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Becton Dickinson Directigen EZ Flu A+B assay in the diagnosis of pandemic influenza A H1N1 2009 virus infection in adult patients

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To the editor:

The recent emergence and spread of the pandemic influenza A H1N1 2009 virus demands the evaluation of rapid antigen assays for their ability to detect this novel subtype of influenza A virus. Data on the ability of BD Directigen EZ Flu A+B immunochromatographic (IC) assay (Beckton Dickinson and Company, Sparks, MD, USA) to detect the pandemic influenza A virus strain in fresh clinical samples have been recently published.[1–5] In these studies, the majority of specimens were collected from pediatric patients, and the sensitivities reported ranged from 46·8% to 76·6%. As viral shedding in the upper respiratory tract during influenza virus infection is of greater magnitude in children than in adults, the clinical utility of IC tests may indeed depend on the patient age.[6] We wish to report on our experience regarding the diagnostic and analytical performance characteristics of the Directigen EZ Flu A+B in a cohort of adults (≥18 years old) presenting with an influenza-like syndrome at a tertiary Spanish hospital (Peset Aleixandre, Valencia Spain).

A total 274 nasopharyngeal swabs from unique patients (median age of 50 years, range 18–97 years; 145 women and 129 men) and collected between July and September 2009 were included in the study. The specimens were obtained within 72 hours after the onset of symptoms by means of flexible nasopharyngeal nylon flocked swabs, placed in 3 ml of transport medium (Universal transport medium; Beckton Dickinson) and delivered to the Microbiology laboratory within 1 hours of collection. The specimens were vortexed and tested by the IC assay following the instructions of the manufacturer.[7] Samples were assayed by RT-PCR within 24 hours after reception. Total RNA was extracted by the MagNApure extraction kit in the MagNA Pure robot (Roche Diagnostics, Basel, Switzerland), and RT-PCR was performed by use of the Realtime Ready Influenza A/H1N1 Detection Set on the LightCycler® 2.0 instrument (Roche Diagnostics).[8,9]

The overall positive rate for novel influenza A virus RNA as determined by real-time PCR was 15·3%. Forty-two specimens tested positive by RT-PCR, of which 18 gave a positive IC result. The remaining 232 specimens tested negative by RT-PCR. All these specimens gave a negative result in the IC assay. The overall agreement between the two assays was 91·2% (250/274), and the sensitivity, specificity, positive predictive value, and negative predictive value (adjusted to the prevalence in our cohort) were of 42·8%, 100%, 100%, and 79·8%, respectively. Cycle threshold (Ct) values for samples testing positive by the IC assay (median, 24·1, range, 20·5–33·6) were significantly lower ($P = 0{\cdot}001$, by the Mann–Whitney test) than those for specimens yielding a negative result (median, 31·5, range, 30·2–34·5).

To determine the analytical sensitivity of the IC assay, a local influenza strain (A/Valencia/1/2009H1N1v) isolated in Mardin Darby Canine Kidney cells was used. The viral stock (50% tissue culture infectious dose-$TCID_{50}$-/ml of $\log_{10}$ 7·0) was serially diluted in viral transport medium and tested in duplicate by IC. The limit of detection of the BD Directigen assay was approximately $\log_{10}$ 4·5 $TCID_{50}$/ml, which is in keeping with previous estimations.[3,10] In summary, the sensitivity of the Directigen EZ Flu A+B assay for the diagnosis of pandemic influenza A virus infection is clearly suboptimal and appears to be lower than that reported in studies conducted in either pediatric or mixed children and adult cohorts. Thus, molecular testing should be mandatory when a negative IC result is obtained, particularly in adult patients with a high pretest probability of infection. Nevertheless, given the specificity of the assay, a positive IC result may be safely

used in making decisions regarding the instauration of antiviral treatments or implementation of infection control measures.

Conflicts of interest

The authors declare no conflicts of interest.

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