 20 Minutes: a Multicenter Study. J Clin Microbiol. 2021;59(2).

28. Mancini F, Barbanti F, Scaturro M, Fontana S, Di Martino A, Marsili G, et al. Multiplex Real-Time Reverse-Transcription Polymerase Chain Reaction Assays for Diagnostic Testing of Severe Acute Respiratory Syndrome Coronavirus 2 and Seasonal Influenza Viruses: A Challenge of the Phase 3 Pandemic Setting. J Infect Dis. 2021;223(5):765-74.

29. Marshall NC, Kariyawasam RM, Zelyas N, Kanji JN, Diggle MA. Broad respiratory testing to identify SARS-CoV-2 viral co-circulation and inform diagnostic stewardship in the COVID-19 pandemic. Virol J. 2021;18(1):93.

30. Perlitz B, Slagman A, Hitzek J, Riedlinger D, Mockel M. Point-of-care testing for influenza in a university emergency department: A prospective study. Influenza Other Respir Viruses. 2021;15(5):608-17.

31. Perng CL, Jian MJ, Chang CK, Lin JC, Yeh KM, Chen CW, et al. Novel rapid identification of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by real-time RT-PCR using BD Max Open System in Taiwan. PeerJ. 2020;8:e9318.

32. Zhen W, Smith E, Manji R, Schron D, Berry GJ. Clinical Evaluation of Three Sample-to-Answer Platforms for Detection of SARS-CoV-2. J Clin Microbiol. 2020;58(8).

Colophon

Title: Clinical utility of multiplex PCR assays for the diagnosis of acute respiratory infections during the SARS-CoV-2 pandemic

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At the request of: INAMI/RIZIV and Task Force Vaccination

Disclaimer: This document is based on a rapid review of the scientific literature and guidelines from national and international agencies, and on an ad-hoc consultation of Belgian experts. KCE compiled the evidence in short time frames to respond to urgent questions and could therefore not follow its regular methodological procedures. This work is used to inform guidance of other governmental agencies (like RIZIV/INAMI, Sciensano, CSS/HGR, AFMPS/FAGG and SPF/FOD).

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