Preliminary evaluation of two rapid antigen assays for novel influenza A (H1N1) virus detection in clinical specimens

At the present time, specific real-time reverse transcription-PCR (rRT-PCR) assays remain the reference method for virological detection of the novel pandemic swine-origin influenza A (H1N1) virus in clinical samples (http://www.who.int/csr/resources/publications/swineflu/WHO_Diagnostic_RecommendationsH1N1_20090521.pdf). The rapid worldwide spread of the virus highlighted the need for influenza diagnostic tests that are rapid, commercially available and readily performed in primary health care settings to detect viral antigens in respiratory clinical samples and so aid clinical management of severe cases and people at risk of complications, such as pregnant women, newborn infants or obese patients. However, the sensitivity and specificity of commercially available rapid enzyme immunoassays are questionable. To assess their sensitivity in detecting novel influenza A (H1N1) virus, we conducted an evaluation of two rapid influenza diagnostic tests (RIDTs), Espline Influenza A&B (Fujirebio) and BinaxNOW Influenza A+B (Emergo Europe), recognizing viral nucleoprotein. Twenty-five clinical respiratory samples consisting of nasal swabs collected in September–October 2009 were retrospectively analysed in November 2009 after storage at −80 °C. They had previously tested positive for novel influenza A (H1N1) virus by H1-specific rRT-PCR assays developed by the French National Reference Centers (NRCs) for influenza viruses (B. Lina, Lyon, France; S. Van Der Werf, Paris, France). Five negative specimens were randomly selected for this evaluation. The viral load quantified in each positive specimen by a M gene-specific rRT-PCR assay, also developed by the NRCs, ranged from 1.3 to 1.4 × 10^6 copies ml^{-1} corresponding, respectively, to C_t values in the H1 rRT-PCR method of 29.8 and 17.5. A glyceraldehyde-3-phosphate dehydrogenase-specific rRT-PCR assay was also performed to check the quality of the nucleic acids extracted and the absence of PCR inhibitors. The RIDTs were carried out according to the manufacturers’ instructions directly from 100 μl of the nasal swab expressed in 500 μl of cell culture medium. For the six specimens with high viral titres (M gene copies >3.6 × 10^6 copies ml^{-1} or H1 rRT-PCR C_t values <20), the two RIDTs demonstrated 100% sensitivity in detecting novel influenza A (H1N1) virus when compared with rRT-PCR. However, among the 14 specimens with H1 C_t values from 20 to 27, the sensitivity of the two RIDTs declined substantially and was, respectively, 71.4% and 21.4% for the Espline and Binax tests. Moreover, no sample with an H1 C_t value >27 (n=5) tested positive, suggesting a limit of detection of these tests corresponding to a viral load ranging from 1 × 10^5 to 1 × 10^6 M gene copies ml^{-1} (Fig. 1). Overall, from the 25 specimens positive for novel influenza A (H1N1) virus, the Espline RIDT detected 16 positive samples, demonstrating a sensitivity of 64%, whereas only nine positive samples tested positive by the Binax RIDT, corresponding to a specificity of 36% (Fig. 1). No false-positive results were observed, indicating a specificity of 100% for both tests. Statistical comparison of these results using the McNemar test (a P-value of <0.05 was considered statistically significant) showed that the two RIDTs were significantly less sensitive than the molecular method for novel influenza A (H1N1) virus detection (P=0.004 and P=0.00003, respectively). Moreover, inter-RIDT differences were statistically significant since the Espline test appeared to be significantly more sensitive (16/25) in this study than the Binax test (9/25) for the detection of novel influenza A (H1N1) virus from respiratory specimens (P=0.02). The positive predictive value was 100% for both tests since no false-positive result was detected while Espline and Binax RIDTs negative predictive values were 35.7% and 23.8%, respectively.

In conclusion, these results showed that the RIDTs were capable of detecting novel
influenza A (H1N1) virus directly in respiratory clinical specimens containing high levels of virus corresponding to H1 Ct values <27 or viral loads higher than 1 × 10^7 M gene copies ml^-1. The sensitivity of the rapid antigen tests that we evaluated was estimated at 64 % for Espline and 36 % for Binax when compared to the specific rRT-PCR assay. These results were identical to those previously reported with the same kits for novel and seasonal influenza A (H1N1) virus detection (Chan et al., 2007, 2009; Ginocchio et al., 2009). Moreover, an effect of the storage of the samples on the sensitivity of the RIDTs for detecting low quantities of the target antigens could not be ruled out.

The good sensitivity of the Espline test could make it of great interest as a first-line test during the height of the novel influenza A (H1N1) virus outbreak since no discrimination between virus subtypes is required. Moreover, a positive RIDT result could avoid performing an expensive, time-consuming RT-PCR assay and could be used to improve clinical and therapeutic management of the patients. However, the low negative predictive values observed indicate that a negative result does not rule out infection with novel influenza A (H1N1) virus. In the case of negative RIDT results, testing with rRT-PCR should be performed for patients with influenza-like symptoms presenting high severity of illness or risk for complications. Additional prospective evaluations of the accuracy of present and future RIDTs in detecting novel influenza A (H1N1) virus should be conducted.

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