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Title page

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Comparative evaluation of the CerTest VIASURE Flu A, B & RSV Real Time RT-PCR Detection Kit on the BD MAX System versus a Routine In-house Assay for Detection of Influenza A and B virus during the 2016/17 Influenza Season

Running title: Evaluation of the VIASURE Influenza A, B & RSV PCR assay

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Abstract

Background: Diagnosing of influenza rapidly and accurately helps clinicians to initiate appropriate treatment options and isolation protocols. Unnecessary antimicrobial treatment and laboratory testing can also be reduced. Assess commercial alternatives to in-house assays that may not only reduce laboratory technician “hands on” time but also the laboratory turnaround time is of interest.

Objectives: We evaluated the performance of the VIASURE Flu A, B & RSV Real Time RT-PCR Detection Kit (CerTest Biotec) for detecting Influenza A and B viruses.
Study Design: During the 2016/17 influenza season 532 clinical samples were tested with the VIASURE assay on the BD MAX™ system versus an in-house real time RT-PCR assay with discrepant results resolved by a real time RT-PCR assay at a national reference laboratory.

Results: The VIASURE assay on the BD MAX showed a sensitivity of 99.5% (95% CI: 97.3 – 100) and a specificity of 99.1% (95% CI: 97.4 - 99.8) for detection of Influenza A virus. The positive predictive and negative predictive values were 98.5% (95% CI: 95.8 - 99.7) and 99.7% (95% CI: 98.3 – 100) respectively. Influenza B virus detection could not be evaluated due to a low positivity rate. The BD MAX platform offered the flexibility of several daily runs, shorter hands-on-time and shorter turnaround time than the in-house assay.

Conclusions: The VIASURE assay on the BD MAX performed well and is now implemented in our clinical laboratory.

Keywords: Molecular diagnostics, influenza, respiratory, commercial assay

Background

Fast and accurate diagnosis of influenza can reduce unnecessary antimicrobial treatment, laboratory testing and isolation of patients[1,2]. It is of interest to assess commercial alternatives to in-house assays that may not only reduce the turnaround time in the laboratory.

Objectives

We evaluated the performance of a multiplex real-time RT-PCR test from CerTest (CerTest Biotec S.L., San Mateo de Gállego, Spain) for detecting Influenza A and B on the BD MAX™ system (Becton, Dickinson, Franklin Lakes, NJ, USA), a combined extraction and PCR platform.

Study design
The department of clinical microbiology, Lillebaelt Hospital Vejle, serves four smaller hospitals with a total bed-count of approximately 700 and provides services to general practitioners in an area with a population of approximately 300,000. Respiratory samples (throat swabs and sputum samples) for routine diagnostics for influenza A and B viruses collected from primary health care and the four hospitals during January 23rd 2017 to February 20th 2017 were consecutively included. Nine samples positive for Influenza A or B from a Quality Control for Molecular Diagnostics (QCMD) panel were also tested.

The in-house assays for Influenza A and B were based on published assays [3,4]; a lysis step preceded nucleic acid extraction using the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega Corporation, Madison, WI, USA) on the Maxwell MDx system. Real-Time PCR was performed on the ABI 7900 system (Applied Biosystems, Foster City, California) using the SuperScript III Platinum One-Step Quantitative RT-PCR kit with the ROX reference dye (Thermo Fisher Scientific, Slangerup, Denmark). The protocol used 0.8µM (each) primer and 0.2µM probe for Influenza A and B (InfA_F 5’ACA AGA CCA ATY CTG TCA CCT CTG A 3’; InfA_R 5’ GTC TAC GYT GCA GTC CYC G 3’; InfA_probe 5’ FAM TYA CTG GGC ACG GTG AGC GTG BHQ1 3’; InfB_F 5’ ACG GTG GAT TAA ATA AAA GCA AGC 3’; InfB_R 5’ CCA GCA ATA GCT CCG AAG AAA 3’;InfB_probe 5’ FAM-TGG GCA ATT TCC TAT GGC-MGB 3’). Cycling conditions were as follows: step 1, 1 cycle of 50°C for 15 min (RT); step 2, 1 cycle of 95°C 2 min; 45 cycles of (95°C for 15 s, 60°C for 60 s). Beta-2-microgloblin was used as an internal nucleic acid extraction and PCR control [5]. Positive and negative controls were included in every run. A sample was considered positive if below or equal to a cut-off threshold of 40 cycles [3].

The VIASURE Flu A, B & RSV Real Time RT-PCR Detection Kit (CerTest Biotec S.L.) is shipped as freeze dried pellets containing primers, probes and internal positive controls. The pellets and rehydration buffer were transferred to BD MAX Conical Tubes and sealed using an Axygen Plate Max sealer (Corning, Vordingborg, Denmark) and stored at room temperature. Samples were extracted and amplified on the BD MAX system using the BD MAX ExK TNA-3 kit (Becton, Dickinson, Franklin Lakes, NJ, USA) by adding 200 µl volume of
sample to the sample buffer tube and following the manufacture’s protocols. PCR was performed on the BD MAX PCR cartridge with the following thermocycler profile: Step 1, 1 cycle of 45°C for 15 min (RT); step 2, 1 cycle of 95°C 2 min; 45 cycles of (95°C for 10 s, 63°C for 50 s) (plus detection). Positive and negative controls (included in the VIASURE kit) were included once a week. The cut-off threshold was 40 cycles in accordance with the manufacture’s recommendation.

Samples with inhibition were re-analysed following the same procedure as above. Samples with discrepant results were sent to a reference laboratory for confirmatory testing (Statens Serum Institut, Copenhagen, Denmark)[6].

For statistical analysis a comparative standard was defined as the composite result of the in-house assay and the VIASURE test with the reference laboratory results used as a resolver for discrepant results. Inhibited samples were included as negatives. R version 3.4.1 with the binom package version 1.1-1 was used to calculate confidence intervals by the Person-Klopper method.

Results

In total, 531 throat swabs in ESwab medium (Copan, Italy), and one sputum sample sent in a sterile container were included. Twenty six (4.9%) samples failed on the BD MAX system, mainly due to technical errors. After re-testing, three (0.6%) showed inhibition. Four (0.8%) samples showing inhibition using the in-house assay despite repeated testing.

Of the 532 samples 189 proved positive for influenza A virus (table 1) and three for influenza B virus by the in-house assay. One sample was found to be negative in the VIASURE assay but positive for influenza A in the in-house assay (C<sub>T</sub> value 35.7) and by the reference laboratory. Three samples were found to be positive for influenza A in the VIASURE assay (C<sub>T</sub> values 36.6-39.0) and negative in the in-house assay and by the reference laboratory. Fifteen samples found negative in the in-house assay, were found positive for influenza A by the VIASURE assay and confirmed positive by the reference laboratory. For eight of these
samples $C_T$ values were in the range of 40.6-43.1 in the in-house assay. The $C_T$ range for the same eight samples run on the BD MAX system was also at the high end of $C_T$ values (31.9-39.5).

The VIASURE assay on the BD MAX displayed a sensitivity of 99.5% and a specificity of 99.1% for detection of influenza A virus (table 2). The PPV and NPV were 98.5% and 99.7% respectively. Three samples were positive for influenza B, with complete concordance between the in-house and VIASURE assays. However with such a low positive rate, assay performance could not be evaluated properly for influenza B.

Correct results were found for four influenza A positive and five influenza B positive QCMD-panel samples tested with 100% agreement between the two assays.

Thirty-five samples tested positive for respiratory syncytial virus (RSV) on the VIASURE assay, of which three also were positive for influenza A virus. Confirmatory testing for RSV was not performed using other systems, but a comparative study is planned.

Total turnaround time (TAT) for a full run of 24 samples on the BD MAX was 4 h 15 min. In comparison, a run of 24 samples using the in house assay takes about 4 hours and 50 minutes in total. During the influenza season our lab provided runs for influenza four times daily, typically with four to ten samples per run. A run of four samples using VIASURE takes 2 h 15 min in total from start of preparation to result, compared to the in-house assay where TAT, regardless of the number of samples, would be at least 4 h 50 min.

**Discussion**

This is the first report of the performance of the CerTest VIASURE Multiplex Influenza A, B and RSV assay on the BD MAX system. As the 2016/2017 influenza season was dominated by Influenza A (H3N2) with very low prevalence of influenza B [6], only the true performance of influenza A testing could be evaluated sufficiently. However there were no discrepancies between the influenza B results when testing clinical
sample specimens and the QCMD samples. Compared to the in-house results combined with those from the reference laboratory, the VIASURE assay on the BD MAX performed very well.

With the need for several daily runs of influenza testing, and taking into account the current and projected sample number for our department, we found the VIASURE assay on the BD MAX platform to be cost effective and suitable to our requirements.

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**Ethical approval:** Not required

**Competing interests:** None declared

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CerTest provided assay reagents and sponsored extraction reagents for analysis on the BD MAX. CerTest had no influence on study design, analysis or interpretation of results. We thank Thea Kølsen Fischer, head of the National Influenza Center, Statens Serum Institut, Denmark, and the technical staff involved in testing of samples included in the study. The authors thank Jonathan Howard Sydenham, BA (Hons), MSPAW, for language assistance and proof-reading.
References


### Table 1. Distribution of primary test results for influenza A on 532 samples

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>VIASURE/BD MAX</th>
<th>In-house Real Time PCR</th>
<th>Reference laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>188</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>3</td>
<td>+</td>
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<tr>
<td>325</td>
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</tr>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*+, positive; - negative; ND, not carried out.
Table 2. Performance of the VIASURE assay for Influenza A for 532 sample specimens compared to a composite standard of in-house real-time RT-PCR and real-time RT-PCR results from a reference laboratory

<table>
<thead>
<tr>
<th>VIASURE on BD MAX</th>
<th>Composite standard</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
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<td>3</td>
<td>206</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>325</td>
<td>326</td>
<td></td>
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<tr>
<td>Total</td>
<td>204</td>
<td>328</td>
<td>532</td>
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</table>

% (95% CI)

- Sensitivity: 99.5 (97.3 - 100)
- Specificity: 99.1 (97.4 - 99.8)
- Positive predictive value: 98.5 (95.8 - 99.7)
- Negative predictive value: 99.7 (98.3 - 100)