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Comparison of the Idylla™ Respiratory (IFV-RSV) panel with the GeneXpert Xpert® Flu/RSV assay: a retrospective study with nasopharyngeal and midturbinate samples

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ABSTRACT

The objective of this study was to compare the performance of the Idylla™ Respiratory (IFV-RSV) panel to the GeneXpert Xpert® Flu/RSV assay and establish the performance of a midturbinate swab compared to nasopharyngeal sampling. Considering GeneXpert® assay as imperfect reference standard, a positive percentage agreement between both assays of 98–100% for influenza A and 96–99% for influenza B could be calculated when 354 nasopharyngeal and 325 midturbinate swabs were retrospectively analyzed. Comparing midturbinate samples to nasopharyngeal specimens of 321 subjects, positive percentage agreement varied from 42% to 94% depending on both target virus and assay used. Negative percentage agreements ranged from 98% to 100% for both methods and sample type comparison. The Idylla™ assay showed excellent performance compared to the GeneXpert® assay for the detection of influenza virus. The study also showed a slightly better performance for nasopharyngeal sampling compared to the use of a midturbinate swab.

1. Introduction

Influenza viruses are among the most important human pathogens contributing to seasonal respiratory tract infections. Nonspecific symptoms including fever, headache, cough, or sore throat make it difficult to differentiate between infections with other respiratory viruses such as respiratory syncytial virus (RSV) (Falsey et al., 2014; Stohr, 2002). Antigen detection methods are often used to aid in diagnosis; however, new rapid molecular detection methods have shown superior sensitivity (DiMaio et al., 2012). As the results are often available approximately 1 h after sample loading, these molecular-based tests can have a positive impact on patient management when available for routine use. Different sampling upper respiratory techniques exist such as the use of midturbinate, nasopharyngeal or oropharyngeal swabs, or nasal washes and nasopharyngeal aspirates. It has been well described that aspirates and washes are superior to the other specimens for the detection of respiratory viruses (Blaschke et al., 2011). They are however not the method of choice because of their more invasive nature compared to the use of swabs. Few studies have compared the performance of different sampling methods, which seem to be depending on the technique used, material composition, and target virus (Daley et al., 2006; Grijalva et al., 2014; Spencer et al., 2013). In general, nasopharyngeal swabs (NPS) are used because they are also the manufacturers' validated sample type for several diagnostic tests.

Idylla™ Respiratory (IFV-RSV) Panel (Janssen Pharmaceutica NV, Belgium) is a new molecular assay for the detection of influenza A [H1, H3, H1N1 (2009)], influenza B, and RSV (RSV-A and RSV-B) which runs on the Idylla™ system (Biocartis NV, Belgium). In addition to identification of different influenza A subtypes, presence of the H275Y mutation in the neuraminidase gene, which is the most common mutation conferring oseltamivir resistance in influenza A/H1N1 strains, is also detected. The GeneXpert Xpert® Flu/RSV assay (Cepheid, Sunnyvale, CA) is a molecular detection method for influenza and RSV of which the performance characteristics have already been studied and compared to in-house RT-PCR assays, with sensitivities varying from 90% to 100% and specificities in the 99–100% range (Popowitch and Miller, 2015; Salez et al., 2015).

The objective of this study was to compare the Idylla™ Respiratory (IFV-RSV) panel, of which no published data are available, with the GeneXpert Xpert® Flu/RSV assay and to investigate the performance of the less invasive sampling method, midturbinate swab (MTS), in comparison to NPS.

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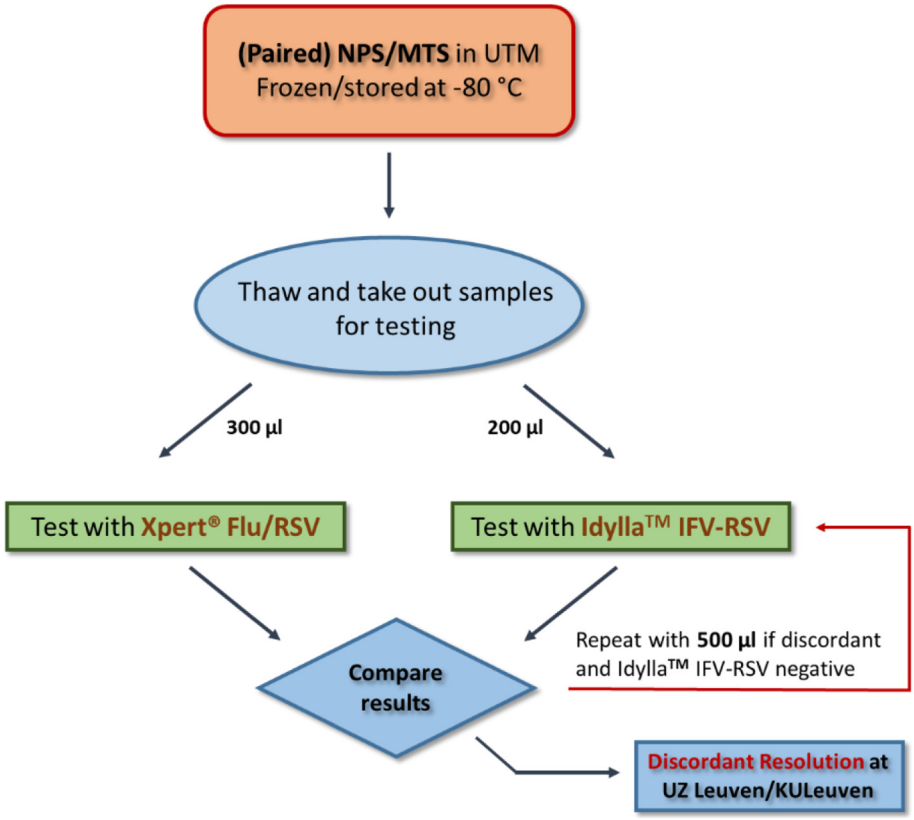

Fig. 1. Study design.

2. Materials and methods

2.1. Sample collection

During the influenza season of 2015–2016, samples collected from patients presenting with influenza-like illness at the Heilig Hart Hospital Lier (Lier, Belgium) were stored at −80 °C after routine testing. A total of 354 nasopharyngeal and 325 mid-turbinate swabs (COPAN diagnostics Inc., Murrieta, CA) were collected in 3 mL Universal Transport Medium (UTM) from 358 different patients. Both NPS and MTS were available for 321 paired samples. Equal gender distribution (M 51%, F 49%) was found among each sample type. Age distribution was as follows: 45% (162/358) children ≤6 years, 39% (139/358) older adults ≥65 year, and 16% (57/358) patients aged 7–64 years old.

2.2. Sample processing

Paired samples, nasopharyngeal and midturbinate, were thawed, mixed, and analyzed simultaneously or within a maximum time span of 1 h for both assays. All samples were brought to room temperature 2 h prior to testing. A sample volume of 300 µL UTM was needed for analysis with GeneXpert®, whereas 200 µL of UTM was used for Idylla™ testing. In case of a discordant result between the 2 molecular platforms with no target virus detected by Idylla™ or between the 2 sample types both analyzed with the Idylla™ panel, retesting with the Idylla™ IFV-RSV panel was performed with an increased volume of 500 µL UTM. Retesting with one of the molecular platforms did also occur when no result was generated due to a system error. Samples were refrozen and stored at −80 °C after definitive results were obtained. In case of discordance,

Table 1
Comparative results between Idylla™ Respiratory (IFV-RSV) panel and GeneXpert Xpert® Flu/RSV assay.

Sample type	Target virus	TP	FP	TN	FN	Total	PPA[a] (95% CI)	NPA[a] (95% CI)
NPS	Flu A	59	3	292	0	354	100.00% (93.94–100.00)	98.98% (97.06–99.79)
	Flu B	102	0	248	4	354	96.23% (90.62–98.96)	100.00% (98.52–100.00)
	RSV	11	4	339	0	354	100.00% (71.51–100.00)	98.83% (97.04–99.68)
MTS	Flu A	53	1	269	1	324	98.15% (90.11–99.95)	99.63% (97.95–99.99)
	Flu B	92	4	227	1	324	98.92% (94.15–99.97)	98.27% (95.63–99.53)
	RSV	4	0	320	0	324	100.00% (39.76–100.00)	100.00% (98.85–100.00)

Flu A = influenza A; Flu B = influenza B; TP = true positives; FP = false positives; TN = true negatives; FN = false negatives.
[a] PPA and NPA are calculated using the GeneXpert Xpert® Flu/RSV assay as reference standard.

Table 2
Discordance resolution by RT-PCR at UZ Leuven/KU Leuven.

Sample type	# Samples	Result Idylla™	Result GeneXpert®	UZL RT-PCR result	KUL RT-PCR result
NPS	4	Neg	Flu B	3/4 Flu B, 1/4 Flu A	RSV Neg
	2	Flu A	Neg	Flu A	RSV Neg
	1	Flu A & B	Flu B	Flu A & B	RSV Neg
	2	RSV B	Neg	Flu Neg	RSV B
	1	Flu B-RSV B	Flu B	Not tested for influenza	RSV B
	1	Flu B-RSV A & B	Flu B	Not tested for influenza	RSV Neg
MTS	1	Flu A	Neg	Flu A	RSV Neg
	3	Flu B	Neg	2/3 Flu B, 1/3 FLU Neg	RSV Neg
	1	Flu A&B	Flu A	Flu A & B	RSV Neg
	1	Neg	Flu A	Flu A	RSV Neg
	1	Neg	Flu B	Flu A & B	RSV Neg

Neg = negative; UZL = UZ Leuven; KUL = KU Leuven.

samples were sent for confirmative testing with an in-house RT-PCR to the Laboratory for Molecular Diagnosis (CEMOL) at University Hospitals Leuven (UZ Leuven, Leuven, Belgium) for influenza virus or to the Laboratory of Clinical and Epidemiological Virology at the Rega Institute for Medical Research of the Catholic University of Leuven (KU Leuven, Leuven, Belgium) for RSV. An overview of the study design is shown in Fig. 1.

2.3. Statistical analysis

Results obtained by the GeneXpert Xpert® Flu/RSV assay were considered as imperfect reference standard when comparing both molecular assays. When comparing different sampling techniques, results obtained from NPS samples were considered as reference. Positive percentage agreement (PPA) and negative percentage agreement (NPA) were calculated using Medcalc® version 16.1 (Medcalc Software, Mariakerke, Belgium).

3. Results

A total of 679 specimens were analyzed with both the Idylla™ Respiratory (IFV-RSV) panel and the GeneXpert Xpert® Flu/RSV assay. Only 6 samples (0.88%) with the Idylla™ panel and 14 samples (2.06%) with GeneXpert® assay needed repeat testing because of an internal failure or error report. All of the samples yielded valid results on repeating with the exception of 1 blood-contaminated MTS. The GeneXpert® system typed the sample as negative, whereas the error could not be resolved with the Idylla™ assay. No confirmative testing was performed, and the sample was excluded from data analysis.

Out of the 354 nasopharyngeal specimens, 343 produced concordant results when analyzed simultaneously on both molecular platforms. A discordance for influenza A, influenza B, or RSV was found in 11 samples. PPA of 100.00% for influenza A, 96.23% for influenza B, and 100.00% for RSV was calculated, with an NPA of 98.98%, 100.00%, and 98.83%, respectively. Similar performance characteristics were found when comparing the results of the 325 midturbinate specimens. Here, 1 sample was discarded because of the unresolved error with the Idylla™ Respiratory (IFV-RSV) panel, whereas 317 results were concordant, and only 7 samples showed a discordance in at least 1 target virus. PPA of 98.15% for influenza A, 98.92% for influenza B, and 100.00% for RSV was calculated, with an NPA of 99.63%, 98.27%, and 100.00%, respectively. Both the tests were able to detect RSV; it should be noted that only a small number of positive samples were included, so no conclusions can be made regarding the sensitivity of the assay. Idylla™ detected RSV in 19 samples, of which 18 were confirmed by either RT-PCR or the GeneXpert®. The GeneXpert Xpert® Flu/RSV assay typed 15 specimens as RSV positive. A detailed overview of these results is presented in Table 1.

In-house RT-PCR testing for discordant resolution typed 10 samples in favor of the Idylla™ Respiratory (IFV-RSV) panel and 8 in favor of the GeneXpert Xpert® Flu/RSV assay. As shown in Table 2, results of the RT-PCR method were mostly in line with the assay which detected the virus.

For comparing different sampling techniques, 321 paired specimens were analyzed. With both molecular assays, 302 pairs produced concordant results. Considering nasopharyngeal sampling as imperfect reference standard, a PPA of 88.52% for influenza A, 94.06% for influenza B, and 42.86% for RSV was calculated with the Idylla™ Respiratory (IFV-RSV) panel, and an NPA of 100.00%, 99.54%, and 100.00% was found, respectively. Similar for the GeneXpert Xpert® Flu/RSV assay, the PPA/NPA was 89.83%/99.62% for influenza A, 88.57%/100.00% for influenza B, and 75.00%/100.00% for RSV. A detailed overview is presented in Table 3.

4. Discussion

Accurate identification of patients infected with influenza virus is crucial for timely initiation of antiviral therapy and isolation procedures. Influenza antiviral treatment should be started within the first 48 h after onset of symptoms in order to obtain a benefit (Dobson et al., 2015). In this study, the Idylla™ Respiratory (IFV-RSV) panel with a turnaround time (TAT) of 50 min was compared to the GeneXpert Xpert® Flu/RSV assay with a TAT of 63 min as reference method. No published data are available of the performance characteristics of the Idylla™ Respiratory (IFV-RSV) panel. Previous studies have already shown sensitivities and specificities in the 90–100% range for both influenza A and influenza B when the GeneXpert Xpert® Flu/RSV assay was compared to laboratory-developed RT-PCR

Table 3
Concordance of the MTS with the NPS.

Diagnostic assay	Target virus	TP	FP	TN	FN	Total	PPA[a] (95% CI)	NPA[a] (95% CI)
Idylla™	Flu A	54	0	259	7	320	88.52% (77.78–95.26)	100.00% (98.59–100.00)
	Flu B	95	1	218	6	320	94.06% (87.2–97.79)	99.54% (97.48–99.99)
	RSV	3	0	313	4	320	42.86% (9.90–81.59)	100.00% (98.83–100.00)
GeneXpert®	Flu A	53	1	261	6	321	89.83% (79.17–96.18)	99.62% (97.89–99.99)
	Flu B	93	0	216	12	321	88.57% (80.89–93.95)	100.00% (98.31–100.00)
	RSV	3	0	317	1	321	75.00% (19.41–99.37)	100.00% (98.84–100.00)

[a] PPA and NPA are calculated using the NPS as reference standard.

Table 4
Concordance of the MTS with the NPS in samples from patients ≤6 years.

Diagnostic assay	Target virus	TP	FP	TN	FN	Total	PPA[a] (95% CI)	NPA[a] (95% CI)
Idylla™	Flu A	37	0	118	1	156	97.37% (86.19–99.93)	100.00% (96.92–100.00)
	Flu B	60	1	92	3	156	95.24% (86.71–99.01)	98.92% (94.15–99.97)
	RSV	3	0	150	3	156	50.00% (11.81–88.19)	100.00% (97.57–100.00)
GeneXpert®	Flu A	37	0	119	0	156	100.00% (90.51–100.00)	100.00% (96.95–100.00)
	Flu B	59	0	92	5	156	92.19% (82.70–97.41)	100.00% (96.07–100.00)
	RSV	3	0	153	0	156	100.00% (29.24–100.00)	100.00% (97.62–100.00)

[a] PPA and NPA are calculated using the NPS as reference standard.

(Popowitch and Miller, 2015; Salez et al., 2015). To our knowledge, there were also no studies available which compared the 2 molecular panels to date. Beside assay comparison, the performance characteristics of an MTS were also investigated as an alternative sampling method to the more invasive NPS.

The Idylla™ Respiratory (IFV-RSV) panel showed excellent performance for the detection of influenza virus. Resolution of discordant results does not suggest a higher sensitivity for either assay. This should however be interpreted with care as the RT-PCR method was only used to resolve discordances and not as a reference standard for every sample analyzed. Two samples appeared false positive after RT-PCR analysis, possibly the result of the extra freeze–thaw cycle that the sample underwent before RT-PCR testing, which is known to damage viral RNA (Ryba et al., 2012). Samples were refrozen before sending them in batch for confirmative testing.

Almost all samples found positive for RSV with the Idylla™ platform were confirmed by a second molecular detection method. There are however insufficient positive RSV specimens to make any statistical conclusions regarding the sensitivity. The absence of false-negative results and the large number of concordant negative samples do indicate a similar specificity as the GeneXpert Xpert® Flu/RSV assay for the detection of RSV.

The Idylla™ Respiratory (IFV-RSV) panel allows the operator to choose between 2 sample volumes: 200 μL and 500 μL. Low volumes are preferred because multiple tests can be performed on the same specimen and no additional sampling is needed. In this study, only 0.74% (5/679) of samples were found positive after the use of 500 μL of UTM. This includes 1 NPS and 2 MTS samples positive for influenza A, and 2 MTS samples positive for influenza B, all obtained from children <9 years of age. This suggests no significant overall increase in sensitivity with a larger sample volume. This however needs to be confirmed with a higher number of samples because, in the current protocol, retesting with a higher volume was only performed in some cases of discordance.

The use of MTS samples yielded lower concordant results when comparing to NPS samples as reference standard and indicates a lower sensitivity. When considering only children below 6 years of age, comparable results are observed, which are shown in Table 4.

A small increase in sensitivity is shown; however, confidence intervals between the latter group and all samples overlap. No significant difference is found. A lower sensitivity with MTS is also reflected in Ct values found with the GeneXpert Xpert® Flu/RSV assay. Higher Ct values indicate lower amount of viral RNA. There was a mean elevation of 1.1 (Flu A2 channel) up to 6.1 (Flu B channel) in Ct value when testing was performed on MTS. These results support previous studies which made use of different detection methods and swabs of different manufacturers (Grijalva et al., 2014; Spencer et al., 2013). It seems insufficient to only swab the anterior part of the nasal cavity, but it is recommended to go as deep as the nasopharynx to have the highest sensitivity in patients with influenza-like symptoms for diagnosis. An explanation could be that the amount of collected infected epithelial cells is possibly higher when entering deeper in the nasal cavity (Daley et al., 2006).

This study is unique as it is the first to compare the Idylla™ Respiratory (IFV-RSV) panel against an already well-studied molecular detection method, the GeneXpert Xpert® Flu/RSV assay. It is also the first study which provides a head-to-head comparison of the flocked MTS and NPS of Copan® with 2 molecular detection methods with large number of samples. A limitation of this study is the use of frozen specimens. Viral loads can decrease upon thawing and may not reflect those in fresh samples of symptomatic patients. Therefore, the decreased sensitivity with MTS may possibly not be clinically relevant.

5. Conclusion

In conclusion, the Idylla™ Respiratory (IFV-RSV) panel can be used as an excellent alternative for the GeneXpert Xpert® Flu/RSV assay for the detection of influenza viruses with the advantage of the detection of the oseltamivir resistance gene and a shorter TAT. In this study, the use of a MTS as an alternative sampling method to the validated NPS suggests a possible drop in sensitivity when only sampling the anterior part of the nasal cavity.

Conflict of interest

None.

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