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Prospective evaluation of three rapid molecular tests for seasonal influenza in patients presenting at an emergency unit

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ABSTRACT

Background: For infection control measures, rapid accurate diagnostics on admission of patients with suspected seasonal influenza is crucial.
Objective: Prospective comparison of three rapid molecular tests for detection of influenza A/B RNA.
Study design: Outpatients presenting at the Medical emergency department of Graz University Hospital with influenza-like illness and a requirement for hospitalization (n = 312) were studied. Nasopharyngeal swabs were collected with the 3 mL-version of the UTM™ Viral Transport Medium (Copan). Specimens were tested for influenza A and B RNA using the Alere™ i Influenza A & B (Abbott), the cobas® Influenza A/B (Roche), and the Xpert® Xpress Flu/RSV (Cepheid) tests. Results were compared to those obtained from the same specimen by the Influenza A/B R-GENE® (bioMerieux) test based on real-time PCR as reference method.
Results: Overall sensitivities of the Abbott, Roche, and Cepheid tests were 90.5%, 96.0%, and 97.0%, overall specificities 99.4%, 97.6%, and 98.2% respectively. With the Abbott and the Cepheid tests, all specimens gave valid results, while the Roche test showed invalid results in 37 (12.1%) specimens. Total time to result for the Abbott, Roche, and Cepheid tests was 18 min, 22 min, and 32 min respectively.
Conclusions: The Abbott test lacked sensitivity, the Roche test was impaired by a high number of invalid results. Overall, despite the longest total time to result, the Cepheid test showed the best performance to detect influenza virus RNA in symptomatic patients presenting at an emergency unit in this study.

1. Background

Seasonal influenza is a challenge for hospital infection control. Patients are required to be separated from other patients to prevent nosocomial spreading. Rapid diagnosis of seasonal influenza for patients with appropriate clinical symptoms upon admission is thus crucial. Furthermore, timely diagnosis can prevent unnecessary additional diagnostic tests and changed management plans in 61% of cases [1]. Clinical diagnosis during influenza season using cough and fever as predictors has a positive predictive value of 79%–86.8% [2,3]. However, clinical diagnosis can be difficult due to many other circulating respiratory viruses. Immunocompromised hosts are at higher risk for complications and may present with fewer signs and symptoms [4]. Clinical diagnosis alone is therefore insufficient for infection control and treatment purposes [5].

Conventional real-time PCR for detection of influenza A/B RNA shows high sensitivity and specificity, but is impaired by an increased time to result and often not available on weekends and during the night. A rapid diagnostic test with high sensitivity and specificity providing the result while the patient is still in the emergency room is thus required. Previously used rapid diagnostic antigen tests such as the BinaxNOW® Influenza A & B (Abbott Molecular, Des Plaines, IL, USA) had a low sensitivity of 44.4–71.0 and 25.0–37.2% for influenza A and B, respectively [6,7]. Recently the FDA has reclassified rapid antigen tests proposing minimum performance standards including a sensitivity of > 80% for influenza A and B when molecular methods are used as the comparator [8].

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Table 1
Features of molecular tests for detection of influenza A/B viral RNA used in this study.

Characteristics	Test name			
	Alere™ i Influenza A & B	cobas® Liat® Influenza A/B	GeneXpert Xpert®Xpress Flu/RSV	Influenza A/B R-GENE®[a]
Manufacturer	Abbott Molecular	Roche Molecular Systems	Cepheid	bioMerieux
Target regions	PB2 of influenza A RNA, PA of influenza B RNA	M of influenza A RNA, NS of influenza B RNA	M, PB2, and PA of influenza A RNA, M and NS of influenza B RNA	Matrix protein gene of influenza A RNA and influenza B RNA
Sample input volume (µL)	200	200	300	500
Amplification / detection method	Nicking endonuclease amplification reaction (isothermal)	Duplex real-time PCR	Multiplex real-time PCR	Duplex real-time PCR
Control(s)/run	Internal procedural control	Internal process control	Sample processing control, probe check control	Positive control and negative extraction and amplification controls

[a] This test was used as reference assay in combination with the CELL Control R-GENE® kit (bioMérieux).

2. Objectives

Rapid molecular tests for seasonal influenza appear to be promising tools to optimize hospital infection control and patient management. The aim of the present study was to evaluate three different rapid molecular tests for detection of influenza A/B RNA. Results obtained were compared to those by conventional real-time PCR as gold standard. Additionally, time to result and hands-on time were measured for each test system.

3. Study design

3.1. Molecular assays

For the evaluation study, the Alere™ i Influenza A & B (Abbott Molecular, Des Plaines, IL, USA), cobas® Influenza A/B for use on the cobas® Liat® System (Roche Molecular Systems, Branchburg, NJ, USA), and the Xpert® Xpress Flu/RSV for use on the GeneXpert® System (Cepheid, Sunnyvale, CA, USA) were employed. Characteristics of the assays are shown in Table 1. All assays were performed according to the manufacturer's package instructions. Results were assigned as "positive", "negative", or "invalid". In addition to run controls, each package of the Abbott kit includes external positive and negative control swabs that have to be analyzed prior to the use of a new kit package. Before using a new lot of the Roche kit, the "Add Lot" procedure must be performed using the cobas® Influenza A/B Quality Control Kit that includes positive and negative control samples. The in vitro diagnostics (IVD)/Conformité Européenne (CE)-labeled Influenza A/B R-GENE® (bioMérieux S.A., Marcy l´Etoile, France) was employed as reference assay. Specimens were extracted on the NucliSENS® easyMAG® (bioMerieux) instrument using the specific B protocol. Amplification and detection was performed on the LC 480 II (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Additionally, the CELL Control R-GENE® kit (bioMérieux) was applied for each sample. This assay includes an amplification premix detecting the human hypoxanthine phosphor-ribosyl transferase 1 gene and thus checking for the presence of human cells in the sample.

3.2. Design of the study

The study was approved by the local Ethics Committee (No. 29-560 ex 2016/17). All adult patients presenting to the Emergency Department of the Medical Department during influenza season 2017/2018 suffering from acute febrile respiratory tract infection and at least one risk factor for complications of seasonal influenza (usually requiring hospital admission) were included in the study (Table 2). Inclusion criteria corresponding to those defined by the Infectious Diseases Society of America were used [9].

One deep nasopharyngeal swab, integral part of the Copan UTM™ (Copan, Brescia, Italy) collection system, was obtained from each patient. For laboratory analysis, swabs were removed from the UTM™ tube containing 3 mL of viral transport medium and aliquots analyzed with the rapid molecular assays at the Clinical Institute of Medical and Laboratory Diagnostics in random order. The remaining sample material was blinded and transferred to the Molecular Diagnostics Laboratory for reference testing. All specimens were stored at 4 °C tests and processed within 72 h.

Table 2
Inclusion criteria corresponding to those defined by the Infectious Diseases Society of America [6]: acute respiratory tract infection and at least one of the following conditions.

High risk for complications of seasonal influenza
○Nursing home resident
○Aged > 65 years
○Pregnant women and women up to 2 weeks post-partum
○Chronic pulmonary disease (e.g. asthma, chronic obstructive pulmonary disease, cystic fibrosis)
○Significant cardiovascular disease (except isolated arterial hypertension)
○Chronic renal dysfunction
○Chronic hepatic disease
○Diabetes mellitus
○Active malignancy
○Hemoglobinopathy including sickle cell disease
○Current immunosuppressive treatment or immunosuppressive disorders
○Patients with neuromuscular disorders or cognitive dysfunction compromising the handling of respiratory secretions
● Other indication for hospital admission (e.g. heart failure, renal failure)
● Hospital-acquired respiratory tract infection (onset within 72 h following discharge)

Sample size calculation was performed with PASS 15 (NCSS, East Kaysville, Utah, USA) using the Wilson score estimating a prevalence of 0.4, a sensitivity of 0.95, specificity of 0.95, precision of 0.05 and a dropout-rate of 5% and yielded a dropout inflated enrolment sample size of 258 [10]. Sensitivity and specificity of assays evaluated were calculated. Comparison of distributions was performed using McNemar's Test for paired samples. The turnaround time was measured for all assays evaluated in this study including the hands-on time. Data were analyzed using SPSS version 23 (IBM, Armonk, NY, USA).

4. Results

Based on the result of the reference assay, seven of 312 specimens were excluded from analysis due to a negative cellular control. The reference assay gave 43 positive results for influenza A RNA, 94 positives for influenza B RNA, and 168 negatives.

Sensitivities and specificities of the three rapid molecular tests evaluated in this study are shown in Table 3. The Abbott test showed a significantly lower overall sensitivity when compared to the Roche and the Cepheid tests. This was mainly due to the low sensitivity for influenza A RNA, while the sensitivity for influenza B RNA did not differ significantly from the alternative tests. The specificity was comparable to that found with the alternative tests. With ROC analysis, the lowest

Table 3
Sensitivities, specificities, area under the curve (AUC) and p-values of McNemar's test for paired samples of three rapid molecular tests using Influenza A/B R-GENE® as reference assay.

	Test name		
	Alere™ i Influenza A & B	cobas® Liat® Influenza A/B	GeneXpert Xpert®Xpress Flu/ RSV
No. specimens included for analysis	305	268[a]	305
Results for Influenza A RNA			
Sensitivity (%)	79.1	100	97.7
Specificity (%)	99.6	98.7	99.6
AUC	0.89	0.99	0.99
McNemar's test (*p* value)	< 0.05	0.25	1.00
Results for Influenza B RNA			
Sensitivity (%)	95.7	93.1	96.8
Specificity (%)	100	99.5	99.1
AUC	0.97	0.96	0.97
McNemar's test (*p* value)	0.13	0.38	1.00
Overall results			
Sensitivity (%)	90.5	96.0	97.0
Specificity (%)	99.4	97.6	98.2
AUC	0.93	0.97	0.97
McNemar's test (*p* value)	< 0.05	1.00	1.00

[a] 37 specimens gave invalid results and were excluded from analysis.

AUC was found with the Abbott test. The Roche test yielded invalid results in 37 specimens, remaining invalid on repeat testing. All of these specimens were found to be positive with the reference assay (36 positives for influenza B RNA and one positive for influenza A RNA). The majority of these positives showed low cycle threshold values with the reference assay indicating a high influenza RNA concentration. Results for sensitivity and specificity were comparable to those obtained with the Cepheid test.

The overall time required was 18 min per sample with the Abbott test when a single sample was tested (Table 4). The Roche test could be performed within 22 min, the Cepheid test within 32 min. Hands-on time was found to be 8 min for the Abbott test and 2 min for the Roche test and 1.5 min for the Cepheid test (Table 4).

5. Discussion

Rapid diagnosis of influenza RNA is essential for patient management during influenza season. In this study, three different rapid molecular assays for the diagnosis of influenza A and B from clinical specimens of patients presenting at an emergency unit were evaluated and compared.

When compared to the Roche and the Cepheid tests, the Abbott test showed a considerably lower sensitivity for influenza A RNA than for influenza B RNA. This has also been shown in a recent study using the Cepheid test as reference assay (sensitivity of 93.8% for influenza A RNA and 100% for influenza B RNA) [11]. In another study evaluating the Abbott test using the Cepheid test as reference assay, sensitivities of 93.8% and 91.2% for influenza A and B RNA, respectively where found; however, the Abbott test showed very low specificities of 62.5% and 53.6% [12]. Another study using conventional real-time PCR as reference method found a low sensitivity (77.8%) for influenza A RNA and an even lower sensitivity (75%) for influenza B RNA with the Abbott test [6]. However, it must be taken into consideration that only 12 influenza B RNA positives were found in that study. The Cepheid test showed sensitivities of 97.7% and 96.8% for influenza A RNA and influenza B RNA in the present study. These results are comparable with those obtained by recent studies [13,14].

The major drawback of the Roche test in the present study was the very high percentage of invalid results in influenza B RNA-positive specimens. This has also been reported in a recent study with a high rate of 6.5% invalid results [14]. In that study, 6 out of 13 results remained invalid on repeat testing; in the present study, all 37 invalid results remained invalid. In contrast, another recent study did not report any invalid result when using the Roche test for 178 specimens [1]. Two other studies reported rates of invalid results with the Roche test of 1% and 1.6% [15,16]. In the present study, 29 of 36 specimens that had given invalid results with the Roche test but were found to be positive for influenza B RNA with the reference assay were available for retesting with the cobas® Influenza A/B and RSV test (Roche) that was brought on the market recently. All 29 samples were found to be positive when employing this test. In addition, these specimens were subjected to sequencing at Roche Molecular Systems (Pleasanton, CA, USA). Polymorphisms that based on bioinformatics analysis should not prevent amplification of the target region were found. The test did not provide a false negative result for any of the samples and amplification occurred but the script to accurately identify samples as influenza B positive could not interpret the signal resulting in invalids. Roche is taking this information into consideration for future script updates of the test. For the Roche Influenza A/B & RSV test, its specific script was able to accurately identify the samples as influenza B positive.

All tests evaluated in this study could be completed within 32 min; however, the total time to result was higher for the Cepheid test when compared to the Abbott and the Roche tests. When hands-on times were compared, the Abbott test required an additional manual step in comparison to the other assays and thus appeared as suboptimal for use as a point of care testing device. In contrast to our study, discordant results were found in 15 of 159 specimens due to erroneous automatic interpretation with the Cepheid test when compared to conventional multiplex RT-PCR in a recent study [17]. Authors suggest careful analysis of amplification curves and endpoint analysis when employing the Cepheid test.

An important strength of our study is that all tests were performed from the identical specimen thus ensuring comparability of results. Seven of 312 specimens (2.2%) lacked the cellular control signal with the reference assay indicating an inadequate specimen collection. The rapid molecular tests do not use a cell control with the quality of the specimen remaining uncertain and results possibly false-negative. Another strength of the present investigation is that all samples were collected with specially designed swabs (Copan UTM™ 3 m L-flocked swabs) for which the manufacturer guarantees nucleic acid stability for 96 h. Furthermore, specimens were processed within 72 h after specimen collection avoiding storage at −70 °C which might lead to RNA degradation followed by decreased sensitivity [18]. Another interesting aspect of our investigation is the relatively high rate of influenza B infections which was a characteristic of the influenza season 2017/2018 in Europe [19].

In conclusion, while specificity was found to be high with all three assays evaluated, the sensitivity was found to be highest with the Cepheid test. The Abbott test showed a considerably lower sensitivity for influenza A, while the Roche test was impaired by a high rate of invalid test results. All tests could be completed within about 30 min

Table 4
Mean times required for analysis per single sample.

	Test name		
	Alere™ i Influenza A & B	cobas® Liat® Influenza A/B	GeneXpert Xpert®Xpress Flu/RSV
Total time (min)	18	22	32
Hands-on time (min)	8	2	1.5

with the Abbott test requiring the longest hands-on time. Overall, despite the longest total time to result, the Cepheid test showed the best performance to detected influenza virus RNA in symptomatic patients presenting at an emergency unit in this study.

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Competing interests

None declared.

Ethical approval

The study was approved by the Ethics Committee of the Medical University of Graz, Austria (No. 29-560 ex 2016/17).

CRediT authorship contribution statement

Thomas Valentin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Petra Kieslinger:** Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft. **Evelyn Stelzl:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Brigitte I. Santner:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft. **Andrea Groselj-Strele:** Conceptualization, Formal analysis, Investigation, Methodology, Software, Supervision, Visualization, Writing - original draft. **Harald H. Kessler:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Beate Tiran:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing.

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References

[1] G.T. Hansen, J. Moore, E. Herding, T. Gooch, D. Hirigoyen, K. Hanson, et al., Clinical decision making in the emergency department setting using rapid PCR: results of the CLADE study group, J. Clin. Virol. 102 (2018) 42–49.
[2] A.S. Monto, S. Gravenstein, M. Elliott, M. Colopy, J. Schweinle, Clinical signs and symptoms predicting influenza infection, Arch. Intern. Med. 160 (2000) 3243–3247.
[3] G. Boivin, I. Hardy, G. Tellier, J. Maziade, Predicting influenza infections during epidemics with use of a clinical case definition, Clin. Infect. Dis. 31 (2000) 1166–1169.
[4] M.J. Memoli, R. Athota, S. Reed, L. Czajkowski, T. Bristol, K. Proudfoot, et al., The natural history of influenza infection in the severely immunocompromised vs nonimmunocompromised hosts, Clin. Infect. Dis. 58 (2014) 214–224.
[5] S.A. Call, M.A. Vollenweider, C.A. Hornung, D.L. Simel, W.P. McKinney, Does this patient have influenza? Jama 293 (2005) 987–997.
[6] B. Hazelton, T. Gray, J. Ho, V.M. Ratnamohan, D.E. Dwyer, J. Kok, Detection of influenza A and B with the Alere i Influenza A & B: a novel isothermal nucleic acid amplification assay, Influenza Other Respir. Viruses 9 (2015) 151–154.
[7] C.H. Cho, M.K. Woo, J.Y. Kim, S. Cheong, C.K. Lee, S.A. An, et al., Evaluation of five rapid diagnostic kits for influenza A/B virus, J. Virol. Methods 187 (2013) 51–56.
[8] D.A. Green, K. StGeorge, Rapid antigen tests for influenza: rationale and significance of the FDA reclassification, J. Clin. Microbiol. 56 (2018).
[9] S.A. Harper, J.S. Bradley, J.A. Englund, T.M. File, S. Gravenstein, F.G. Hayden, et al., Seasonal influenza in adults and children–diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America, Clin. Infect. Dis. 48 (2009) 1003–1032.
[10] K. Hajian-Tilaki, Sample size estimation in diagnostic test studies of biomedical informatics, J. Biomed. Inform. 48 (2014) 193–204.
[11] Nguyen Van JC, F. Camelena, M. Dahoun, B. Pilmis, A. Mizrahi, J. Lourtet, et al., Prospective evaluation of the Alere i Influenza A&B nucleic acid amplification versus Xpert Flu/RSV, Diagn. Microbiol. Infect. Dis. 85 (2016) 19–22.
[12] K.C. Chapin, E.J. Flores-Cortez, Performance of the molecular Alere I influenza A&B test compared to that of the xpert flu A/B assay, J. Clin. Microbiol. 53 (2015) 706–709.
[13] S.M. Novak-Weekley, E.M. Marlowe, M. Poulter, D. Dwyer, D. Speers, W. Rawlinson, et al., Evaluation of the Cepheid Xpert Flu Assay for rapid identification and differentiation of influenza A, influenza A 2009 H1N1, and influenza B viruses, J. Clin. Microbiol. 50 (2012) 1704–1710.
[14] L. Ling, S.E. Kaplan, J.C. Lopez, J. Stiles, X. Lu, Y.W. Tang, Parallel validation of three molecular devices for simultaneous detection and identification of influenza a and B and respiratory syncytial viruses, J. Clin. Microbiol. 56 (2018).
[15] F.S. Nolte, L. Gauld, S.B. Barrett, Direct comparison of Alere i and cobas liat influenza a and B tests for rapid detection of influenza virus infection, J. Clin. Microbiol. 54 (2016) 2763–2766.
[16] K.A. Stellrecht, S.M. Nattanmai, J. Butt, V.P. Maceira, A.A. Espino, A.J. Castro, et al., Effect of genomic drift of influenza PCR tests, J. Clin. Virol. 93 (2017) 25–29.
[17] I. Engelmann, E.K. Alidjinou, M. Lazrek, A. Dewilde, D. Hober, Necessity to critically review the automatic results of the Xpert Flu assay, Diagn. Microbiol. Infect. Dis. 88 (2017) 26–30.
[18] P. Jokela, T. Vuorinen, M. Waris, R. Manninen, Performance of the Alere i influenza A&B assay and mariPOC test for the rapid detection of influenza A and B viruses, J. Clin. Virol. 70 (2015) 72–76.
[19] Control. WHOaECfDPa. Influenza intensity, spread and dominant virus type/subtype, 2017/18 season overview. Available at: http://flunewseurope.org/.