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Short communication

Prospective comparison of RT-PCR/ESI-MS to Prodesse ProFlu Plus and Cepheid GenXpert for the detection of Influenza A and B viruses

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A B S T R A C T

RT-PCR/ESI-MS has previously demonstrated the capability to detect and identify respiratory viral pathogens in nasopharyngeal swabs. This study expands on previous research by performing a prospective evaluation of RT-PCR/ESI-MS to detect and identify Influenza A and B viruses compared to Prodesse ProFlu Plus and combined ProFlu Plus and Cepheid Xpert Flu. ProFlu Plus was also used as a gold standard for comparison for respiratory syncytial virus detection. Using ProFlu Plus as a gold standard, RT-PCR/ESI-MS had sensitivity and specificity of 82.1% (23/28) and 100% (258/258), respectively, for Influenza A, 100% (16/16) and 99.6% (269/270), respectively for Influenza B, and 88.6% (39/44) and 99.6% (241/242) for any Influenza virus. Using matching results from ProFlu Plus and Xpert Flu as a gold standard, RT-PCR/ESI-MS had 85.2% (23/27) and 100% (259/259) sensitivity and specificity respectively for Influenza A, 100% (14/14) and 99.6% (270/272), respectively for Influenza B virus. Overall, RT-PCR/ESI-MS was not as sensitive as the combined gold standard of ProFlu Plus and Xpert Flu, although it has the capability of detecting other respiratory viruses.

Influenza viruses represent a seasonally significant source of varying morbidity and mortality based on the strain of virus circulating in worldwide populations (Thompson et al., 2003; Mahony, 2010; Mahony et al., 2011). From 2010 to 2011, Influenza, along with rhinovirus/enterovirus, was the most frequently detected respiratory virus in outpatient visits for people with Influenza like illness (Fowlkes et al., 2014). Diagnostic assays, particularly molecular diagnostic assays, are useful when applied to Influenza virus surveillance because they can assist disease management, aid in clinical decision making and can be used for pathogen discovery (Mahony, 2010; Mahony et al., 2011; Swayne and Spackman, 2013).

RT-PCR/ESI-MS is a complex diagnostic technology that has been evaluated for Influenza virus detection and genotyping with the Pan-Influenza detection kit (Jeng et al., 2012; Murillo et al., 2013; Tang et al., 2013). Briefly, reverse transcription PCR electro-spray ionization mass spectrometry (RT-PCR/ESI-MS) is a commercially developed (Ibis Biosciences, San Diego, CA, United States) technology that analyzes PCR products via mass spectrometry and produces accurate base pair compositions of generated PCR products. The base pair compositions of the PCR products are plotted in three dimensional space and compared against a database of known organisms. Organism identifications are made based on alignment of base pair compositions of PCR products with base pair compositions of known organisms. Based on the ability of the technology to detect and identify viruses, bacteria and fungi on one platform, it is a potentially flexible and useful approach to detect and identify pathogens from clinical samples. Additional evaluations have been performed with broad respiratory virus primers for RT-PCR/ESI-MS via Respiratory Virus Surveillance (RVS) kits (Ibis Biosciences, San Diego, CA, United States) v2.0–2.5, which detect Influenza A and B viruses, Respiratory syncytial virus, Coronaviruses, Adenoviruses, Metapneumovirus and Parainfluenza Virus 1–3, with various gold standard assays (Chen et al., 2011a,b; Forman et al., 2012; Hardick et al., 2014).

This study sought to expand on previous studies through comparison of the RVS 2.5 kit against two molecular diagnostic methods, Prodesse ProFlu Plus (Hologic/Gen Probe, San Diego, CA, United States) and Cepheid Xpert Flu (Cepheid, Incorporated Sunnyvale CA, United States), for the determination of sensitivity, specificity and Kappa statistic specifically for Influenza A and B viruses in prospectively collected nasopharyngeal swabs from an adult population.

From November of 2012 through March of 2013, as part of an ongoing, IRB-approved study (Dugas et al., 2014) that examined the

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Table 1
Sensitivity and specificity of RT-PCR/ESI-MS compared to ProFlu Plus and combined ProFlu Plus and Xpert Flu.

Comparison method	Virus	Sensitivity	Specificity	Kappa (95% C.I.)
ProFlu Plus	Influenza A	82.1% (23/28)	100% (258/258)	0.89 (0.80–0.99)
	Influenza B	100% (16/16)	99.6% (269/270)	0.97 (0.9–1)
Combined ProFlu Plus and Xpert Flu	Influenza A	85.2% (23/27)	100% (259/259)	0.91 (0.83–0.99)
	Influenza B	100% (14/14)	99.6% (270/272)	0.93 (0.83–1)

impact of Xpert Flu on clinical decision making in a population (age range 18–89, median age = 50) that met the Centers for Disease Control and Prevention criteria for anti-viral therapy at the Emergency Medicine department, nasopharyngeal swabs (N = 286, MicroTest M4RT, Remel, Lenexa, KS, United States) were obtained for Xpert Flu evaluation and confirmatory testing with Prodesse ProFlu Plus. Both Xpert Flu and Prodesse ProFlu Plus were performed per manufacturer instructions using 300 μl and 200 μl sample per test, respectively.

Multiple aliquots of each nasopharyngeal swabs, one for Xpert Flu testing, one for ProFlu Plus testing and one for RT-PCR/ESI-MS testing were made in 500 μl volumes and stored at −80 °C prior to testing with RT-PCR/ESI-MS (Ibis Biosciences/Abbott Molecular, San Diego CA, Des Plaines, IL, United States). Nucleic acid extraction was performed on each 250 μl thawed aliquot using the Arrow Viral NA kit (Diasorin, Stillwater, MN, United States) per manufacturer instructions, and each extract was amplified and analyzed with the RVS 2.5 kit via RT-PCR/ESI-MS. Positive control reactions from the NATtrol Respiratory Validation Panel 3 (Zeptometrix Corporation, Buffalo, NY, United States) were included in each extraction and amplification run. Positive reactions were defined as reactions having a Q score ≥0.9, where the Q score is a measure of the confidence of an identified positive. Xpert Flu and ProFlu Plus results were not available to the operator until after all RT-PCR/ESI-MS testing had been completed. The RT-PCR/ESI-MS testing was performed under a Johns Hopkins University IRB-approved study.

RVS 2.5 kit results were first compared to ProFlu Plus as a gold standard, followed by combined Xpert Flu and ProFlu Plus as a gold standard. For combined Xpert Flu and ProFlu Plus results, positive and negative reactions were defined as reactions that were in agreement for both assays. The test data were used to calculate sensitivity, specificity and Kappa analysis for the RVS 2.5 kit compared to each gold standard.

For Influenza A virus, when ProFlu Plus was used as the gold standard, sensitivity and specificity were 82.1% (23//28) and 100% (258/258, Kappa = 0.89, 95% C.I., 0.80–0.99) respectively, while for Influenza B sensitivity and specificity were 100% (16/16) and 99.6% (269/270, Kappa = 0.97, 95% C.I., 0.90–1), respectively (Table 1).

For combined gold standard results, sensitivity and specificity of RT-PCR/ESI-MS for Influenza A virus were 85.2% (23/27) and 100%, respectively (259/259, Kappa = 0.91, 95% C.I., 0.83–0.99), while for Influenza B, sensitivity and specificity were 100% (14/14) and 99.6% (270/272, Kappa = 0.83–1), respectively (Table 1).

Additionally, the RVS 2.5 kit provided basic genotype information about Influenza A and B viruses. All Influenza A viruses detected were H3N2 viruses, although there was variation in H3N2 subtype, with A/Managua/2/2007 being the most prevalent genotype at 53.2% (12/23) of Influenza A positive samples, which is consistent with the genotype of circulating Influenza A viruses in Maryland for the 2012–2013 respiratory virus season. For Influenza B, there was variation in the RT-PCR/ESI-MS designation for the virus, but in terms of reference genotype, Yamagata was the most prevalent genotype at 88.2% (15/17) of Influenza B positives, and Victoria was 11.7% (2/17) of Influenza B positives, which was consistent with Influenza B viruses circulating in Maryland during the 2012–2013 respiratory virus season.

Lastly, the RVS 2.5 kit detected additional respiratory viruses (N = 24) in the study patients. Coronavirus was the most prevalent non-Influenza virus detected at 4.5% (13/286), with 76.9% (10/13) as Human coronavirus OC43 and 23% (3/13) as Human coronavirus HKU1. Additionally, Metapneumovirus, Adenovirus and Parainfluenza viruses 1–3 were all detected at 0.7% (2/286) for each.

RT-PCR/ESI-MS results compared favorably to both ProFlu Plus and a combined gold standard of ProFlu Plus and Xpert Flu with >80% sensitivity and >90% specificity respectively for Influenza A and B viruses, regardless of the comparison method. Kappa analysis indicated excellent agreement, ≥0.89, between RT-PCR/ESI-MS and all comparison methods for Influenza A and B viruses.

RT-PCR/ESI-MS detected additional respiratory viruses in the study patients that were not detected by the comparison assays because ProFlu Plus which only detects Influenza A, B and RSV, while Xpert Flu is exclusively an Influenza A/B assay.

RT-PCR/ESI-MS provided basic genotype information for Influenza A and Influenza B subtype detected. All Influenza A were the H3N2 subtype, and Influenza B were primarily Yamagata (88.2%). This additional information could be useful in prospective surveillance studies with technology for discovery of potential Influenza A and Influenza B variant genotypes within at risk populations, and could be used for early detection of potentially pandemic strains of Influenza as well as to gather epidemiologic data regarding the composition of Influenza A and B subtypes in a respiratory season.

The results here are similar to previous RT-PCR/ESI-MS studies, although comparison methods in this study were different than in Chen et al. (2011a,b), Forman et al. (2012) and Hardick et al. (2014), as this study primarily evaluated Influenza detection capability with RVS 2.5. Previous evaluations compared conventional viral culture methods and direct fluorescent antibody (DFA) (Chen et al., 2011a,b), or xTag RVP and DFA (Hardick et al., 2014) or to xTag RVP alone (Forman et al., 2012). Additionally, previous studies used archived nasopharyngeal swabs (Chen et al., 2011a,b; Hardick et al., 2014) or prospective pediatric samples (Forman et al., 2012) while this study analyzed prospective nasopharyngeal swabs from exclusively adult emergency department patients, and compared RT-PCR/ESI-MS performance to two molecular diagnostic methods.

There were some limitations to the study, notably the relatively low number of Influenza A (N = 27) and Influenza B (N = 14) positive samples. However, limited resources prevented further enrollment which was required to conclude when the primary study was completed (Dugas et al., 2014).

There was also the limitation of performing confirmatory testing on samples that tested positive for respiratory viruses other than Influenza A, Influenza B and RSV. However, as previously mentioned, other studies examining the performance of RT-PCR/ESI-MS to detect and identify respiratory viruses have indicated sufficient sensitivity and specificity of this technology to detect Coronavirus, Metapneumovirus, Adenovirus and Parainfluenza 1–3 (Chen et al., 2011a,b; Forman et al., 2012; Hardick et al., 2014).

RT-PCR/ESI-MS is a highly complex technology that, although not suited toward point of care diagnostic testing, has multiplex detection capability similar to other methods such as xTAG RVP, and can provide basic genotype information for respiratory viruses.

Genotype information is helpful in surveillance and epidemiology of respiratory viruses, particularly Influenza A and B, for outbreak control and recognition of possible new strains.

More substantial evaluations of RT-PCR/ESI-MS technology using the RVS 2.5 kit are required before the technology can be directly applied to clinical use, but the evaluation presented here illustrates that RT-PCR/ESI-MS can be a useful diagnostic method for detection and identification of Influenza A and Influenza B viruses. The lower sensitivity in this study could be explained by differences in the limit of detection between assays, as ProFlu Plus and Xpert Flu report limits of detection as low as 5 PFU/ml, while RT-PCR/ESI-MS has a reported limit of detection of approximately 150 copies of target per well (Chen et al., 2011a,b).

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