Evaluation of three commercial multiplex assays for the detection of respiratory viral infections

Sandi Radko, J. Ian Stuart, George Zahariadis*

Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada

A B S T R A C T

Background: Timely identification of respiratory virus infection is essential to mitigate inappropriate antibiotic use and to implement appropriate treatment and/or infection control procedures. As such, multiplexed PCR assays have become standard in many virology laboratories.

Objectives: To compare the Seeplex RV15 (test of record) with two newer generation multiplex assays, the Anyplex II RV16 and the xTAG respiratory virus panels.

Study design: Two hundred and three retrospective and 36 prospective respiratory samples were tested by all three assays. Samples were deemed to be positive if they tested positive for a virus by at least two of the three respective assays. Negative samples also had to test negative by at least two of the three assays. Inconclusive samples were those that showed band signal intensity between 0 and 100 on the RV15, but had not been previously tested on the RV16 or xTAG.

Results and conclusions: Overall sensitivity and specificity of all three assays were similar (∼85% and 100%, respectively). Given each assay can identify multiple different viruses, the targets reported by one assay did not always agree with each target from another assay. Partial discordant rates were 47% and 21% for positive and negative samples, respectively. These higher than expected partial discordant rates may be due to primer or chemistry differences amongst the three multiplex assays.

1. Background

Respiratory viral infections are a significant cause of morbidity and mortality globally (Esposito et al., 2013; Huijskens et al., 2013). Early identification of respiratory pathogens permits rapid implementation of appropriate infection control precautions, decreased antibiotic use and where appropriate, initiation of antiviral therapies (Barenfanger et al., 2000; Heinonen et al., 2011). Traditional laboratory methods such as viral culture and direct fluorescent-antibody testing are time consuming and lack sensitivity, and are no longer the method of choice (Gharabaghi et al., 2011; She et al., 2010). Currently, molecular methods are now standard and are employed in most virology laboratories. Multiplexed assays have enabled the detection of several viral targets and permit the simultaneous identification of co-infections in patient specimens (Esper et al., 2011). There are currently several multiplex assays available commercially. Three such Health Canada approved assays include the Seeplex RV15 ACE Detection Kit (RV15) (Seegene, South Korea), the Anyplex II RV16 (RV16) (Seegene, South Korea) and the xTAG Respiratory Viral Panel (xTAG) (Luminex, United States)

The RV15 is a multiplex assay based on dual priming oligonucleotide (DPO) technology (Bruijnesteijn van Coppenraet et al., 2010). The list of fifteen detectable viruses include: influenza A virus (INF A), influenza B virus (INF B), respiratory syncytial viruses A and B (RSVA and RSVB), adenovirus (ADV), human metapneumovirus (hMPV), coronavirus OC43 (CoV OC43), parainfluenza viruses (PIV) 1–4, rhinovirus (RhV) A to C, enterovirus (EV), and Bocaviruses (BoV).

The RV16 is based on Tagged Oligo Cleavage Extension (TOCE™) technology, which makes it possible to detect multiple pathogens in a single fluorescence channel using real time PCR (Kim et al., 2013). The RV16 can detect a total of 16 viruses including serotypes of each virus. The RV16 viral panel is identical to the RV15 viral panel with the additional detection of CoV 229E and CoV NL63 viruses.

The Luminex xTag system is a liquid-bead-suspension-array that is based on multiplex PCR (Jokela et al., 2012). Its viral panel includes influenza A virus (INF A) H1, H3, H1N1, influenza B virus (INF B), respiratory syncytial viruses (RSV), adenovirus (ADV), human metapneumovirus (hMPV), coronavirus (CoV) 229E, CoV NL63, CoV OC43, HKU1, parainfluenza viruses (PIV) 1–4, rhinovirus (RhV)/enterovirus (EV), and Bocaviruses (BoV). It is important to note that,

* Corresponding author at: Faculty of Medicine, Memorial University of Newfoundland Dr. L.A. Miller Centre, Suite 1, 100 Forest Road, St. John's, NL A1A 3Z9 Canada.
  E-mail address: geozah71@gmail.com (G. Zahariadis).

bocaviruses were previously not included on the viral panel for xTAG; however, version 2 being analyzed in this study has this viral target as part of its testing panel.

Thus, this study aims to compare the diagnostic performance of the RV16 and xTAG assays to that of the RV15, as the test assay of record.

2. Study design

2.1. Specimens

Patient respiratory specimens including nasopharyngeal swabs (NPS) and bronchoalveolar lavage (BAL) fluid samples submitted for RV15 testing were collected between November 2012 and June 2013. NPS samples were collected using flocked swabs (Starwab Multitrans Collection and Transport System). A total of 239 samples were collected. Of these samples, 203 were retrospective and 36 were prospective. Retrospective samples were RV15-test-positive sample aliquots that were stored at −80 °C. After undergoing one freeze-thaw cycle, the retrospective samples were tested simultaneously on the Anyplex II RV16 and xTAG. Prospective samples were tested as they were received by the laboratory and were simultaneously tested on all three assays. The patient demographics in this study were as follows: 104 female patients ranging from 0 to 95 years old and 135 male patients ranging from 0 to 94 years old. The mean ages of the female and male patients in this study were 42.8 and 33.6 years old, respectively.

2.2. Nucleic acid extraction and internal control

Nucleic acid extraction for all assays was performed on the MagnaPur Compact (Roche, Switzerland). The initial input volume for all Seegene RV15 and RV16 extractions was 700 μL (300 μL Lysis Buffer and 400 μL patient sample) with a final elution volume of 100 μL. Samples for the Luminex MagPix assay had an initial input volume of 400 μL (200 μL Lysis Buffer and 200 μL patient sample) with a final elution volume of 50 μL. MS2 bacteriophage was added as an internal control prior to extraction for both the Luminex MagPix and Seegene RV16 assays as per manufacturer's instructions. The Seegene RV15 internal control and specific primer were added following extraction, according to manufacturer's instructions.

2.3. RV15 testing

Samples were tested using the Seegene Seeplex RV15 One Step ACE Detection Kit. Each sample was simultaneously amplified in three separate reactions with corresponding primer sets specified as "A", "B" and "C". The final volume of the PCR reaction mixture was 50 μL, containing 40 μL of One-Step RT-PCR Master Mix and 10 μL of the sample's eluate. Master Mix composition was as per manufacturer's instructions, as were thermocycling conditions using the SeeAmp thermocycler. Reaction mixtures were vortexed prior to thermocycling, to allow for thorough reaction mixing. The amplified PCR products were analyzed by agarose gel electrophoresis using the Caliper gel based detection platform (Life Sciences, United States). Following analysis, the Seegene Viewer software assigned a positive or negative result for each virus present in the sample tested against the viral panel.

2.4. RV16 testing

Samples were tested using the Seegene RV16 Detection kit. Reverse transcription was performed, followed by cDNA synthesis on the SeeAmp thermocycler using manufacturer specifications.

PCR was then conducted in a final volume of 20 μL and reactions were analyzed on the CFX96 Real-Time PCR System (BioRad) using manufacturer's instructions. The Catcher Melting Temperature Analysis (CMTA) was achieved by cooling the samples down to 55 °C for 30 s and then heating the mixtures from 55 °C to 85 °C. During the heating

period, fluorescence (F) and Temperature (T) were measured continuously. Curves of the negative derivative of the fluorescence over temperature versus temperature (-dF/dT versus T) were generated by the CFX96 to determine the samples melting point. The melting points were interpreted by using Seegene Viewer software as either " + " or " − " for each virus and its subtype. The RV 15 and RV16 limit of detection is 50 copies for each virus type, as reported by the manufacturer. The manufacturer product insert does indicate that for the RV15 some strain variations can cause the sensitivity for Enterovirus and Rhinovirus to vary 10–100 fold.

2.5. xTAG RVP fast v2

Reverse transcription and cDNA amplification were conducted on the Luminex MagPix as per manufacturer instructions, using an Eppendorf thermocycler with ramp speeds of 1.2 °C/sec followed by reverse transcription, cDNA amplification, and bead hybridization.

After hybridization, plates were transferred to the Luminex MagPix for detection. Each well was analyzed for bead hybridization using TDAS RVP FAST 2.20 software and cutoff thresholds for each virus were determined, based on previously determined thresholds by the manufacturer. Samples were considered positive for a virus or its serotype if the threshold was met or exceeded.

2.6. Definitions and data analysis

Samples were considered positive if the sample tested positive for the same virus by at least two of the three assays. Samples were deemed to be negative if they tested negative by at least two of the three assays. Inconclusive results were previously defined by the RV15 as positive specimens if the band signal intensity was between 0 and 100. In specimens where the virus or viruses identified were not concordant between all the three assays, conflicting results were classified based on discordant virus identities and analyzed accordingly. Agreement, sensitivity and specificity were calculated for each assay.

3. Results

3.1. Sample classification

The distribution of viruses detected is shown in Table 1 and using our definition of positive or negative result being agreement in two out of three assays, a total of 161 positive specimens were identified. Of these, 32 (19%) specimens were co-infected with more than one type of virus with 26 and 6 samples having dual and triple co-infections, respectively.

Eighty-six positive samples (53%) showed complete agreement among all three assays. Seventy-five (47%) positive specimens had

Table 1
Virus Distribution.

Virus	Number of Positive Specimens
Adenovirus	12
Bocavirus	10
Coronavirus	23
Human Metapneumovirus	15
Inflluenza A	20
Influenza B	11
Parainfluenza 1	1
Parainfluenza 2	2
Parainfluenza 3	14
Parainfluenza 4	2
Rhino/Enterovirus	50
RSV A	31
RSV B	10
Total	201