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Standardizing the influenza neuraminidase inhibition assay among United States public health laboratories conducting virological surveillance

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ABSTRACT

Background: Monitoring influenza virus susceptibility to neuraminidase (NA) inhibitors (NAIs) is vital for detecting drug-resistant variants, and is primarily assessed using NA inhibition (NI) assays, supplemented by NA sequence analysis. However, differences in NI testing methodologies between surveillance laboratories results in variability of 50% inhibitory concentration (IC_{50}) values, which impacts data sharing, reporting and interpretation. In 2011, the Centers for Disease Control and Prevention (CDC), in collaboration with the Association for Public Health Laboratories (APHL) spearheaded efforts to standardize fluorescence-based NI assay testing in the United States (U.S.), with the goal of achieving consistency of IC_{50} data.

Methods: For the standardization process, three participating state public health laboratories (PHLs), designated as National Surveillance Reference Centers for Influenza (NSRC-Is), assessed the NAI susceptibility of the 2011–12 CDC reference virus panel using stepwise procedures, with support from the CDC reference laboratory. Next, the NSRC-Is assessed the NAI susceptibility of season 2011–12 U.S. influenza surveillance isolates (n = 940), with a large subset (n = 742) tested in parallel by CDC. Subsequently, U.S. influenza surveillance isolates (n = 9629) circulating during the next three influenza seasons (2012–15), were independently tested by the three NSRC-Is (n = 7331) and CDC (n = 2298).

Results: The NI assay IC_{50}s generated by respective NSRC-Is using viruses and drugs prepared by CDC were similar to those obtained with viruses and drugs prepared in-house, and were uniform between laboratories. IC_{50}s for U.S. surveillance isolates tested during four consecutive influenza seasons (2011–15) were consistent from season to season, within and between laboratories.

Conclusion: These results show that the NI assay is robust enough to be standardized, marking the first time IC_{50} data have been normalized across multiple laboratories, and used for U.S. national NAI susceptibility surveillance.

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1. Introduction

Neuraminidase (NA) inhibitors (NAIs) are currently the only class of antiviral drugs recommended by Centers for Disease Control and Prevention (CDC) for the control of influenza infections, due to widespread resistance to the M2 blockers (adamantanes)

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(Deyde et al., 2007). In 1999, orally administered oseltamivir and inhaled zanamivir were approved by the United States (U.S.) Food and Drug Administration (FDA) for control of influenza type A and B infections. In December 2014, a third NAI, intravenous peramivir (Shetty and Peek, 2012), was FDA-approved. Peramivir is also licensed in Japan, South Korea and China, while inhaled laninamivir (Yamashita et al., 2009) is approved in Japan. Intravenous zanamivir has been provided for compassionate use in recent years, and is undergoing evaluation for treatment of hospitalized patients with severe influenza (https://clinicaltrials.gov/show/NCT01231620).

During initial post-marketing years, monitoring influenza virus susceptibility to NAIs was carried out by a central laboratory contracted by the Neuraminidase Inhibitor Susceptibility Network (NISN) which utilized chemiluminescence- and fluorescence-based NI assays (Wetherall et al., 2003). Later, monitoring NAI-susceptibility became an integral part of virological surveillance within the WHO Global Influenza Surveillance and Response System (WHO-GISRS), where both functional (NA inhibition) and sequence-based (pyrosequencing, real time RT-PCR, Sanger) assays have been utilized to conduct drug susceptibility monitoring worldwide (Monto et al., 2006; Meijer et al., 2014). Following the FDA's approval of zanamivir and oseltamivir, CDC implemented the NI assay, first using a chemiluminescence-based (Mungall et al., 2004), then a fluorescence-based methodology (Okomo-Adhiambo et al., 2013). The NI assay testing is done for the purpose of monitoring changes in the baseline NAI susceptibility of circulating influenza viruses.

Historically, there has been significant variability in IC_{50} data (the drug concentration required to inhibit 50% of viral NA enzyme activity), due to factors such as variations in assay choice and/or assay conditions. The lack of standardization in NI assay methodologies and the resulting IC_{50} variability has been a challenge in sharing and interpreting IC_{50} data among laboratories. In 2012, in efforts to harmonize the interpretation and reporting of IC_{50} data, the WHO Expert Working Group for GISRS on Surveillance of Antiviral Susceptibility (WHO-AVWG) agreed on criteria to define influenza viruses as exhibiting normal, reduced (RI) or highly reduced (HRI) NA inhibition, based on the fold change of their IC_{50} compared to reference IC_{50} values (WHO, 2012). These criteria have been helpful in interpretation and reporting of NI assay data generated by different WHO Collaborating Centers (Okomo-Adhiambo et al., 2014; Takashita et al., 2014, 2015a), and for providing annual global updates (Meijer et al., 2014; Takashita et al., 2015b). Viruses of N1 subtype carrying the H275Y substitution in the NA (H274Y in N2 subtype) and A(H3N2) viruses carrying E119V or R292K substitutions consistently demonstrate HRI by oseltamivir in NI assays (Meijer et al., 2014; Okomo-Adhiambo et al., 2014; Takashita et al., 2015b).

In the summer of 2011, the CDC spearheaded efforts to standardize influenza NI testing within the U.S., in collaboration with the Association for Public Health Laboratories (APHL) and several state public health laboratories (PHLs), with the goal of minimizing NI assay IC_{50} data variability within and among surveillance laboratories, as well as increasing the US capacity to monitor the NAI susceptibility of influenza viruses. Three PHLs, designated as National Surveillance Reference Centers for Influenza (NSRC-Is), namely, the California Department of Public Health (CDPH), Richmond, CA; Unified State Laboratories, Public Health (USLPH), Taylorsville, UT; and the Wisconsin State Laboratory of Hygiene (WSLH), Madison, WI, participated in the "Project for Standardization of NAI Susceptibility Testing," through a step-wise procedure developed by the CDC. In April 2012, Maryland Department of Health and Mental Hygiene (MD DHMH) Laboratories Administration also participated in an additional NI Standardization Project, but was not selected as a NSRC-I.

After successfully completing the standardization process, the three NSRC-Is performed NI assay testing on U.S. influenza surveillance specimens collected during the 2011–12 season, and three subsequent seasons, 2012–13, 2013–14, and 2014–15. The NI assay testing activity was added to an ongoing APHL contract for influenza virus isolation, collectively referred to as the "VI/NI Project." At CDC, standardized IC_{50} data generated by the NSRC-Is were further analyzed based on criteria of the WHO-AVWG (WHO, 2012) to identify viruses with RI or HRI, which were genetically analyzed by pyrosequencing and/or conventional NA sequence analysis to determine underlying NA changes responsible for elevated IC_{50}s. Viruses with markers previously associated with resistance to NAI(s) were reported as NAI-resistant to the WHO GISRS and in the U.S. influenza virological surveillance Report (FluView) (http://www.cdc.gov/flu/weekly/).

This report describes results of the NI assay standardization project performed on the 2011–12 CDC reference virus panel by three NSRC-Is (CDPH, USLPH and WSLH), as well as NI assay data for U.S. influenza surveillance isolates they tested during seasons 2011–15. These results suggest that NAI drug susceptibility data generated in the NI assay can be interpreted and shared in a consistent, reproducible manner when detailed procedures and reference materials are used in assay implementation.

2. Materials and methods

2.1. Training

Prior to the standardization process, participating laboratories received training on the fluorescent NI assay by CDC laboratorians, through lectures and hands-on instruction. The training course, conducted in April 2011, was sponsored by the APHL, CDC and National Laboratory Training Network (NLTN), and comprised pre- and post-test knowledge assessment; background information on influenza and drug susceptibility testing; NI assay workflow; reagent and drug preparation; determination of virus dilutions for use in the NI assay; identification of acceptable NI assay results; equipment operation; IC_{50} data analysis and interpretation.

2.2. Viruses

The 2011–12 CDC reference panel (Table 1) comprising wildtype and variant virus pairs of seasonal influenza types/subtypes, as well as surveillance isolates circulating during the 2011–14 influenza seasons were propagated in Madin–Darby canine kidney (MDCK) cells provided by CDC. Since October, 2014 (season 2014–15) influenza A(H3N2) viruses are propagated in MDCK-SIAT1 cells, also provided by CDC. Note, an updated CDC reference virus panel version 2, for NAI susceptibility testing, was introduced in October, 2012 (season 2012–13), and comprises viruses different from those in the 2011–2012 panel, with exception of A/Washington/01/2007(H3N2) wildtype, A/Texas/12/2007(H3N2) NA-E119V, B/Memphis/20/96 wildtype and B/Memphis/20/96 NA-R152K (Supplementary Table S1). Laboratories conducting influenza virological surveillance using NI assays can obtain this panel from the Influenza Reagent Resource (IRR) upon registration and approval (Catalogue No. FR-1176) (http://www.influenzareagentresource.org/Catalog.aspx).

2.3. Neuraminidase inhibitors

Oseltamivir carboxylate, the active compound of the ethyl ester prodrug oseltamivir phosphate was kindly provided by Hoffmann-La Roche (Basel, Switzerland), zanamivir by GlaxoSmithKline (Uxbridge, UK), and peramivir by BioCryst Pharmaceuticals