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Evaluation of 3 rapid influenza diagnostic tests during the 2012–2013 epidemic: influences of subtype and viral load

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ABSTRACT

This article evaluates the performance of 3 rapid influenza diagnostic tests (RIDTs), in correlation with the influenza subtypes and the viral load. A total of 236 samples were prospectively analyzed with BinaxNOW Influenza A/B, Directigen EZ Flu A and B, and bioNexia Influenza A+B. The results were compared to cell cultures and real-time polymerase chain reaction. Positive samples were further subtyped. Thirty-seven samples were positive for influenza A, and 57, for influenza B. For A(H1N1), the sensitivities were 71.42% for BinaxNOW, 78.57% for Directigen, and 67.85% for bioNexia. Eight samples were positive for A(H3N2), and only the bioNexia test had 1 false-negative result. Lowest sensitivities were observed for influenza B/Yamagata, (56.86% for BinaxNOW and Directigen and 39.21% for bioNexia). The 3 evaluated RIDTs were more efficient at detecting influenza A (H3N2) than for A(H1N1) and B/Yamagata. Highest viral loads in the samples were associated with better rate of detection.

1. Introduction

Rapid diagnosis of influenza infections is critical during epidemic season to allow treatment initiation and patient isolation. The diagnosis of influenza can be made using several methods. Cell culture is relatively sensitive for recovering influenza viruses, but this method needs viable viruses in the sample and is rather slow; the delay in obtaining results often varies between 3 and 14 days. Molecular methods are the most sensitive techniques available and can detect non-viable viruses by targeting a conservative area of the matrix gene (WHO Global Influenza Network, 2011); however, these techniques have high financial costs, are not available in all facilities, and require a well-optimized workflow during epidemics to be able to obtain results in a clinically relevant timeframe. Rapid influenza diagnostic tests (RIDTs) offer a fair alternative; these tests based on the principle of immunochromatography are easy and rapid to perform, as they generally yield a result in 10–15 minutes. They can detect non-viable viruses as they are targeted at their nucleoprotein. Additionally, these tests are very specific. However, they lack the sensitivity of the cell culture and molecular methods (Kumar and Henrickson, 2012). Moreover, an evaluation of some Food and Drug Administration–approved RIDTs published in November 2012 stated that some subtypes of influenza viruses could be less detectable than others depending on the RIDT used (Beck et al., 2012). In the present study, the performances of 3 RIDTs were evaluated in comparison to the cell culture method and influenza A/B real-time polymerase chain reaction (PCR) analysis during the 2012–2013 influenza epidemic. All influenza A– and influenza B–positive samples were retrospectively subtyped to determine if some subtypes were less detectable than others.

2. Materials and method

2.1. Samples and population

The threshold of flu epidemic 2012–2013 in Belgium was crossed in week 52 of 2012, and the epidemic was declared in week 1 of 2013 and lasted 12 weeks (Thomas et al., 2013). From January 18th to February 18th 2013 (week 3 to week 8 of 2013), a total of 236 clinical samples were prospectively collected. The samples were taken from 118 female and 111 male patients between the ages of 8 days and 86 years old (mean age: 13.25 years; median: 1.35 years). All samples for which the practitioners prescribed a test for influenza were included whether the patients' symptoms met the case definition of influenza like illness or not (ECDC, 2005–2014). The interval between the time of collection of the sample and the onset of the first symptoms was not standardized, and patients coming later to the hospital could still have a sample taken if the practitioner estimated it could be useful. The samples were taken during 2 separate periods, and the gap between the 2 periods corresponded to a shortage of RIDTs due to insufficient production by the suppliers. The samples included 154 nasopharyngeal aspirates, 71 nasopharyngeal swabs, 5 throat swabs, 3 bronchoalveolar washes, 1 tracheal aspirate, 1 sputum, and 1 nasal swab. Swabs were collected with

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FLOQSwab (Copan, Brescia, Italy) and transported in 1.5 mL of veal infusion broth (Difco, Becton Dickinson, Sparks, MD, USA) supplemented with bovine albumin (Sigma Aldrich, St. Louis, MO, USA).

2.2. RIDTs

The 3 RIDTs evaluated were BinaxNOW Influenza A/B (Alere Inc., Waltham, MA, USA), Directigen EZ Flu A and B (Becton Dickinson, Franklin Lakes, NJ, USA), and bioNexia Influenza A+B (bioMérieux, Marcy l'Etoile, France). These tests are only validated for nasopharyngeal swabs and aspirates, which constitute 95.7% of the samples in this study, but are routinely performed on other types of samples with a warning concerning the reliability of the result. The samples were analyzed only once with each test according to the recommendations of the manufacturers. Viral transport medium with the nasopharyngeal swabs as well as the nasopharyngeal aspirates were diluted up to 3 mL with fresh viral transport medium to allow sufficient sample volume to perform all of the routine analyses.

2.3. Cell cultures

Portions of the diluted specimens were used to inoculate confluent Vero (African green monkey kidney), MRC5 (human lung), and LLC-MK_2 (rhesus monkey kidney) cell cultures (Vircell, Santa-Fé, Spain) in 24-well or 6-well tissue culture plates (Greiner-Bio One, Frickenhausen, Germany); these cultures were incubated at 36 °C in a 5% CO_2 atmosphere for 2 weeks for the Vero and LLC-MK_2 cells and 3 weeks for the MRC5 cells. The media was replaced weekly. The cultures were examined every 2–3 days using an inverted microscope. The combination of these 3 cell lines allows the recovery of most of the significant human respiratory viruses (Ginocchio and Harris, 2011). LLC-MK_2 cell line is utilized in our laboratory mainly for the recovery of influenza virus, whereas the use of MDCK cell line (canine kidney) is more common. In our experience, both cell lines perform as well for the recovery of influenza virus but LLC-MK_2 is better for parainfluenza virus, which motivated our choice (Frank et al., 1979). Hemadsorption was performed on the LLC-MK_2 cells at the end of the second week of incubation.

2.4. Molecular testing

An Influenza A/B real-time PCR was performed on the frozen aliquot of every culture-negative sample and on the samples that tested positive for a non-influenza virus (Fig. 1). First, 400 μL of the frozen aliquot was purified according to the QIAsymphony automated extraction protocol using the QIAsymphony DSP Virus/Pathogen Midi extraction kit (Qiagen, Germantown, MD, USA). Then, analyses was performed on a Lightcycler 480 using the Taqman EZ RT-PCR kit (Applied Biosystems, Paisley, United Kingdom); the primer and probe sequences were designed by Ward et al. (2004).

Finally, for all positive samples (culture-positive and culture-negative/PCR-positive samples), the frozen extracts were sent to the National Reference Centre for Influenza to confirm the typing and for subtyping analysis. The typing of influenza A/B was performed with an in-house duplex quantitative real-time PCR (qRT-PCR) using an Mx3005p qPCR System (Agilent Technologies, Stockport, United Kingdom) and the SuperScript III Platinum One-Step qRT-PCR kit (Invitrogen, Carlsbad, NM, USA). The primers and probes were these used for universal detection of influenza A in the CDC protocol for influenza A(H1N1)pdm09 (CDC, 2009), and the primers and probes used to detect influenza B were based on the works of van Elden et al. (2001). The subtyping of influenza A was performed using qRT-PCR and the SuperScript III RT/Platinum Taq Mix, with primers and probes for H1 (CDC, 2009) and H3 (Overduin et al., 2012). To determine the lineage of the

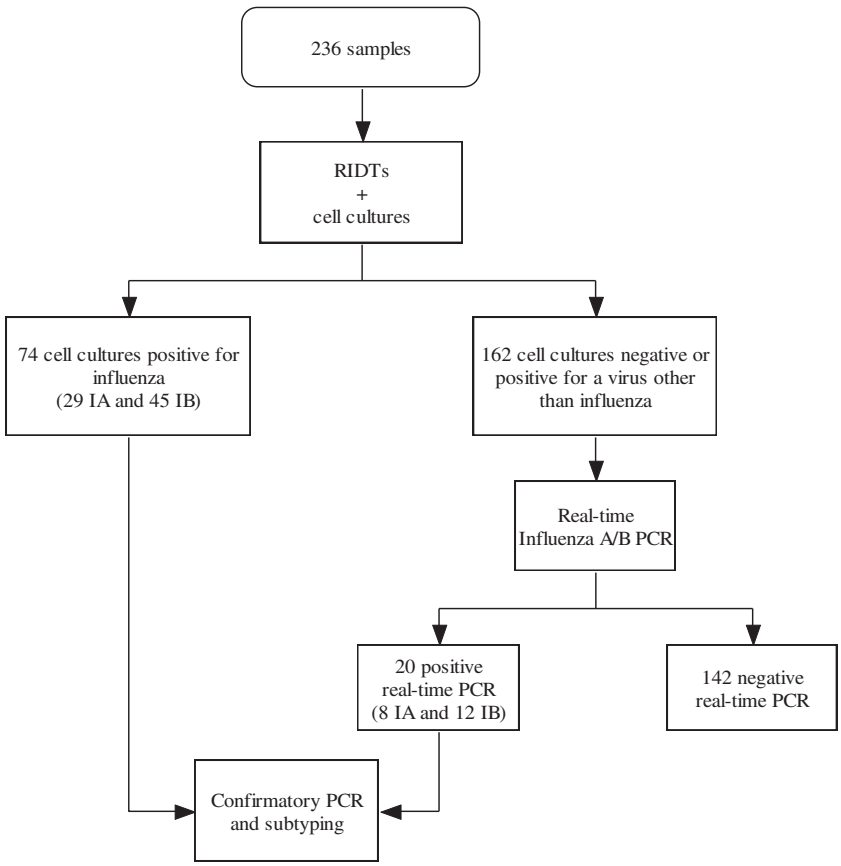

Fig. 1. Study work scheme. IA = influenza A; IB = influenza B.