European multicenter evaluation of Xpert® Xpress SARS-CoV-2/Flu/RSV test

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Abstract
Rapid diagnostics for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are paramount for reducing the spread of the current pandemic. During additional seasonal epidemics with influenza A/B and respiratory syncytial virus (RSV), the clinical signs and symptoms cannot be distinguished easily from SARS-CoV-2. Therefore, a new assay combining four targets in the form of the new Xpert Xpress SARS-CoV-2/Flu/RSV assay was evaluated. The assay was compared to the Xpert Xpress SARS-CoV-2, Xpert Xpress Flu/RSV, Seegene Flu/RSV, influenza A/B r-gene® and RSV/hMPV r-gene®. A total of 295 nasopharyngeal and throat swabs were tested at four institutes throughout Europe including 72 samples positive for SARS-CoV-2, 65 for influenza A, 47 for influenza B, and 77 for RSV. The sensitivity of the new assay was above 95% for all targets, with the highest for SARS-CoV-2 (97.2%). The overall correlation of SARS-CoV-2 Ct values between Xpert Xpress SARS-CoV-2 assay and Xpert Xpress SARS-CoV-2/Flu/RSV assay was high. The agreement between Ct values above 30 showed the multiplex giving higher Ct values for SARS-CoV-2 on average than the singleplex assay. In conclusion, the new assay is a rapid and reliable alternative with less hands-on time for the detection of not one, but four upper respiratory tract pathogens that may circulate at the same time.

Keywords
COVID-19, influenza, multiplex PCR, pandemic, respiratory syncytial virus, SARS-CoV-2

1 | INTRODUCTION

A year into the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, the number of cases remain high and with new mutations resulting in increased infectiousness and transmission, this will most likely remain of concern at least until a significant percentage of the population is vaccinated or naturally immunized.¹,² A fast and accurate SARS-CoV-2 diagnostic remains important to identify infected persons, reduce transmission and gather data for surveillance purposes. A shortage of consumables, protective materials, and polymerase chain reaction (PCR) platform capacity is thus seen. During winter, other respiratory viruses such as influenza A/B and respiratory syncytial virus (RSV), which...
are usually epidemic in Europe between November and April, may co-
incide with SARS-CoV-2.3,4 However, recent data from Australia show
that those seasonal epidemics have been reduced during the SARS-CoV-
2 pandemic, possibly as a result of isolation, restricted travel, and social
distancing.5 These respiratory viruses often show initial similar symptoms
and cannot be differentiated on clinical presentation alone. However,
they do require different isolation regimens, depending on the patient
category, such as children, elderly, and the immunocompromised and the
course of the diseases evolve differently according to each virus.6–9
Additionally, treatment options differ and surveillance of individual pa-
thogens is relevant for public health decision making.10,11 To distinguish
between four of the most common and clinically relevant seasonal airway
pathogens and to be able to identify coinfections rapidly, a multiplex
PCR, the Xpert Xpress SARS-CoV-2/Flu/RSV (multiplex) has been made
available.12,13 The aim of this study was to evaluate this new multiplex
PCR assay in a European multicenter setting.

2 | MATERIALS AND METHODS

2.1 | Study sites and samples selection

Samples from archived collections were selected at four institutes
throughout Europe. The Radboud University Medical Center in Nij-
megen the Netherlands, the Medical University of Graz in Austria,
the Institute of Medical Virology, the University of Zurich/University
Hospital Zurich in Switzerland, and the University Hospital of Re-
ness, in France. Each institute selected samples positive for
SARS-CoV-2, influenza-A, influenza B, and RSV. Those samples have
previously been tested on a range of different platforms. A total of
295 clinical samples were included. The range of Ct values is shown in Table 1.

2.2 | Reference method

2.2.1 | Radboud University Medical Center

Samples consisted of nasopharyngeal and/or throat swabs in UTM or
GLY medium (Table 1) and stored at −80°C. SARS-CoV-2 was tested
using Xpert Xpress SARS-CoV-2 (singleplex) and Xpert Xpress Flu/
RSV was used for the influenza A, B, and RSV positive samples. All
samples underwent one freeze-thaw cycle before use in the valida-
tion of the SARS-CoV-2/Flu/RSV cartridge. Three samples included
were co-infections. Two of the samples selected as influenza A po-
sitive were also RSV positive. One influenza A sample was also SARS-
CoV-2 positive.

2.2.2 | Medical University of Graz

Left-over routine samples that had been obtained by a deep or-
opharyngeal swab with the Copan UTMM™ (Copan) collection system
and had been stored at −80°C were used for this study. All SARS-
CoV-2 diagnostics were performed using the Xpress Xpress SARS-
CoV-2 cartridge. Influenza A and B was tested using the Influenza A/
B R-GENE® (bioMerieux SA) assay, RSV using the RSV/hMPV
R-GENE® (bioMerieux) assay. After extraction on the NiciSSENS®
EMAG® (bioMerieux) platform using the specific B protocol, ampli-
fication and detection were performed on the LC 480 II (Roche Di-
agnostics International Ltd.). Additionally, the CELL Control
R-GENE® (bioMerieux) kit was used for each sample. This assay in-
cludes an amplification premix detecting the human hypoxanthine
phosphor-ribosyl transferase 1 gene and thus checking for the pre-
sence of human cells in the sample. Four samples included were
coinfections, three were influenza A with RSV and one sample was
influenza B with RSV.

2.2.3 | Institute of Medical Virology, University of
Zurich/University Hospital Zurich

Samples of nasopharyngeal and throat swabs tested for SARS-CoV-2
were collected in an in-house virus transport medium (HEPES,
DMEM, FCS, antibiotics) and stored before use at −80°C, samples
tested for influenza A/B and RSV were stored at −20°C. All samples
underwent one freeze-thaw cycle before use in the validation of the
SARS-CoV-2/Flu/RSV cartridge. All samples, SARS-CoV-2, influenza
A/B, and RSV were tested using Xpert Xpress SARS-CoV-2 or Xpert
Xpress Flu/RSV. Three out of 10 RSV positive samples were tested
previously using an in-house PCR.14 Samples with coinfections were
not included.

2.2.4 | Rennes University Hospital

All samples were nasopharyngeal swabs collected in either TranSwab
or eSwab medium and stored at −80°C until use. SARS-CoV-2 was
tested using Xpert Xpress SARS-CoV-2 (singleplex). influenza A/B
and RSV were tested using Seegene Allplex respiratory panel 1
(Eurobio) as recommended by the manufacturer. Seven included
samples had coinfections as determined using the Seegene techni-
que. Five samples were both influenza A and RSV positive, one
sample was influenza B and RSV positive. One sample was influenza
A and B positive.

2.3 | Xpert Xpress SARS-CoV-2/Flu/RSV

The Xpert SARS-CoV-2/Flu/RSV assay was performed at each par-
ticipating institute according to the manufacturer’s protocol and
using research use only cartridges. Briefly, 300 µl of the sample was
added to the cartridge, with a run time of 36 min on a GeneXpert
platform. The cartridge performs sample preparation, RNA isolation,
reverse transcription, and PCR in one single test, without additional
hands-on time. All discrepant results were repeated if sufficient
| Study site                  | Sample type and medium | Reference platform                                      | Number of samples tested using the reference method |
|----------------------------|------------------------|--------------------------------------------------------|----------------------------------------------------|
|                            |                        |                                                        | SARS-CoV-2+ | Influenza A+ | Influenza B+ | RSV+ | Negative |
|                            |                        |                                                        | N           | Range Ct (average) | N           | Range Ct (average) | N           | Range Ct (average) | N           | Range Ct (average) |
| Radboudumc, Nijmegen       | NPS in UTM/GLY         | Xpert Xpress SARS-CoV-2                                 | 21          | 20           | 10           | 22          | 21          | 10           | 9            |
|                            |                        | Xpert Xpress Flu/RSV                                    | N2: 18–42 (30) E: 15–41 (22)                         | 18–37 (26) | 19–32 (24) | Unknown | 18–39 (26) | n.a.          |
| Medical University of Graz | OPS in Copan UTM™      | Xpert Xpress SARS-CoV-2                                 | 20          | 19           | 12           | 24          | 16          | 12           | 10           |
|                            |                        | Influenza A/B r-gene®                                   | N2: 14–38 (26) E: 12–34 (24)                         | 19–33 (26) | 17–27 (24) | 17–38 (28) | 14–30 (34) | n.a.          |
|                            |                        | RSV/hMPV r-gene®                                       |                                                        |            |            |            |            |               |
| Institute of Medical Virology, University of Zurich | NPS/throat                   | Xpert Xpress SARS-CoV-2                                 | 11          | 10           | 10           | 10          | 10           | 10           | 9            |
|                            |                        | Xpert Xpress Flu/RSV                                    | N2: 16–44 (27) E: 18–42 (29)                         | 23–39 (31) | 25–45 (35) | 27–39 (34) | n.a.          |
| Rennes University Hospital | NPS in TranSwab of eSwab | Xpert Xpress SARS-CoV-2                                 | 20          | 16           | 15           | 21          | 14–30 (31) | 20           |
|                            |                        | Seegene FluA/B/RSV Allplex panel 1                      | N2: 14–38 (23) E: 12–35 (21)                         | 21–30 (23) | 14–36 (27) | 14–31 (19) | n.a.          |
|                            |                        |                                                        | Total 72     | Total 65     | Total 47     | Total 77    | Total 49     |

Note: For SARS-CoV-2 the reference method Xpert Xpress SARS-CoV-2 reports two target genes (E- and N2-gene), similar for influenza A (reporting A1 and A2) in Xpert Xpress Flu/RSV.

Abbreviations: NPS, nasopharyngeal swab; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; n.a., not available.
sample material was available. Targets for SARS-CoV-2, influenza A/ 
B, and RSV are similar to the individual cartridges (singleplex SARS- 
CoV-2 and the Flu/RSV). The multiplex assay reports four separate  
Ct values for SARS-CoV-2, Flu A, Flu B, and RSV. The main difference  
with Xpert SARS-CoV-2 is that the multiplex assay reports 1 Ct value  
for both N2 and E targets (mix) and the singleplex assay reports 2 Ct  
values (E and N2).

3 | STATISTICAL ANALYSIS

Positive (PPA) and negative (NPA) percentage agreement of Xpert  
Xpress SARS-CoV-2/Flu/RSV was calculated using cross tables and  
analysed using IBM SPSS Statistics, version 25.0. To evaluate the  
agreement between tests, linear regression analysis with Passing- 
Bablok fit, a Shapiro–Wilk test for normality of the difference, and a  
Bland–Altman plot were calculated using Analyse-it Software, Ltd.  
and SPSS (version 25; IBM Corporation). For Xpert Xpress SARS- 
CoV-2 the average of E- and N2-gene Ct values were calculated and  
compared to the Ct values of the Xpert Xpress SARS-CoV-2/Flu/RSV.  

4 | RESULTS

For all 121 SARS-CoV-2 positive samples tested with the new Xpert  
Xpress SARS-CoV-2/Flu/RSV, a PPA of 97.2% and NPA of 100% was  
observed compared to the reference assay performed at each institute  
(Table 2). Two discrepant results were found; both samples tested
positive with the reference method (Xpert Xpress SARS-CoV-2) but negative with the multiplex assay. Upon retesting, one sample tested negative with both assays (Table 3). The other sample showed Ct values of 44.1 (E-gene) and 42.9 (N2-gene) with the singleplex assay.

Agreement of SARS-CoV-2 Ct values for Xpert Xpress SARS-CoV-2 and Xpert Xpress SARS-CoV-2/Flu/RSV are shown in Figure 1. The correlation between both tests was high ($R^2 = 0.89$). Normal distribution of the differences was confirmed using the Shapiro–Wilks test to

| Study Site | Target            | Comparator method & Result | Ct value comparator | Xpert Xpress SARS-CoV-2/Flu/RSV | Comments |
|------------|-------------------|---------------------------|---------------------|---------------------------------|----------|
| Zurich     | SARS CoV-2        | Xpert Xpress SARS-CoV-2   | E-gene: 44.1        | Not Detected                    | High Ct values with Xpert Xpress SARS-CoV-2 |
|            |                   | Positive                  | N2-gene: 42.9       |                                 |          |
| Rennes     | SARS-CoV-2        | Xpert Xpress SARS-CoV-2   | E-gene: 31.1        | Not Detected                    | Sample was negative on retesting with singleplex cartridge. |
|            |                   | Positive                  | N2-gene: 33         |                                 |          |
| Zurich     | RSV               | Xpert Xpress Flu/RSV      | RSV: 38.2           | Not Detected                    | High Ct values with Xpert Xpress Flu/RSV, Close to limit of detection |
|            |                   | RSV positive              |                     |                                 |          |
| Graz       | RSV               | Influenza A/B r-gene®     | Negative            | Positive Ct value 42            | High Ct values with Xpert Xpress SARS-CoV-2/Flu/RSV |
|            | negative, Xpert Xpress SARS-CoV-2/Flu/RSV R® negative |                     |                     |                                 |          |
| Graz       | RSV               | Influenza A/B r-gene®     | Negative            | Positive Ct value 40.9          | High Ct values with Xpert Xpress SARS-CoV-2/Flu/RSV |
|            | negative, RSV/hMPV R-GENE® negative |                     |                     |                                 |          |
| Rennes     | RSV               | Seegene FluA/B/RSV       | RSV: 38.0           | Not Detected                    | High Ct values with Seegene, Close to limit of detection |
|            |                   | RSV positive              |                     |                                 |          |
| Nijmegen   | RSV               | Xpert Xpress Flu/RSV      | RSV: 40.1           | Not Detected                    | High Ct value, Close to limit of detection |
|            |                   | RSV positive              |                     |                                 |          |
| Zurich     | Flu A             | Xpert Xpress Flu/RSV      | Influenza A1: 38.2  | Not Detected                    | High Ct value with Xpert Xpress Flu/RSV, Close to limit of detection |
|            |                   | Influenza A2: negative    |                     |                                 |          |
| Zurich     | Flu A             | Xpert Xpress Flu/RSV      | Influenza A1: 35.8  | Not Detected                    | High Ct value with Xpert Xpress Flu/RSV, Close to limit of detection |
|            |                   | Influenza A2: 37.9        |                     |                                 |          |
| Nijmegen   | Flu A             | Xpert Xpress Flu/RSV      | Influenza A1: 37.4  | Flu A Not Detected              | High Ct value with Xpert Xpress Flu/RSV, Close to limit of detection |
|            |                   | Influenza A2: 0          |                     | SARS-CoV-2: 37*                 |          |
|            |                   | SARS-CoV-2 positive      |                     |                                 |          |
| Rennes     | Flu B             | Seegene FluA/B/RSV       | Influenza A: 29.0   | Flu A Not Detected              | Mixed infection, Flu A detected in both samples and Flu B not detected with 4 Plex |
|            |                   | Influenza B positive     |                     |                                 |          |
| Rennes     | Flu B             | Seegene FluA/B/RSV       | Influenza B: 36     | Not Detected                    | High Ct value with Seegene, Close to limit of detection |
|            |                   | Influenza B positive     |                     |                                 |          |

Note: Depicted for each target. Ct values of comparator method reported. The green/orange background indicate the details on individual samples showing discrepancies between tests. *One SARS-CoV-2 sample was detected using the in-house PCR and Xpert Xpress SARS-CoV-2 as was done for all other samples, performed using COBAS 4800 with RT-PCR described by Corman et al.15

Abbreviations: RSV, respiratory syncytial virus; RT-PCR, reverse-transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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5 | DISCUSSION

Early and rapid detection of SARS-CoV-2 remains vital to reduce the current pandemic and optimize patient care. The Xpert Xpress SARS-CoV-2 test has been available since March 2020. In previous seasons, the Xpert Xpress Flu/RSV had been successfully used to rapidly identify influenza and RSV in patients with respiratory infections.13,16–18

For seasons with high influenza and RSV incidence coinciding with the SARS-CoV-2 pandemic, the combined SARS-CoV-2/Flu/RSV cartridge was developed. Overall, we found the PPA to be above 95% for all tested targets, with the highest percentage for SARS-CoV-2 (97.2%). The correlation between testing was found to be high, which is to be expected when using two near-identical platforms with similar targets. However, the Bland–Altman plot suggested that samples with Ct values above 30 show higher levels in the multiplex compared to the singleplex assay, and this is not equally distributed compared to samples below Ct 30. As the number of samples at this higher Ct value is relatively low, a greater sample size would be needed to identify if this is a common occurrence.

Two samples were discordant for detection of SARS-CoV-2 between the singleplex and multiplex assays. One of the samples was negative on retesting in the singleplex assay. This could either mean that freeze-thawing steps degraded the sample although this would be relatively surprising as an initial Ct value of 33 is relatively low. Alternatively, misclassification or sample mix-up on initial testing before selection of the sample could be the cause. The other discordant case occurred in a sample with a low viral load, which could be near the limit of detection of the Xpert assay. This finding was similar for samples positive for influenza A, B, and RSV by Xpert Flu/RSV but negative by Xpert SARS-CoV-2/Flu/RSV. These discordant cases could be caused by several freeze-thaw steps as these samples were not retested using the reference method at the same time as testing with the multiplex assay.

In this study, throat and oropharyngeal swabs were used which are not part of the claim for the Xpert assay. However, these sample types are widely used in the field and are, in some countries, part of national guidelines, which is why it is important to assess the performance of the assay in this setting. Additionally, several types of transport medium were used for collection of samples. These were UTM/GLY medium, Copan UTM, and TranSwab or eSwab (Table 1). Validation of transport medium was not performed in the current assay however all transport media has previously been validated for its use on the GeneXpert platform at the individual sites, where it has been used in different cartridges or on other reference platforms (Table 1) without reduction of sensitivity.

Overall, the results obtained from the new SARS-CoV-2/Flu/RSV cartridge show good agreement with results obtained from previous testing. The faster turn-around time of only half an hour is of major significance for fast and accurate treatment as well as for public health decisionmaking during the current SARS-CoV-2 pandemic.
CONFLICT OF INTERESTS
The authors have declared that there are no conflict of interests.

AUTHOR CONTRIBUTIONS
Femke Wolters conducted the research, performed analysis, and wrote the manuscript. Maria Grünberg, Michael Huber, Harald H. Kessler, Florian Prüller, Lanja Saleh, Christine Fébreau, Janette Rahamat-Langendoen, and Vincent Thibault conducted research, analyzed the data locally, and were responsible for proofreading the manuscript. Willem J. G. Melchers supervised the study and proofread the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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