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Rapid diagnosis of influenza: An evaluation of two commercially available RT-PCR assays

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KEYWORDS
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Summary *Objective:* To evaluate the sensitivity and specificity of influenza virus detection by two commercial reverse transcriptase PCR methods compared with a reference real-time PCR.
Methods: 122 clinical specimens were tested on Xpert® Flu and RealStar® Influenza Screen & Type. A reference real-time RT-PCR, at a specialist laboratory was chosen as the gold standard for comparison.
Results: RealStar® Influenza Screen & Type had higher sensitivity for influenza A and influenza B respectively (92.3% and 88.2%) when compared to Xpert® Flu (78.8% and 76.5%). Both tests had excellent specificity.
Conclusions: The simplicity and speed of the Xpert® Flu system could allow it to be used in the near-patient setting; however in circumstances where excluding a diagnosis of influenza may be critical, negative specimens may need to be repeated using a more sensitive assay.

Introduction

In 2009 a novel influenza virus A/H1N1v was identified in Mexico and soon reached pandemic status.[1] During the seasonal influenza period of 2010/11, influenza A/H1N1v and influenza B were the predominant circulating viruses in England and Wales.[2] Several methods have been used to obviate the need for protracted viral cultures in influenza diagnosis. These include antigen testing, immunofluorescence and molecular testing. Recent reports have highlighted sensitivities of only 10—50% for rapid antigen-based tests.[3] Immunofluorescence requires highly trained laboratory staff

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that may be overwhelmed by the increase in workload during an epidemic. Molecular diagnosis of influenza is attractive, offering timely and accurate results which can aid clinical management and infection control.[4] Standardised commercial assays would allow in-house testing without the requirement for referral of specimens to specialist centres, thus speeding up the availability of results.

Materials

Recent studies have separately evaluated the performance of Xpert® Flu (Cepheid, Sunnyvale, CA, USA) and RealStar® Influenza Screen & Type (Alere, Hamburg, Germany). Xpert® Flu is a simple test that runs on GeneXpert® system (Cepheid, Sunnyvale, CA, USA). It simultaneously detects and differentiates influenza A, influenza B, and influenza A/H1N1v (A/H1N1v) viruses in about 80 min. It involves a cartridge-based PCR system for performing nucleic acid extraction, PCR amplification, and real-time detection. It automatically sets the temperatures and number of cycles without intermediate sample-handling steps. This avoids the need to transport specimens onto a new plate on another platform. Therefore sampling errors can be reduced and it may be suitable for point of care testing (POCT) by non-laboratory trained healthcare staff. In addition, all internal controls for Xpert®Flu are included within the cartridge system.

RealStar® Influenza Screen & Type is a batch based assay that can be run on a range of PCR platforms. It would not be suitable for near-patient testing as it requires a number of specimen handling steps which would need to be performed by a trained biomedical scientist. The entire process for a complete set of results takes 4 h. Both these assays have shown specificity of up to 100% but with variable sensitivity in recent validation studies.[5–8] However none of these studies directly compared the two commercial assays. We therefore sought to compare these two commercially available RT-PCR assays, using as a reference real-time RT-PCR (reference method) offered by a specialist laboratory and widely used for the diagnosis of influenza in the United Kingdom.

Method

All specimens sent to our laboratory for influenza detection between 13th and 27th December 2010 were included in the study. Only 122 of the 150 specimens had sufficient sample for the evaluation as each had to be tested on both the commercial assays and the reference method. The 122 specimens comprised 97 nasopharyngeal swabs, nine nasopharyngeal aspirates, nine endotracheal aspirates, four nasal swabs and three unknown specimen type. Specimens were originally tested on receipt using the Xpert® Flu system and were then stored and frozen in MicroTest™ M4RT® (Remel, Lenexa, KS, USA) at −20 °C. These specimens were then thawed and subsequently used for further testing in the other two assays. 71 specimens were from female patients and 51 from males. Patients' ages ranged from 1 month to 91 years with a median age of 34 (mean 37).

RealStar® Influenza Screen & Type was run on an ABI Prism® 7500 PCR System (Applied Biosystems, Cheshire, UK). RNA was extracted using the Arrow NA (NorDiag, Oslo, Norway) prior to amplification. Specimens were barcode labelled and tested in batches; each batch had a negative control and positive controls for influenza. Each test was performed according to manufacturer's instructions, by one of two biomedical scientists blinded to previous results. The following thermal profile was used: 50 °C for 10 min, 95 °C for 2 min followed by 45 cycles at 95 °C for 15 s, 55 °C for 45 s and 72 °C for 15 s.

The reference method's total nucleic acid extraction was performed using the MDx automated biorobot (Qiagen, Düsseldorf, Germany). The RT-PCR was performed on ABI 7500 fast real-time PCR (Applied Biosystems, Cheshire, UK) using a multiplex format. The one-step RT-PCR thermal profile was as follows: 50 °C for 15 min, 95 °C for 2 min, 95 °C for 15 s for 45 cycles, followed by one cycle at 60 °C for 40 s. Internal controls were also included throughout the process to control for extraction and PCR. Barcode identification was included throughout the reference method process to reduce the chance of incorrect specimen testing.

Results

For diagnosis of influenza A, the reference method identified 52 cases, of which Xpert® Flu detected 41(78.8%). There were no cases detected by Xpert® Flu that were not positive in the reference method. RealStar® Influenza Screen & Type detected 51 positives of which only 48 were identified by the reference method (92.3%). Fig. 1 shows the concordance of the different tests for influenza A for illustrative purposes. The reference method confirmed all 52 influenza A positive cases as influenza A/H1N1v on a separate A/H1N1 specific PCR, while Xpert® Flu confirmed 39 of its 41 positive cases. RealStar® Influenza Screen & Type identified 51 cases, one of which failed to confirm as influenza A/H1N1v and was negative by the reference method.

For influenza B, the reference method identified 17 cases, of which Xpert® Flu detected 13 (76.5%). RealStar® Influenza Screen & Type detected 15 cases all of which were confirmed by the reference method. Table 1 shows the sensitivity, specificity, positive and negative predictive values for the two commercial assays using the reference method for comparison.

Discussion

When evaluating molecular tests for the diagnosis of infectious disease it is difficult to choose a gold standard with which to compare results. In this case we chose the reference test used by the Health Protection Agency Laboratories which was developed in June 2009.[9] Using this as a standard we found that both RealStar® Influenza Screen & Type and Xpert® Flu had reduced sensitivity in detecting A/H1N1v and influenza B when compared to the reference method. Sensitivity was greater for RealStar® Influenza Screen & Type than Xpert® Flu in detecting influenza A (92.3% cf 78.8%) and influenza B (88.2% cf 76.5%).

Another major difficulty with this type of study is the problem of lack of simultaneous testing. However this is