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Acute respiratory illnesses (ARIs) caused by respiratory viruses including influenza viruses and respiratory syncytial virus (RSV) affect hundreds of millions of people per year and are the most common causes of viral infections in the respiratory tract in humans (Berry et al., 2015; Fendrick et al., 2003). Each year, approximately 500 million cases of ARI are reported in the United States, with direct and indirect costs approaching $40 billion annually (Berry et al., 2015; Fendrick et al., 2003).

The 2009 H1N1 pandemic strain of influenza and the H3N2 seasonal variant that circulated during the 2014–2015 season serve as examples of the importance of continued respiratory virus surveillance efforts, and the importance of using accurate and inclusive diagnostics in respiratory virus management (Flannery et al., 2016; Mahony, 2010; Mahony et al., 2011). In addition, given the emergence of Middle East respiratory syndrome (MERS) coronavirus and subsequent outbreaks thereof in the Middle East and South Korea, it is important that diagnostic assays for respiratory viruses are rapid and deployable at or near the point of care (POC) (Bhadra et al., 2015; Raj et al., 2014). Such diagnostic platforms are becoming more common and are capable of detecting a wide array of respiratory pathogens (Zumla et al., 2014).

We evaluated a novel, portable, near-POC diagnostic platform, the Mobile Analysis Platform (MAP), by assessing the capability of the MAP to detect influenza A, influenza B, and RSV in externally extracted clinical samples, and by establishing the platform’s limit of detection (LOD) for RSV and MERS in clinical matrices. The MAP is a small, portable device integrating disposable assay-specific microfluidic cards (Fig. 1). The MAP system is equipped with a set of subsystems to allow for automatic assay processing. This includes A) a barcode reader to input both specimen and microfluidic card serial numbers; B) a motorized system to crush liquid reagent

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Clinical samples were evaluated with the Mobile Analysis Platform (MAP) to determine platform performance for detecting respiratory viruses in samples previously characterized using clinical reverse transcriptase polymerase chain reaction assays. The percent agreement between MAP and clinical results was 97% for influenza A (73/75), 100% (21/21) for influenza B, 100% (6/6) for respiratory syncytial virus (RSV), and 80% (4/5) for negative specimens. The approximate limit of detection of the MAP was 30 copies/assay for RSV and 1500 copies/assay for Middle East respiratory syndrome coronavirus.

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packs on command; C) a pneumatic system (air pressure and vacuum) to move liquids within the cards via peristaltic pumping using vacuum-activated midstream valves (specimens are thus mixed with various reagents prepackaged into the cards in lyophilized beads to move sample and reagents on the microfluidics card); and D) a set of thermal electric coolers to allow for rapid polymerase chain reaction (PCR; 30 min for 40 cycles) PCR cycling of the mixed sample and reagents is performed in a chamber sandwiched between 2 thermoelectric cooling devices; E) heating elements to maintain temperature at a separate hybridization chamber; F) an optics system that includes a LED for dye excitation and CCD camera for imaging the microarray; G) an ARM processor; H) an LCD screen; and I) keypad.

The microfluidics card is disposable and has all the materials required for the assay including A) sample port to load the swab, B) wet reagents held in sealed blister packs, C) lyophilized reagents, and D) a microarray chip. The microarray chip consists of 100-μm spots of specific DNA capture probes. The array has 400 of these features, with 100 features reserved for image alignment. This allows up to 300 separate spots for the assay. PCR products are then digested using uracil DNA glycosylase and hybridized to a spotted microarray and washed. The microarray is then imaged with a digital camera and LED illumination, and the image is analyzed and statistically interpreted via onboard analysis software. The entire process is fully automated, and results are presented as positive/negative/invalid declarations on the screen of the MAP unit. Multiple primer pair/probe combinations are employed for each analyte, each of which is designed to offer maximum breadth of coverage within the known diversity of the targeted viral genus.

A total of 130 clinical respiratory virus samples were evaluated, including both nasopharyngeal swab samples resuspended in VTM (N = 85) and nasal wash samples (N = 45) composed of true positives for influenza A (N = 93), influenza B (N = 21), and RSV (N = 7), and samples that were negative for these analytes (N = 9). Samples were acquired through institutional review board-approved studies (IRB00052743, NHRC.2015.0033) from adults ≥18 years old that were symptomatic for a respiratory virus infection. Influenza A and B samples previously tested positive via the Cepheid Xpert Flu assay (Cepheid, Sunnyvale, CA) or CDC Human Influenza Virus Real-Time PCR Diagnostic Panel (US Centers for Disease Control and Prevention, Atlanta, GA), while RSV samples previously tested positive via a published real-time PCR assay (Templeton et al., 2004). LOD experiments were performed for RSV and MERS by spiking virus into negative clinical matrix. When available, leftover extracts from initial clinical diagnostic testing were utilized. When these were not available, the same methods were used to generate new extracts from waste aliquots of the specimens. Nucleic acids were extracted using either the Arrow Viral NA Kit (NorDiag) or the Viral Mini Kit (Qiagen). Extracted nucleic acid was loaded onto individual MAP assay cards, which were then run and analyzed on portable MAP devices according to the manufacturer’s instructions. Primer sequences for the target organisms can be found in Table 1, with reagent concentrations for the array cards listed in Table 2.

Cycling conditions were as follows: RT step, 5 min at 52 °C; Hotstart step, 30 s at 95 °C; and extended touchdown PCR and fast PCR, 1 cycle of 7 s at 95 °C, 1 s at 55 °C, 30 s at 69.5 °C, 2 s at 80 °C followed by 1 cycle of 7 s at 95 °C, 1 s at 53.5 °C, 30 s at 67 °C, 2 s at 80 °C followed by 1 cycle of 7 s at 95 °C, 1 s at 51 °C, 30 s at 65 °C, 2 s at 80 °C followed by 1 cycle of 7 s at 95 °C, 1 s at 50 °C, 30 s at 63 °C, 2 s at 80 °C followed by 1 cycle of 7 s at 95 °C, 1 s at 49 °C, 30 s at 62 °C, 2 s at 80 °C followed by 35 cycles of 7 s at 95 °C, 1 s at 45 °C, 5 s at 65 °C and 2 s at 80 °C. All ramp rates on approach to 80 °C were dampened to 20% of maximum. Time to result including nucleic acid extraction was 110 min per sample. All results are reported here as they appeared on the automated output of the MAP devices. Samples that generated either an invalid result or an error report were recorded as assay failures and were not repeated.

Percent agreement between MAP and standard-of-care reverse transcriptase PCR results was 97% (73/75) for influenza A-positive samples, with 2 false-negative results and 18 assay failures resulting in invalid or error reports. Percent agreement for influenza B was 100% (21/21) with no assay failures and 100% (6/6) for RSV with 1 assay failure. Agreement was 80% (4/5) for negative samples, with 1 RSV-positive result and 4 assay failures. The LOD established was 30 and 1500 copies of virus/assay for RSV and MERS coronavirus, respectively.

Previous epidemics and pandemics, as well as the emergence of new respiratory viral pathogens, highlight the need for accurate diagnostic platforms capable of being deployed near the POC (Zumla et al., 2014). We performed an evaluation of the MAP prototype to determine its capability to detect and identify influenza A, influenza B, and RSV, and performed LOD experiments in clinical matrix for RSV and MERS. Percent agreement between the MAP assay result and the predicate result were high for all pathogens evaluated (97–100%) when excluding invalid assay card results and error reports (no-test instances), and LOD experiments for RSV and MERS yielded acceptable LODs.

An ideal evaluation would have included enough positive and negative samples to calculate sensitivity and specificity at the lower bound of the
Organism targets, predicted PCR product size, primer name, and primer sequences are listed here.

exclusively the result of variable clinical samples, this was not possible for this evaluation. III (Life Technologies, Carlsbad, CA).

Reagent concentrations for the MAP respiratory virus assay cards.

Composition of each of the 3 lyophilized bead reagents provided with the Map cards, and the resulting reagent concentrations or compositions following resuspension thereof in the chambers of the card in which the beads were packages are represented. Super Script III (Life Technologies, Carlsbad, CA).

which could be overcome by modifying the technology utilized for fluid handling on the MAP unit. Additional improvements to the MAP unit would also include combining the extraction and amplification process into one instrument, which would bring the MAP unit closer to being a POC diagnostic and meeting ASSURED criteria.

Clearly, there are limitations to the study; most notably, sensitivity and specificity for the assay were not calculated due to the low number of negative samples evaluated. Future studies would ideally include a large increase in the number of negative samples evaluated, as well as performing the study in a prospective manner.

While this technology is in early stages of development and, as such, yielded a high rate of invalid (no test) results, percent agreement with clinical laboratory methods approached 97% for completed tests. This technology shows promise as a rapid, accurate, deployable diagnostic technology for automated detection and discrimination of multiple pathogens in clinical sample extracts.

Table 1
Primer targets and sequences for MAP respiratory virus array.

Organism Molecular target Product size (bp)a Primer name Sequence

Influenza A M1 110 VIR13095F TCGGCCGUUGUUCUGGCAGA
VIR13096R /5MAXN/TGCCAGGAGTGGTGGCTGCTTTAACC
Influenza A PA 74 VIR13099F CTUGAGAAUUUUGAAUCUAAGUG
VIR13100R /5MAXN/CACCTTGGAGAAAGGCTGCTCATAT
Influenza B PB1 83 VIR13470F CAGGGCCACAAUAAACACATCCC
VIR13471R/GTAGGCATCAATATTTTGCTCAAGAAGC
Influenza B PA 78 VIR13491F GGAGGAAAUUUCUGGTUCCGTAAT
VIR13492R /5MAXN/TAAGCCTTACATTCCCAATTTATTGT
RSV Matrix protein 83 VIR13361F AAGAUGGGGCAAAUAUGGAAACAUACGUGA VIR13362R /5MAXN/TAGGCCATTGATTGAAAGAGTCGCTGT
RSV Phosphoprotein 84 VIR13355F TCGGCUCUGUUGAAGGAAAGACA VIR13356R /5MAXN/CGTCATTAATGCTTCAGTTCTGATTTTTTCTAT
MERS Orf1A protein 92 VIR13100F CACATTTGAGAAAGCTTGCCCTCAAT VIR13101R /5MAXN b/TGCAGGATTGGTCTTGTCTTTAICCA
MERS N protein 102 VIR13096R /5MAXN b/TGCAGGATTGGTCTTGTCTTTAICCA

Table 2
Reagent concentrations for the MAP respiratory virus assay cards.

UDG (Excipient X4)—100 μL in digestion well
10 U UDG
3% Trehalose
1.5 mmol/L Tris
7.5 mmol/L KCI
0.015 mmol/L EDTA
0.15 mmol/L DTT
0.0015 % BSA
0.015 mmol/L MgCl2
0.4 mmol/L DTT
0.005 % Tween-40
0.03 mmol/L dNTP mixture

Kapa (Excipient X2.2)—150 μL in PCR Master Mix chamber
16 U Kapa
2 % Trehalose
266.67 μmol/L dNTP mixture
4.67 mmol/L MgCl2
1.33 X Buffer A
SSIII (Excipient A)—150 μL in MM chambera
50 U SuperScript III
2 % Trehalose
5 mmol/L Tris
1 mmol/L ammonium sulfate
1.2 mmol/L MgCl2
0.005 % Tween-40
0.4 mmol/L DTT
0.015 μg random primers
0.03 μg Poly-A

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