RESEARCH ARTICLE

Evaluation of the Luminex xTAG Respiratory Viral Panel FAST v2 assay for detection of multiple respiratory viral pathogens in nasal and throat swabs in Vietnam [version 1; referees: 2 approved]

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Abstract

Background: Acute respiratory infections (ARI) are among the leading causes of hospitalization in children ≤5 years old. Rapid diagnostics of viral pathogens is essential to avoid unnecessary antibiotic treatment, thereby slowing down antibiotic-resistance. We evaluated the diagnostic performance of the Luminex xTAG Respiratory Viral Panel FAST v2 against viral specific PCR as reference assays for ARI in Vietnam.

Methods: Four hundred and forty two nose and throat swabs were collected in viral transport medium, and were tested with Luminex xTAG Respiratory Viral Panel FAST v2. Multiplex RT-PCR and single RT-PCR were used as references.

Results: Overall, viral pathogens were detected in a total count of 270/294 (91.8%, 95% CI 88.1-94.7) by the Luminex among reference assays, whilst 112/6336 (1.8%, 95% CI, 1.4-2.1) of pathogens were detected by the Luminex, but not by reference assays. Frequency of pathogens detected by Luminex and reference assays was 379 and 292, respectively. The diagnostic yield was 66.7% (295/442, 95% CI 62.1-71.1%) for the Luminex assay and 54.1% (239/442, 95% CI, 49.3-58.8%) for reference assays. The Luminex kit had higher yields for all viruses except influenza B virus, respiratory syncytial virus, and human bocavirus.

Open Peer Review

Referee Status: ✔ ✔

Invited Referees
1
2

version 2
published
30 Apr 2018

version 1
published
05 Sep 2017

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and human bocavirus. High agreements between both methods [mean (range): 0.91 (0.83-1.00)] were found for 10/15 viral agents.

Conclusions: The Luminex assay is a high throughput multiplex platform for rapid detection of common viral pathogens causing ARI. Although the current high cost may prevent Luminex assays from being widely used, especially in limited resource settings where ARI are felt most, its introduction in clinical diagnostics may help reduce unnecessary use of antibiotic prescription.

**Keywords**
Luminex, RVP FAST v2, Vietnam

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Author roles: Thi Ty Hang V: Data Curation, Formal Analysis, Writing – Original Draft Preparation; Thi Han Ny N: Formal Analysis; My Phuc T: Project Administration; Thi Thanh Tam P: Project Administration; Thao Huang D: Project Administration; Dang Trung Nghia H: Investigation; Tran Anh Vu N: Investigation; Thi Hong Phuong P: Investigation; Van Xang N: Investigation; Dong N: Investigation; Nhu Hiep P: Investigation; Van Hung N: Investigation; Tinh Hien T: Visualization; Rabaa M: Validation, Visualization; Thwaites GE: Conceptualization, Validation, Visualization; Baker S: Methodology, Resources, Visualization; Van Tan L: Methodology, Writing – Review & Editing; van Doorn HR: Conceptualization, Supervision, Writing – Review & Editing;

Competing interests: No competing interests were disclosed.

How to cite this article: Thi Ty Hang V, Thi Han Ny N, My Phuc T et al. Evaluation of the Luminex xTAG Respiratory Viral Panel FAST v2 assay for detection of multiple respiratory viral pathogens in nasal and throat swabs in Vietnam [version 1; referees: 2 approved]
Wellcome Open Research 2017, 2:80 (doi: 10.12688/wellcomeopenres.12429.1)

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Grant information: This work was supported by the Wellcome Trust [093724].
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

First published: 05 Sep 2017, 2:80 (doi: 10.12688/wellcomeopenres.12429.1)
Introduction
Acute respiratory infections (ARI) are the leading cause of morbidity and mortality in infants and children worldwide, especially in Southeast Asia (including Vietnam) and Africa\textsuperscript{1-2}. In Vietnam, there is a high burden of ARI in children in the first year of life, who are more likely to be admitted to intensive care and have a longer hospital stay than children with other infectious diagnoses\textsuperscript{3}.

Viruses are the most common causes of ARI\textsuperscript{4-9}. Rapid identification of causative agents is therefore of clinical and public health significance, and may reduce the widespread inappropriate use of antibiotics.

The advances in molecular diagnostics have provided powerful means for detection of viruses in terms of sensitivity, specificity and turnaround time\textsuperscript{10-12}. The introduction of multiplex PCR for detection of a panel of respiratory pathogens enables faster results, higher throughput and lower cost\textsuperscript{13-14}.

The Luminex xTAG Respiratory Viral Panel (RVP) FAST v2 assay (Luminex Molecular Diagnostics, Toronto, ON, Canada) is a qualitative multiplex molecular diagnostic assay for simultaneous detection of 19 viral types and subtypes within two hours in a single reaction. It was approved in September 2012 by the US Food and Drug Administration (FDA). Previous reports have shown that the sensitivity of Luminex RVP FAST system varied between settings, while the specificity was consistently high\textsuperscript{15-19}. Here, we aimed to evaluate the diagnostic performance of the Luminex xTAG RVP FAST v2 (hereafter called Luminex) against a combination of a number of published reference assays on clinical specimens collected from patients with ARI from four provincial hospitals in Vietnam.

Methods
Study samples and study procedure
This is a retrospective study. Respiratory samples were derived from the Vietnam initiative on Zoonotic infections (VIZIONS) study\textsuperscript{20}. This study was approved by the local ethical committee and the Oxford Tropical Research Ethics Committee (OxTREC Approval No. 15-12). The Institutional Review Boards of the hospital sites (Daklak, Dong Thap, Hue, and Khanh Hoa) submitted the official document approvals to the Hospital for Tropical Diseases (HTD) local ethics committee. The HTD then gave ethical approval (approval no. CS/ND/13/28). Written informed consent was obtained from all patients or from parents/legal guardians if the patient was a child prior to enrolment into the study. In and outpatients with a clinical diagnosis of ARI were recruited from clinical specimens collected from patients with ARI from four provincial hospitals in Vietnam.

Nucleic acid extraction
Equal volumes of VTM from nose and throat swabs were pooled and subjected to total nucleic acid extraction after addition of internal control (EAV - equine arteritis virus for reference assays and bacteriophage MS2 for the Luminex assay) using the MagNApure 96 platform (Roche Diagnostics, Mannheim, Germany), according to the manufacturer’s instructions. Extracted nucleic acids were eluted in 50 ul of elution buffer and stored at -80°C for further analyses.

Luminex xTAG RVP FAST v2 set
The Luminex assay includes reagents to detect Influenza A virus (InfA: generic, H1N1 (1977), H1N1pdm09, H3N2), Influenza B virus (InfB), Respiratory Syncytial Virus A & B (RSVA, RSVB), enteroviruses including rhinoviruses (ENT/Rhi), human parainfluenza viruses 1–4 (PIV1–4), human metapneumovirus (hMPV), adenovirus (ADV), human coronavirus NL63 (hCoV NL63), hCoV HKU1, hCoV 229E, hCoV OC43, and human bocavirus (hBoV). Bacteriophage lambda was included in every run to control the amplification and assay performance. The assay comprised a PCR amplification and hybridization step, and was performed according to the manufacturer’s instructions. Signal acquisition presented as MFI (median fluorescence intensity) was done on the Luminex MAGPIX instrument. Data was read and reported by the TDAS RVP FAST software, version 2.2.

Reference assays
The reference assays included an RT-PCR for RSVA and RSVB\textsuperscript{21}, the CDC RT-PCR obtained by protocol transfer agreement with the US CDC for universal detection of influenza A virus, influenza A virus subtype H1 (1977), influenza A virus subtype H3, and InfB [Table 1, CDC Realtime RTPCR (rRTPCR) Protocol for Detection and Characterization of Influenza, Revised April 4, 2006]; and the 4-tube real time multiplex RT-PCR\textsuperscript{22}. Because the influenza A viruses, InfB and RVS targets were tested in the above specific RT-PCRs, they were not tested in the 4-tube assay. Detection of Influenza A virus H1N1pdm09 was performed by an in-house RT-PCR assay using primer set targeting HA (haemagglutinin) gene segment 4 (forward primer, 5'-GTATCCCAAGGAGCTTCTCGA-3’; reverse primer, 5’-CATGACCTGACCTTTTGGTT-3’; and probe, 5’-FAM-AAGTTTCATGCCCCAATCATGACTCGA-BHQ1-3’ [FAM, 6-carboxyfluorescein, BHQ1, black hole quencher 1]). The reference was considered to be positive if any one of the reference assays was positive.

Data analysis and statistical analysis
Performance of the Luminex assay was evaluated as diagnostic yields and positive rate of pathogens detected by the Luminex compared to reference assays with 95% confidence interval using 2x2 tables. The calculation of these was performed with Intercooled Stata 9.2 (Stata, College station, TX, USA). Agreement between the TDAx and reference assays was determined by
Kappa statistic using SPSS version 23 (IBM Corp. SPSS Statistic, NY, USA). McNemar test (SPSS version 23) was used to examine whether there was any difference of diagnostic rates of individual viruses between the Luminex and reference assays. Statistical significance was set at $P<0.05$.

**Results**

**Baseline results**
A total of 442 samples collected between November 2012 and April 2014 were analysed for the evaluation of the Luminex assay. Three hundred forty-eight samples were from children ($\geq 15$ years old) and 94 samples from adults. The male/female ratio was 0.62 (273): 0.38 (169). The median age of children was 1 year (Interquartile range, IQR: 1, 2) and of adults 46 years (IQR: 34, 72). Overall, 302 specimens (68.3%) were positive by either Luminex or reference assays or both. One hundred forty samples (31.7%) remained undiagnosed. ENT/Rhi was the most frequently detected pathogen by the two techniques (159 over 403 total count of all pathogens, 40%), followed by hBoV (n = 45, 11%) and PIV3 (n = 42, 10%). Less frequently detected were ADV (n = 32, 8%), hMPV (n = 29, 7%), InFA (n = 22, 6%), hCoV (n = 22, 6%), RSV A and B (n = 21, 5%), PIV4 (n = 12, 3%), InFB (n = 10, 2.5%), PIV1 (n = 6, 1%) and PIV2 (n = 3, 0.5%).

**Comparison of the Luminex xTAG RVP FAST v2 test and references**
Diagnostic yields of Luminex and reference assays were 66.7% (295/442, 95% CI, 62.1-71.1%) and 54.1% (239/442, 95% CI, 49.3-58.8%), respectively. The frequency of pathogens detected by Luminex and reference assays was 379 and 292, respectively. Shown in Figure 1 is number of cases that were positive for individual viruses detected by the Luminex and reference assays. The Luminex assay had a higher detection rate for most viruses, but significant differences were seen in ENT/Rhi (99% versus 57%, $P=0.001$, McNemar test) and PIV4 (100% versus 42%, $P=0.01$, McNemar test). In contrast, for hBoV, RSV and InFB, the reference assays had higher detection rates (78% versus 96%, $P=0.04$; 90% versus 100%, $P>0.05$; and 90% versus 100%, $P>0.05$; respectively). Regarding mixed-infection, the Luminex assay also detected more co-infections compared to the reference assays: 68 versus 47. The maximum number of pathogens detected in a single patient was 4. Parechoviruses were detected in four samples by the reference assays, but were not included in the Luminex assay. HCoV were not subtyped by reference assays.

**Evaluation of the test performance and agreement between assays**
Table 2 shows the diagnostic performance of the Luminex assay on clinical swabs against reference assays. Overall, 270 pathogens [91.8% (95% CI, 88.1-94.7)] detected by reference assays were also detected by Luminex assay (true positive rate or “sensitivity” against reference assays as gold standard). There were 112 pathogens detected by Luminex but not detected by references, corresponding to 1.8% (95% CI: 1.4-2.1) detection rate. In addition, there were 24 pathogens detected by reference assays but not detected by the Luminex, corresponding to 0.3% (95% CI: 0.2-0.6) detection rate.

For individual targets, positive detection by Luminex among references was more often for 13 targets, ranging from 88.8% to 100% (ENT/Rhi, PIV3, ADV, hMPV, InFA matrix, H1N1 pdm09, H3N2, hCoV, RSV A, PIV4, InFB, PIV1, PIV2), whilst it was less often positive for hBoV and RSVB (76.7% and 76.9%, respectively). Remarkably, 43.4% (69/159) of ENT/Rhi were detected by Luminex but were negative by reference assays.

Among the 442 clinical swabs, concordance between the two techniques was noted in 372 samples and discordance was recorded in 70 samples.

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**Table 1. Primers and probe sequences of CDC influenza A/B virus PCR.**

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Oligo sequence (5’&gt;3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FluA Forward</td>
<td>GACCRATCCTGTCACCCTGTGAC</td>
</tr>
<tr>
<td>FluA Reverse</td>
<td>AGGGCATTYTGGACAAAACGTCTA</td>
</tr>
<tr>
<td>FluA probe</td>
<td>FAM-TGCAGTCCCTGCTCACTGGGCACG-BHQ1</td>
</tr>
<tr>
<td>1977 seasonal H1 Forward</td>
<td>AACTACTAGGACACTGCTGCTGGAA</td>
</tr>
<tr>
<td>1977 seasonal H1 Reverse</td>
<td>CCATTGGTCATTTGAGGCTGAGT</td>
</tr>
<tr>
<td>1977 Seasonal H1 Probe</td>
<td>FAM-TGAYCCAAAGCC“T” (BHQ1)CTACTCAGTGCAAGAC</td>
</tr>
<tr>
<td>FluA H3 Forward</td>
<td>AAGCATTCCYATGACAAAACC</td>
</tr>
<tr>
<td>FluA H3 Reverse</td>
<td>ATTGCRCAATATGCTCTCTAGT</td>
</tr>
<tr>
<td>FluA H3 Probe</td>
<td>FAM-CAGGATCACATATGGGCTCTGTCAGAGAC-BHQ1</td>
</tr>
<tr>
<td>FluB Forward</td>
<td>TCCCAACCTCACTTCCGACGG</td>
</tr>
<tr>
<td>FluB Reverse</td>
<td>CGGTGCTCTTGGACCAAATTGG</td>
</tr>
<tr>
<td>FluB probe</td>
<td>FAM-CCAATTCGAGCAGCTGAAAAGTCGCTGAG-BHQ1</td>
</tr>
</tbody>
</table>

Note: FAM: 6-carboxyfluorescein (FAM); BHQ1: Blackhole Quencher 1.  
1: Taqman® probe is internally quenched at a modified “T” residue with BHQ1.
Table 2. Diagnostic performance of the Luminex RVP FAST v2 in comparison with reference assays.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>+ L/ ref</th>
<th>+ L- ref</th>
<th>-L/ ref</th>
<th>-L- ref</th>
<th>Positive rate by Luminex and ref (% [95% CI])</th>
<th>Positive rate by Luminex only (% [95% CI])</th>
<th>Kappa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENT/Rhi</td>
<td>89</td>
<td>69</td>
<td>1</td>
<td>283</td>
<td>98.8 (93.9-99.9)</td>
<td>19.6 (15.6-24.1)</td>
<td>0.62 (0.54-0.69)</td>
</tr>
<tr>
<td>hBoV</td>
<td>33</td>
<td>2</td>
<td>10</td>
<td>397</td>
<td>76.7 (61.4-88.2)</td>
<td>0.5 (0.06-1.7)</td>
<td>0.83 (0.73-0.92)</td>
</tr>
<tr>
<td>PIV3</td>
<td>32</td>
<td>7</td>
<td>3</td>
<td>400</td>
<td>91.4 (76.9-98.2)</td>
<td>1.7 (0.6-3.5)</td>
<td>0.85 (0.77-0.92)</td>
</tr>
<tr>
<td>ADV</td>
<td>19</td>
<td>11</td>
<td>2</td>
<td>410</td>
<td>90.5 (69.6-98.8)</td>
<td>2.6 (1.3-4.6)</td>
<td>0.67 (0.53-0.80)</td>
</tr>
<tr>
<td>hMPV</td>
<td>20</td>
<td>8</td>
<td>1</td>
<td>413</td>
<td>95.2 (76.2-99.9)</td>
<td>1.9 (0.8-3.7)</td>
<td>0.80 (0.68-0.91)</td>
</tr>
<tr>
<td>InFA matrix*</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>439</td>
<td>100 (29.2-100)</td>
<td>0.0 (0.0-0.0)</td>
<td>1.00 (1.00-1.00)</td>
</tr>
<tr>
<td>H1N1pdm09</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>432</td>
<td>100 (66.4-100)</td>
<td>0.2 (0.0-1.2)</td>
<td>0.95 (0.85-1.05)</td>
</tr>
<tr>
<td>H3N2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>432</td>
<td>100 (69.1-100)</td>
<td>0.0 (0.0-0.0)</td>
<td>1.00 (1.00-1.00)</td>
</tr>
<tr>
<td>hCoV (OC43, NL63, HKU1)</td>
<td>16</td>
<td>4</td>
<td>2</td>
<td>420</td>
<td>88.8 (65.3-98.6)</td>
<td>0.9 (0.2-2.3)</td>
<td>0.83 (0.71-0.94)</td>
</tr>
<tr>
<td>RSVA</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>432</td>
<td>100 (55.4-99.7)</td>
<td>0.0 (0.0-0.0)</td>
<td>0.95 (0.84-1.05)</td>
</tr>
<tr>
<td>RSVB</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>429</td>
<td>76.9 (46.2-94.9)</td>
<td>0.0 (0.0-0.0)</td>
<td>0.87 (0.73-1.00)</td>
</tr>
<tr>
<td>PIV4</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>430</td>
<td>100 (47.8-100)</td>
<td>1.6 (0.6-3.2)</td>
<td>0.58 (0.30-0.85)</td>
</tr>
<tr>
<td>InFB</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>432</td>
<td>90.0 (55.4-99.7)</td>
<td>0.0 (0.0-0.0)</td>
<td>0.94 (0.83-1.04)</td>
</tr>
<tr>
<td>PIV1</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>436</td>
<td>100 (29.2-100)</td>
<td>0.6 (0.1-1.9)</td>
<td>0.66 (0.30-1.01)</td>
</tr>
<tr>
<td>PIV2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>439</td>
<td>100 (29.2-100)</td>
<td>0.0 (0.0-0.0)</td>
<td>1.00 (1.00-1.00)</td>
</tr>
<tr>
<td>Overall</td>
<td>270</td>
<td>112</td>
<td>24</td>
<td>6224</td>
<td>91.8 (88.1-94.7)</td>
<td>1.8 (1.4-2.1)</td>
<td>0.67 (0.61-0.73)</td>
</tr>
</tbody>
</table>

L: Luminex, ref: reference assays, * positive for M gene but negative for H1-1977, H1N1pdm09 and H3N2.
70 samples, showing substantial agreement (overall kappa 0.67, 95%CI 0.61–0.73). Table 2 also shows the test agreement of all viral pathogens. Almost perfect agreement was recorded in 10 pathogens, hBoV, PIV3, InFA matrix, H1N1pdm09, H3N2, hCoV, RsvA, RsvB, InFB and PIV2 (kappa 0.83 – 1.00). Substantial agreement (kappa 0.62–0.80) was seen in hMPV, ENT/Rhi, ADV and PIV1, while agreement in PIV4 was moderate (kappa 0.58).

Compared to reference assays, our study found an increased detection rate by the Luminex for most targets, and significant difference was seen in Ent/Rhi and PIV4 (+L/-ref= 69, p<0.001; and +L/-ref= 7, p=0.01, respectively; Figure 1). Especially a high number of +L/-ref for Ent/Rhi agent shows a considerable difference with other studies in previous xTAG Luminex studies15,18,21–23; it probably again reveals that Luminex is a strong assay in detection of this viral agent. Meanwhile, detection rate for hBoV was significantly higher in reference assays than in the Luminex (10 versus 2, p=0.04; Figure 1), which is similar to other previous studies15,18 (sensitivity of Luminex for this viral agent was rather low, 41.4% and 20.0% in these studies, respectively).

Though the Luminex assay may be of benefit to diagnostics and cost of treatment2,13,15,17,26, it requires a specific instrument for detection and data acquisition. Therefore, it may not be appropriate to laboratories with limited equipment. However, with a highly automated system and the capacity to test up to 94 specimens within two hours (not including nucleic acid extraction and hands-on time), this high throughput Luminex RVP FAST method would be useful in large hospitals where they could have high input of respiratory samples to run by batch.

Globally, the fear of clinical worsening for patients with ARI usually results in empiric antibiotic prescription, even though doctors are aware that most ARI are caused by viruses. One of the factors contributing to this is the long interval between sampling and reporting of test results. The Luminex assay may be part of the solution to this with its fast turnaround time.

The Luminex has a number of weaknesses: it cannot distinguish enterovirus and rhinovirus, it is not quantitative, it comprises a two-tube step for RT-PCR and DNA hybridization, including an open-tube step for transferring PCR product from RT-PCR tube to hybridization tube, which brings a risk for contamination, and it is expensive. A significantly lower positivity rate of the Luminex assay for hBoV found in this study (P=0.04) is also a weakness of this kit, which may need further clinical evaluation.

Limitations of this study are the lack of a true gold standard as is commonly seen when evaluating diagnostic assays and low numbers of positive samples for several ‘uncommon’ viruses (such as PIV1, PIV2). This low number of positive samples may conceal true diagnostic rates for these viruses.

In conclusion, our study shows the Luminex RVP FAST has a good diagnostic performance for detection of multiple respiratory viruses. Results from this study provided an additional evaluation on the utility of this commercial test. Though the cost of Luminex assay is rather high, Luminex RVP FAST platform could become affordable in large hospitals where samples are high input, reducing cost of the test by batch run. Once the per assay cost of this assay become more affordable, the above advantages and the short turnaround time could contribute to improving patient management and changing the prescription culture in countries like Vietnam.

Discussion
Fast and reliable diagnostic tests are a practical need in helping physicians to make appropriate treatment decisions and are a useful tool in research and surveillance. We report here the evaluation of a Luminex assay on respiratory swabs collected from patients admitted to four provincial hospitals in Vietnam. Lacking a true “gold standard”, we combined a number of published PCR assays as reference tests for evaluation.

Overall positivity of Luminex among reference assays was high (91.8%, CI 95% 88.1–94.7). Ten viral targets had almost perfect agreement (kappa 0.81–1.00) between both assays. However, 5 targets had lower agreement, in which 4 viral targets were at substantial agreement (0.61–0.80; hMPV, K=0.80, ENT/Rhi, K=0.62; PIV1, K=0.66; ADV, K=0.67) and one target was at only moderate agreement (PIV4, K=0.58). These 5 targets were all more often detected by Luminex (number of +L/-ref cases ranged from 3 – 69; Table 2) than by reference assays. This suggests that detection using Luminex is superior to reference assays for these targets.

1 Data File

Dataset 1: Respiratory Viral Diagnostic Result from 442 patient swabs of the Luminex and reference assays.
- Site: Abbreviation of provinces where hospitals involved in the study, where patients were recruited. (DK: Daklak, DT: Dong Thap, H: Hue, and KH: Khanh Hoa)
- Gender: patient sex, 1: Male, 2: Female
- DateCollection: Date when patient samples were collected
- Flu A type (CDC): Results of influenza A and types from the reference assay: CDC Realtime RTPCR assay
- Flu B (CDC): Results of influenza B from the reference assay: CDC Realtime RTPCR assay
- RSV type: Results of RSV A and RSV B from in-house reference PCR assay
- detected by 4tube multiplexPCR: Results of other 10 viruses from reference assay: in-house 4-tube multiplex PCR
- Luminex result: Results from Luminex assay
- Diagnose: Final diagnosis based on Luminex and reference assays, it was defined “pos” if sample was positive with any of reference assays or Luminex assay, “undiagnosed” if sample was negative with all reference assays and the Luminex assay

https://doi.org/10.6084/m9.figshare.5353630.v1
Data availability

**Figshare:** Evaluation of the Luminex xTAG Respiratory Viral Panel FAST v2 assay for detection of multiple respiratory viral pathogens in nasal and throat swabs in Vietnam

**Dataset 1:** Respiratory Viral Diagnostic Result from 442 patient swabs of the Luminex and reference assays.

https://doi.org/10.6084/m9.figshare.5353630.v1

Competing interests
No competing interests were disclosed.

Grant information
This work was supported by the Wellcome Trust [093724].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
This work was done as part of the Vietnam initiative on Zoonotic infections study (VIZIONS).

We would like to thank the study teams in VIZIONS consortium members at the all collaborators at Dong Thap General Hospital, Khanh Hoa General Hospital, Hue Central Hospital, and Dak Lak General Hospital.

The VIZIONS Consortium members (alphabetical order by surname) from the Oxford University Clinical Research Unit are Bach Tuan Kiet, Stephen Baker, Alessandra Berto, Maciej F. Boni, Juliet E. Bryant, Bui Duc Phu, James I. Campbell, Juan Carrique-Mas, Dang Manh Hung, Dang Thao Huong, Dang Tran Oanh, Jeremy N. Day, Dinh Van Tan, H. Rogier van Doorn, Duong An Han, Jeremy J. Farrar, Hau Thi Thu Trang, Ho Dan Trung Nghia, Hoang Bao Long, Hoang Van Duong, Huynh Thi Kim Thu, Lam Chi Cuong, Le Manh Hung, Le Thanh Phuong, Le Thi Phuc, Le Thi Phuong, Le Xuan Luat, Luu Thi Thu Ha, Ly Van Chuong, Mai Thi Phuc Loan, Behzad Nadjam, Ngo Thanh Bao, Ngo Thi Hoa, Ngo Thi Tue, Nguyen Canh Tu, Nguyen Duc Thuan, Nguyen Dong, Nguyen Khac Chuyen, Nguyen Ngoc An, Nguyen Ngoc Vinh, Nguyen Quoc Hung, Nguyen Thanh Dung, Nguyen Thanh Minh, Nguyen Thi Binh, Nguyen Thi Hong Tham, Nguyen Thi Hong Tien, Nguyen Thi Kim Chuc, Nguyen Thi Le Ngoc, Nguyen Thi Lien Ha, Nguyen Thi Nam Lien, Nguyen Thi Ngoc Diep, Nguyen Thi Nhung, Nguyen Thi Song Chau, Nguyen Thi Yen Chi, Nguyen Thieu Trinh, Nguyen Thu Van, Nguyen Van Cuong, Nguyen Van Hung, Nguyen Van Kinh, Nguyen Van Minh Hoang, Nguyen Van My, Nguyen Van Thang, Nguyen Van Thanh, Nguyen Van Vinh Chau, Nguyen Van Xang, Pham Ha My, Pham Hong Anh, Pham Thi Minh Khoa, Pham Thi Thanh Tam, Pham Van Lao, Pham Van Minh, Phan Van Be Bay, Maia A. Rababa, Motiur Rahman, Corinne Thompson, Guy Thwaites, Ta Thi Dieu Nguan, Tran Do Hoang Nhu, Tran Hoang Minh Chau, Tran Khanh Toan, Tran My Phuc, Tran Thi Kim Hong, Tran Thi Ngoc Dung, Tran Thi Thanh Thanh, Tran Thi Thuy Minh, Tran Thua Nguyen, Tran Tinh Hien, Trinh Quang Tri, Vo Be Hien, Vo Nhut Tai, Vo Quoc Cuong, Voong Vinh Phat, Vu Thi Lan Huong, Vu Thi Ty Hang, and Heiman Wertheim; from the Centre for Immunity, Infection, and Evolution, University Of Edinburgh: Carlijn Bogaardt, Margo Chase-Topping, Al Ivens, Lu Lu, Dung Nyugen, Andrew Rambaut, Peter Simmonds, and Mark Woolhouse; from The Wellcome Trust Sanger Institute, Hinxton, United Kingdom: Matthew Cotten, Bas B. Oude Munnink, Paul Kellam, and My Vu Tra Pham; from the Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center of the University of Amsterdam, Amsterdam, the Netherlands: Martin Deij, Lia van der Hock, Maarthen F. Jebbink, and Seyed Mohammad Jazaeri Farsani; and from Metabiota, CA: Karen Saylors and Nathan Wolfe.

References


Open Peer Review

Current Referee Status: ✓ ✓

Version 1

Referee Report 12 February 2018
doi:10.21956/wellcomeopenres.13459.r30347

Marta Canuti
Department of Biology, Memorial University of Newfoundland, St. John’s, NL, Canada

Thi Ty Hang et al. evaluated the Luminex system’s performance in diagnosing respiratory viral infections in ARI cases in Vietnam and compared their results to those obtained with other reference diagnostic methods. Although more expensive than in-house diagnostic assays, Luminex performed well and it could be of real benefit in laboratories with a high throughput of samples.

The paper is well written, sample size is appropriate and conclusions are supported by the results. I only have minor suggestions.

1. I think Table 2 is a bit difficult to follow. When I read “positive rate” I think about N+/total number of test (while here it is over the N of positives or the N of negatives with the reference method – columns 6 and 7). I think rephrasing the captions for columns 6 and 7 and/or including a note with the used formulas will greatly increase the readability of the table (e.g. something like “Luminex sensitivity”, L+/Ref+/ Ref+ for column 6 and “additional Luminex positive”, L+/Ref- for column 7). Personally, I would also remove column 5, because it adds confusion.

2. Abstract, results: Similarly, the sentence “viral pathogens were detected in a total count of 270/294 by the Luminex among reference assays” is not clear.

3. Results, third paragraph: I would rephrase the sentence “…corresponding to a 1.8% detection rate” into something like “…corresponding to an additional 1.8% positives”. (Same at the following line).

4. Methods: Was the used VTM commercially available or prepared in house? A reference to the provider or a list of component used for its preparation should be included.

5. Results, first paragraph: Shouldn’t it be <=15, instead of >=15?

6. Was the higher number of co-infections detected by the Luminex due to the higher number of Entero/Rhinoviruses identified?

7. Results, paragraph starting with “For individual targets”: I think a word is missing after “more often”.

8. Discussion: I would avoid the repetition of the K values as they are stated 2 paragraphs above.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 28 December 2017

**Bharti Malhotra**
Sawai Man Singh Medical College, Jaipur, Rajasthan, India

Approved with minor modifications, authors to address the following

1. Authors should provide sensitivity & specificity, limit of detection of the reference assay used

2. Authors should provide how the sample size was achieved

3. Was any other reference method used to assess whether additional positive by Luminex were false positive or true positive to truly evaluate the performance of luminex / label it as more sensitive

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes
Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** Mycobacteriology & virology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.