Original Article

Diagnosis of respiratory syncytial virus and influenza A and B with cobas® Liat® from nasopharyngeal aspirations in pediatrics

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ABSTRACT

The cobas® Liat® Influenza A/B and respiratory syncytial virus assay was tested on nasopharyngeal aspirates. The resolution of invalid samples was performed using a preanalytical step. cobas® Liat® can be used on nasopharyngeal aspirates with a preanalytical processing step, with a slightly diminished performances in detecting respiratory syncytial virus but not for influenza.

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1. Introduction

cobas® Liat® (Lab-In-A- Tube) is a CLIA-waived point-of-care test (POCT) reverse transcriptase (RT) polymerase chain reaction (PCR) device that has been available on the market for several years, allowing the molecular diagnosis of influenza A and B (InfA and InfB) and respiratory syncytial virus (RSV) from nasopharyngeal swabs (NPS) in only 20 minutes. The sensitivity of cobas® Liat® is equivalent to that of reference PCR methods and superior to that of viral culture (Akashi et al., 2019; Chen et al., 2015; Gibson et al., 2017; Gosert et al., 2019; Ling et al., 2018; Melchers et al., 2017; Young et al., 2017). In our institution, pediatricians use nasopharyngeal aspirates (NPAs) over NPSs on children because NPSs are considered uncomfortable. However, cobas® Liat® is not validated on this sample type. Some authors reported the use of cobas® Liat® in gargles, sputa and endotracheal secretions but retrospectively and on a limited number of samples not including NPAS (Goldstein and Gunson, 2019). We describe here for the first time the validation of the use of cobas® Liat® on NPAS for routine use on children, comparing cobas® Liat® to Simplexa™ Flu A/B & RSV Direct and to 2 in-house multiplex PCR assays. Despite excellent performance, we achieved a high rate of invalid results with cobas® Liat®. Thus, we aimed to reduce a posteriori the rate of invalid results using a preanalytical protocol.

2. Materials and methods

2.1. Study design

This prospective study was conducted during the winter season of 2017 to 2018 at a single 550-bed hospital site of a public institution in Brussels. We tested all fresh NPAS from children who were consulted in pediatric emergency wards, pediatric consultation and pediatric hospitalization.

2.2. Sample collection

Nasopharyngeal secretions were aspirated through a catheter connected to a mucus trap and fitted to a vacuum source after flushing 3 mL of physiological serum in each nostril. The sample was then brought immediately to the lab.

2.3. Virus detection

Samples were tested with cobas® Liat® using the Influenza A/B and RSV Assay (Roche Diagnostics, Mannheim, DE) and with Liaison MDX...
The results of PoPA and NePA are expressed in percentages with 95% confidence intervals. The respective invalid rates were 9.3%, 11.6%, and 13%. The initial performance of the cobas® kit was 90.6% PoPA for RSV, 100% for InfA, and 100% for InfB. The NePA were 100%. The initial average rate of invalid samples was 32.9% for RSV (71/216), 34.3% for InfA (74/216), and 33.8% for InfB (73/216). The dilution/centrifugation protocol was applied to 66 of 74 samples. Eight of 74 samples were not in sufficient volume to apply the protocol. The invalid rate dropped to 5.3%. The PoPA of cobas® kit dropped to 80.0% for RSV but remained the same before and after the preanalytical processing for the influenza viruses. Table 1 summarizes the methods' performances.

### 4. Discussion

The search for respiratory viruses by microscopic fluorescence has become inadequate because of its poor performance, and the particular expertise needed to interpret the results does not allow 24/7 use. Nonmolecular rapid tests are easy to use and fast, but their sensitivity is quite poor. Molecular methods are considered the gold standard, but their use is often cumbersome and requires extensive infrastructure. They are also rarely used routinely. cobas® kit is a practical method for detecting RSV, InfA, and InfB. The instrument perfectly meets the POCT definition and is categorized as CLIA waived by the FDA. Indeed, it is compact, can be installed in a medical consulting practice and provides results in 20 minutes, which is a reasonable time to make a decision before the patient leaves the consultation as to whether to administer an antibiotic in a clinical presentation of a respiratory infection (Benirschke et al., 2019; Hansen et al., 2018). In addition, the viruses tested are among the most common in pediatric practice. The performance previously described makes it possible to compare cobas® kit to other molecular laboratory instruments, and its cost of use is equivalent. Binnicker et al. compared cobas® kit to Simplexa™, with very good results (Binnicker et al., 2015). Only one study reported a lower sensitivity for cobas® kit than for other molecular methods (Youngs et al., 2019). cobas® kit was also compared with Alere-i, another molecular, non-PCR method for the diagnosis of respiratory viruses, and showed equivalent to significantly higher performance (Leonardi, 2019; Nolte et al., 2016; Valentin et al., 2019). However, the kit is not validated only on NPSs. While NPS collection is easy and common in adults, it is much more traumatic and uncomfortable for children, especially since the median age of our population was 5.5 months, and the youngest was only 4 days old. Ten percent of our population was less than 1-month-old. Our objectives was to develop a rapid test that could be used for infants and young children as well, especially since the median age of our population was 5.5 months, and the youngest was only 4 days old. Ten percent of our population was less than 1-month-old. Our objective was to validate the method on NPA samples, as our pediatricians have a higher degree of control of the technique, and the collection is less unpleasant on very young infants. Although we achieved good performances for cobas® kit, we encountered a diagnostic failure in more than 34% of the samples, which is not acceptable given the analysis cost. Invalid results were also observed with the Simplexa™ kit and were almost negligible with the NRC homemade PCRs, which is validated on all types of respiratory samples due to the external

### Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>RSV</th>
<th>FluA</th>
<th>FluB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liat®</td>
<td>PoPA 90.6% (0.83–0.95)</td>
<td>100.0% (0.91–1.0)</td>
<td>100.0% (0.77–1.0)</td>
</tr>
<tr>
<td>NePA 100.0% (0.94–1.0)</td>
<td>100.0% (0.96–1.0)</td>
<td>100.0% (0.97–1.0)</td>
<td></td>
</tr>
<tr>
<td><strong>κ</strong></td>
<td>0.88</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Liat® with preanalytical protocol and reanalysis</td>
<td>PoPA 80.0% (0.71–0.87)</td>
<td>100.0% (0.91–1.0)</td>
<td>100.0% (0.81–1.0)</td>
</tr>
<tr>
<td>NePA 98.9% (0.94–1.0)</td>
<td>100.0% (0.98–1.0)</td>
<td>100.0% (0.98–1.0)</td>
<td></td>
</tr>
<tr>
<td><strong>κ</strong></td>
<td>0.78</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Simplexa™ Flu A/B &amp; RSV</td>
<td>PoPA 79.0% (0.70–0.86)</td>
<td>100.0% (0.91–1.0)</td>
<td>100.0% (0.81–1.0)</td>
</tr>
<tr>
<td>NePA 100.0% (0.96–1.0)</td>
<td>100.0% (0.98–1.0)</td>
<td>99.4% (0.97–1.0)</td>
<td></td>
</tr>
<tr>
<td><strong>κ</strong></td>
<td>0.78</td>
<td>1.0</td>
<td>0.96</td>
</tr>
<tr>
<td>Homemade PCR (NRC)</td>
<td>PoPA 88.4% (0.81–0.93)</td>
<td>94.9% (0.83–0.99)</td>
<td>93.3% (0.7–0.99)</td>
</tr>
<tr>
<td>NePA 100.0% (0.96–1.0)</td>
<td>100.0% (0.98–1.0)</td>
<td>100.0% (0.98–1.0)</td>
<td></td>
</tr>
<tr>
<td><strong>κ</strong></td>
<td>0.88</td>
<td>0.97</td>
<td>0.96</td>
</tr>
</tbody>
</table>

PoPA = positive percent agreement; NePA = negative percent agreement; κ = κappa coefficient of concordance; N/A = not applicable.

The results of PoPA and NePA are expressed in percentages with 95% confidence intervals.
The dilution of the initial sample did not affect the performance of the method for the influenza viruses despite the use of a matrix not recommended by the manufacturer. However, the performance was even better than that obtained by the reference laboratory for RSV, we observed due to the viscous and sometimes hemorrhagic properties of the samples. This phenomenon is less marked with Simplexa™, probably due to the centrifugation inherent to the amplification process. We retested the invalid samples for which there was still enough volume, applying a simple analytical protocol based on a dilution in UTM-RT medium, centrifugation and pipetting of the supernatant to perform analysis as with an ordinary sample. The application of this protocol resulted in a 6-fold decrease in the invalid rate ($P < 0.05$; chi-square).

The dilution of the initial sample did not affect the performance of the method for the influenza viruses despite the use of a matrix not recommended by the manufacturer. The performance was even better than that obtained by the reference laboratory. For RSV, we observed a diminished PoPA. Simplexa™ showed a lower PoPA than cobas® Liat® only for RSV, with an invalid rate 3 times lower than that of cobas® Liat® when the dilution protocol was not applied. Nevertheless, the turnaround time of Simplexa™ is 70 minutes for 1 to 8 samples. Therefore, it is positioned as a point-of-impact, time-to-result test and not as a POCT. Overall, the lower PoPA for RSV with cobas® Liat® and Simplexa™ than that reported in the literature can be explained by the method chosen for the composite gold standard. We opted for the “any positive method” to not bias the analysis in favor of the assay under evaluation. If we had chosen a method where 2 positive methods validate the positivity, the PoPA of Liat® and Simplexa™ for RSV would have been 98.8% and 93.5%, respectively, with a NePA of 96.2% and 95.3%, which are comparable or superior to the results from other authors (Banerjee et al., 2019; J Banerjee et al., 2018).

5. Conclusion

The preanalytical protocol used to process NPSs before testing with cobas® Liat® significantly reduces the invalid rate. The limitation of our study is that we did not apply this protocol on valid samples at the first run to exclude a reduction in PoPA related to dilution. This approach was not possible for cost reasons and should be validated in future studies. On the other hand, the systematic application of the protocol requires manipulations, which makes the use of cobas® Liat® at the patient’s bedside more challenging and compromises the CLIA-waived status of the method. However, the necessary manipulations remain simple and fast (taking less than 2 minutes) and can be performed in a medical examination room.

Authors’ contributions

Laurent Blairon: study design, molecular analyses, statistical analyses, writing; Marie Tré-Hardy: writing, bibliography; Isabelle Thomas: molecular analyses; Phu-Quoc Lê and Ingrid Beukinga: internal review.

Ethical approval

All the procedures were in accordance with the 1964 Helsinki Declaration and its later amendments.

Informed consent

According to French Health Public Law (CSP Article L1121-1), this type of study did not require specific informed consent or ethics committee approval.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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References

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