Detecting 2009 Pandemic Influenza A (H1N1) Virus Infection: Availability of Diagnostic Testing Led to Rapid Pandemic Response

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Diagnostic tests for detecting emerging influenza virus strains with pandemic potential are critical for directing global influenza prevention and control activities. In 2008, the Centers for Disease Control and Prevention received US Food and Drug Administration approval for a highly sensitive influenza polymerase chain reaction (PCR) assay. Devices were deployed to public health laboratories in the United States and globally. Within 2 weeks of the first recognition of 2009 pandemic influenza H1N1, the Centers for Disease Control and Prevention developed and began distributing a new approved pandemic influenza H1N1 PCR assay, which used the previously deployed device platform to meet a >8-fold increase in specimen submissions. Rapid antigen tests were widely used by clinicians at the point of care; however, test sensitivity was low (40%–69%). Many clinical laboratories developed their own pandemic influenza H1N1 PCR assays to meet clinician demand. Future planning efforts should identify ways to improve availability of reliable testing to manage patient care and approaches for optimal use of molecular testing for detecting and controlling emerging influenza virus strains.

Accurate diagnosis is critical for pandemic influenza recognition, surveillance, and public health interventions. Without available and reliable laboratory testing, early response efforts are fraught with uncertainty and delays. In 2007, the Centers for Disease Control and Prevention (CDC), along with other federal agencies and laboratory partners, began implementing a strategy for improving global diagnostic preparedness for pandemic influenza. This plan included development of new diagnostic tests, guidance to clinicians, increased capacity for critical diagnostic reagents, and enhancement of surveillance for novel influenza virus strains.

In April 2009, the emergence of a transmissible, novel, swine-origin influenza virus among humans prompted public health and clinical laboratories to quickly identify means for diagnosing suspected cases and monitoring the spread of influenza illness. Over the months after the recognition of the 2009 pandemic influenza A H1N1 (pH1N1) virus, testing was performed at various points across the spectrum of clinical laboratories, ranging from high-complexity reference laboratories to point-of-care testing in clinicians' offices. The availability, speed, and accuracy of testing varied considerably and revealed a number of challenges for clinicians and public health officials in providing medical care and responding to the pandemic. In this article, we describe the important role of molecular diagnostic testing, the

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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benefit of predeployed testing capacity in public health laboratories, and the challenges of patient treatment decisions when accurate clinical diagnostic tests are not available.

METHODS

Influenza test results were obtained from ~150 US public health, academic, and hospital laboratories participating in the CDC's virus surveillance activities [1]. These laboratories documented the total number of specimens tested for influenza and the number of specimens positive for influenza by various methods, including rapid influenza diagnostic tests (RIDTs), fluorescent antibody tests, reverse-transcription polymerase chain reaction (RT-PCR) tests, and antigenic characterization of cultured virus strains.

A subset of participating laboratories performed influenza typing and/or subtyping with use of 2 RT-PCR assays on ABI 7500 Fast Dx instruments. The first was the Human Influenza Virus Real-Time RT-PCR Detection and Characterization Panel (CDC 5-Target PCR) [2]. This assay was developed at the CDC, was clinically evaluated in collaboration with the Association of Public Health Laboratories, and was cleared by the US Food and Drug Administration (FDA) on 30 September 2008 for in vitro diagnostic detection of influenza types A and B and 3 subtypes: seasonal A/H1, seasonal A/H3, and A/H5 (ie, H5 Eurasian highly pathogenic avian influenza). If a test identified RNA from an influenza A virus but did not give a positive test result for circulating seasonal A/H1 or A/H3 subtypes (defined as unsubtypable), a sample was required to be forwarded to the CDC for additional testing [3]. Before the recognition of pH1N1 in March 2009, ~45 state public health laboratories and 2 Department of Defense laboratories were qualified to run the CDC 5-Target PCR assay. The second PCR assay used by the surveillance network after the emergence of the pandemic was the Swine Influenza Virus Real-Time RT-PCR Detection Panel (CDC pH1N1 PCR) [4]. The FDA granted an Emergency Use Authorization (EUA) for this test on 27 April 2009. Both assays are cleared for use only in prequalified laboratories in which personnel have received training from the CDC and that possess appropriate equipment. As of 1 March 2010, 146 US public health and Department of Defense laboratories were qualified to perform these tests for virus surveillance purposes.

The distribution of the CDC-manufactured 5-Target PCR kits was initiated at CDC laboratories during September 2008, and during April 2009, FDA authorized transfer of distribution responsibility to the CDC Influenza Reagent Resource, as part of a contract with ATCC, to provide routine and surge quantities of reagents to public health laboratories and test devleopers [5]. Each PCR test kit can provide ~1000 test reactions. In 2008, the CDC collaborated with the Association of Public Health Laboratories to estimate the expected surge quantities of test reagents needed for production during a pandemic by modeling all steps in the testing process at public health laboratories [6]. These estimates were used to determine the amount of reagents stockpiled at the Influenza Reagent Resource for pandemic preparedness.

Nasal swab specimens were also tested as part of a CDC-sponsored clinical trial at the Naval Health Research Center (NHRC, San Diego, CA) with use of an investigational point-of-care in vitro diagnostic device developed by Meso Scale Diagnostics (CDC contract 200-2007-19346) [7]. This device uses electrochemiluminescence to detect antigens for influenza types A and B and subtypes A/H5 and seasonal A/H1 and A/H3 in nasal swab specimens directly in <20 minutes.

Requests from providers to Centers for Medicare and Medicaid Services for reimbursement of influenza diagnostic testing were analyzed to approximate changes in test use during 2004-2009 with use of American Medical Association Current Procedural Terminology codes for virus isolation, fluorescent antibody testing, and antigen detection (86710, 87252, 87253, 87254, 87275, 87276, 87400, 87449, and 87804) [8].

RESULTS

Diagnostic Testing Before pH1N1

From December 2005 until the recognition of pH1N1, 12 sporadic infections with swine influenza A/H1 virus strains had been diagnosed in the United States in persons with recent exposure to pigs [9]. This represented a significant increase from the number of cases diagnosed in previous years and was thought to be attributable primarily to improved virologic surveillance, enhanced testing capacity in state public health laboratories, and use of PCR testing for more specific virus characterization. PCR assays for detecting the hemagglutinin gene from these swine-origin influenza H1N1 virus strains and from other rarely occurring animal-origin influenza A virus strains with pandemic potential had been validated at the CDC and were available for in-house screening of unsubtypable specimens.

Diagnostic Testing for Detection of the First 2 Recognized Cases

On 30 March 2009, a 10-year-old boy with uncomplicated influenza-like illness (ILI) was enrolled in a CDC-sponsored investigation using the Meso Scale Diagnostics investigational point-of-care testing device in San Diego, California in collaboration with NHRC [10, 11]. Two upper respiratory tract specimens were collected by a site clinician. The first nasal swab specimen was tested with the investigational device, which identified an influenza A virus, with the subtype not determined. Because the clinical trial protocol required prompt additional testing of specimens with suspect unsubtypable influenza A test results from the investigational device, the second swab

specimen was transferred to the designated reference laboratory (Marshfield Clinic, Marshfield, WI), where results confirmed an unsubtypable influenza A virus. The remaining sample was forwarded to the Wisconsin State Laboratory of Hygiene (Madison, WI), in accordance with national guidelines, for evaluation of influenza virus strains with pandemic potential [12]. Testing was performed using equipment and procedures established as part of a CDC pandemic preparedness laboratory surveillance initiative, and strains were confirmed as an unsubtypable influenza A virus strain with use of the CDC 5-Target PCR. The CDC received a sample on 15 April, ~2 weeks after initial specimen collection, and characterized the novel influenza A virus with an in-house CDC PCR assay for swine origin influenza gene signatures. Genetic sequencing, completed on 16 April 2009, revealed a novel influenza A H1N1 virus with a combination of gene segments that previously had not been reported in swine or human influenza virus strains in the United States or elsewhere [13].

A second case of uncomplicated ILI in a child from southern California who had illness onset in late March 2009 was also identified as unsubtypable with PCR of a swab specimen collected as part of a CDC partnership surveillance activity for monitoring influenza along the US–Mexico border (Border Infectious Disease Surveillance Project in collaboration with NHRC) [14, 15]. A sample was received at the CDC on 17 April 2009, and testing confirmed presence of a novel influenza A virus similar to the virus from the first case. The CDC reported these and subsequent early cases [10, 16] and recommended increased monitoring to identify additional cases; however, specific testing for pH1N1 was available only at public health laboratories, limiting clinicians' ability to discriminate pH1N1 infections.

Ramping-up Testing

At the onset of the pandemic, 45 public health laboratories were already performing surveillance using the FDA-cleared CDC 5-Target PCR assay and, thus, were able to identify probable cases of pH1N1 with no change in testing practices. A probable case of swine-origin influenza A (H1N1) virus infection was defined as acute febrile respiratory illness in a person positive for influenza A but negative for H1 and H3 by influenza real-time RT-PCR [16]. A review of data from these public health laboratories that were collected over the preceding months had not revealed an increase in the number of unsubtypable influenza A–positive specimens, indicating that the emergence of pH1N1 was likely to be a new phenomenon. The CDC and Association of Public Health Laboratories collaborated to rapidly qualify additional state and local public health laboratories to help meet testing demand.

After completing partial genome sequencing of the pH1N1 virus strain, the CDC posted results on publicly available Web sites beginning on 20 April 2009; 40 sequences comprising the full pH1N1 virus genome were posted by 27 April 2009 [17, 18]. Availability of the sequences enabled diagnostic test developers to begin evaluating whether their current influenza A assays were able to detect the new virus or whether modification might be needed. On the following day, 28 April 2009, the CDC posted the complete CDC pH1N1 PCR assay protocol on the World Health Organization Web site [19]. Early posting of both the sequences and the protocol allowed research and hospital laboratories to produce their own laboratory-developed pH1N1 PCR assays (ie, home brews). As of 29 March 2010, the CDC had posted 4030 pH1N1 gene sequences from >1060 influenza virus isolates.

On 27 April 2009, a day after the US Secretary of Health and Human Services declared a public health emergency, the FDA granted an EUA to allow the CDC to manufacture and distribute the CDC pH1N1 PCR assay for use by public health laboratories [20]. Within 2 weeks after the first recognition of pH1N1, the CDC developed and validated the new PCR assay, began manufacturing the reagents, obtained FDA authorization for distribution and use, and distributed PCR kits domestically and internationally. As represented in Figure 1, distribution of test kits started during the first week of increased specimen collection during late April 2009, in time to meet the increased testing demands in May at public health laboratories. As of 20 February 2010, a total of 2710 PCR kits had been shipped to 459 clinical and public health laboratories in all US states and in 153 countries.

Diagnostic Testing for Influenza Surveillance

From 4 October 2008 until the first report of pH1N1 infections during the week ending on 25 April 2009, the number of specimens from patients with ILI that were submitted to laboratories participating in the CDC influenza surveillance network was 201,121; ~14% of these were positive for influenza A or B virus [21]. During the subsequent single week, from 25 April through 2 May 2009, the total number of specimens submitted per week increased by 8.4-fold, from 4197 to 35,381; seasonal influenza H1N1 detection was 16-fold greater, seasonal influenza H3N2 detection was 26-fold greater, and unsubtypable influenza (later confirmed as pH1N1) detection was 93-fold greater [Figure 1]. The total number of specimens tested was increased by >4000, compared with the fall peak that would occur 6 months later. The number of submitted specimens exceeded all prior weeks on record. The percentage of submitted specimens that tested positive for influenza during the week from 25 April through 2 May 2009 increased from 6.7% to 12.1% [Figure 1]. This finding suggests that the dramatic increase was attributable to collection of specimens from patients with ILI and not otherwise healthy or mildly symptomatic patients.

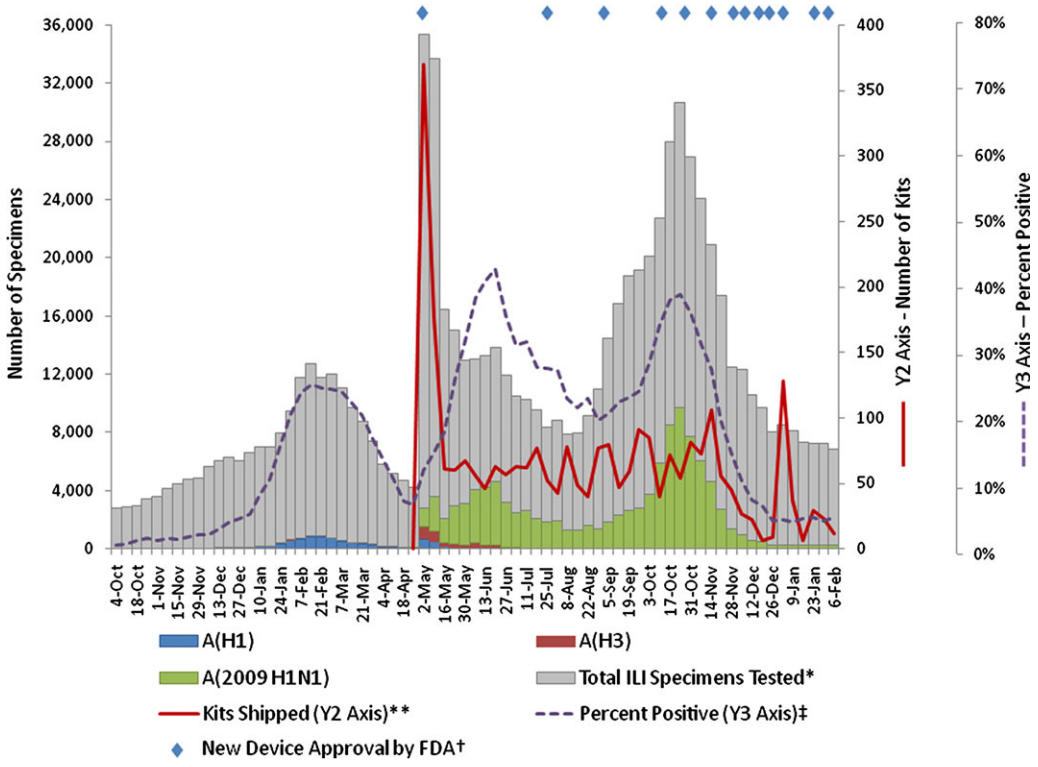

Figure 1. Test kits shipped to laboratories and the respiratory specimen testing from ~150 laboratories participating in the Centers for Diease Control and Prevention (CDC) virus surveillance system (US World Health Organization Collaborating Laboratories and National Enteric and Respiratory Virus Surveillance System), 2008–2010. * Bars representing total influenza-like illness (ILI) specimens tested are not stacked. The remaining bars for subtypes A(H1), A(H3), and A(2009 H1N1) are stacked. ** Kits were shipped as supplies were available. The large number of kits in early January 2010 represents a shipment of updated reagents sent to laboratories to replace older reagents. ‡ Percentage positive was calculated using the total number of tests positive for influenza A and B as the numerator and total specimens submitted to the US virus surveillance system as the denominator. Influenza tests include polymerase chain reaction (PCR), rapid influenza diagnostic tests, fluorescent antibody tests, and virus culture. The percentage positive includes results for all influenza tests; however, only PCR subtype results are shown as bars. The remainder of positive test results (rapid influenza diagnostic tests, fluorescent antibody tests, and virus culture) are presented elsewhere [21]. † Each diamond represents the date that that US Food and Drug Administration issued an Emergency Use Authorization for a new influenza diagnostic device. The first authorization on 27 April 2010 was for the CDC pH1N1 PCR assay; all other devices were sponsored by other entities. An additional 3 approvals occurred in February and March 2010 and do not appear on the graph, nor do authorizations modifying previously authorized devices (eg, modified indications).

During the summer, the numbers of submitted samples decreased, but the percentage of results positive for pH1N1 remained 20%–30%. More specimens were submitted during the summer of 2009 than during the entire preceding 2008–2009 season as a consequence of an unprecedented persistence of influenza activity during a period when only rare sporadic seasonal influenza cases had been reported in the past. During this period of record testing, most public health laboratories began accepting only specimens for PCR testing that were from patients who had severe illness, were hospitalized, died of suspected influenza, or were seen at sentinel outpatient ILI surveillance sites as recommended by the CDC. For this reason, the cases reported in the summer and fall represent a fraction of the cases that might have been reported with a more liberal testing policy [22].

As schools started in August 2009, the number of specimens submitted and the number of pH1N1-positive cases increased considerably [Figure 1]. The appearance of an influenza season this early in the year had not occurred since the influenza H2N2 pandemic of 1957 [23]. At the peak of specimen submissions in the fall pH1N1 wave, the number did not surpass the dramatic increase in the spring but was 2.4-fold greater than the peak during the preceding 2008-2009 influenza season. Overall, 607,344 specimens were submitted for testing to participating laboratories from 2 May 2009 through 6 February 2010; this number was ~3 times the number submitted during the previous season.

Rapid Influenza Diagnostic Tests

Although the CDC pH1N1 PCR assay was quickly made available at 146 public health laboratories in the United States, clinician access to these tests was limited. Many hospital laboratories could provide or obtain other influenza diagnostic alternatives, including immunofluorescent antibody testing, virus culture, or

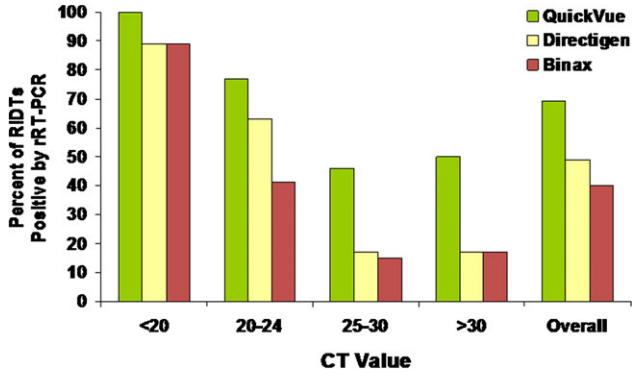

Figure 2. Comparison of the performance of 3 rapid influenza diagnostic tests (RIDTs) with the Centers for Disease Control and Prevention 5-Target real-time reverse-transcription polymerase chain reaction (RT-PCR) assay. Data are from MMWR Morb Mortal Wkly Rep 2009;58(30):826–9 [27]. Three tests were evaluated: (1) Inverness Medical BinaxNOW Influenza A&B (Binax), (2) Becton Dickinson Directigen EZ Flu A+B (Becton Dickinson), and (3) Quidel QuickVue Influenza A+B (Quidel). Cycling time (CT), or threshold cycle, required to detect the virus with use of real-time RT-PCR is shown. Lower CT values indicate higher virus RNA concentrations in the specimens tested.

PCR assays for influenza A and B (both FDA-approved or home brew). The most widely available tests were the RIDTs, at hospital laboratories, physician offices, and noncentralized laboratory settings, such as emergency departments.

When the pandemic virus emerged, 8 FDA-approved rapid tests were available in the United States for rapid influenza testing [24]. In general, these tests are simple to use and detect influenza virus antigens in respiratory specimens within 15 min. Two of these tests are waived under the Clinical Laboratory Improvement Amendments of 1988, allowing their use in many settings that provide point-of-care results, including physician office settings. According to information in the manufacturers' package insert documents, sensitivities for detecting seasonal influenza A virus in clinical specimens ranged from 70% to 90% (generally compared with virus culture results).

Before the emergence of pH1N1, published reports showed lowered performance of RIDTs; sensitivities ranged from 27% to 61%, when compared with PCR testing on the same specimen [25, 26]. In August 2009, the CDC reported results of an evaluation with 3 widely available RIDTs with sensitivities for detection of pH1N1 virus ranging from 40% to 69%, compared with the CDC pH1N1 PCR assay [27]. Similar findings were reported by other investigators [15, 28, 29]. Of note, sensitivity varied among the different RIDTs and was directly proportional to the relative pH1N1 RNA concentration in the original respiratory specimen; essentially, higher virus loads led to a greater likelihood of a positive result. [Figure 2] These findings prompted guidance that a negative RIDT result does not rule out infection with pH1N1 virus and that patients with indications for influenza antiviral treatment should be treated empirically for influenza regardless of a negative RIDT result [22]. In addition, guidance also focused on specimen collection, because performance of RIDTs and other influenza tests is improved when swab samples are collected appropriately to achieve sufficient respiratory secretions within the first 48 h after illness onset, when viral loads are highest.

Early after recognition of the pandemic strain, the CDC recommended isolation of patients with confirmed pH1N1 infection as an infection control measure; however, clinicians using RIDTs were not able to differentiate pH1N1 from other influenza A subtypes that continued to circulate from April through June 2009 [Figure 1]. As the pandemic progressed, the positive predictive value of an influenza A RIDT–positive result indicating pH1N1 became higher and remained high through the fall, because pH1N1 was the predominate circulating strain of influenza. Among all influenza A subtyped specimens, the percentage of pH1N1 increased from 16% on 25 April 2009 to 89% on 23 May 2009 and remained above that level through the most recent report on 10 April 2010 [30].

Although the benefit of a positive RIDT result was evident, continued clinical decision-making based on false-negative results confounded infection control and medical management. For example, additional transmission of illness among campers and at schools may have occurred as the result of persons resuming activities after receiving negative RIDT results [31, 32]. In addition, implementation of infection control precautions were delayed, contact investigations for health care–associated cases of influenza were complicated, and delay in antiviral treatment or respiratory support measures occurred [33, 34]. In a report of obstetric and gynecologic patients with pH1N1 infection in California, RIDT results were falsely negative for 58 (38%) of 153 persons tested [35]. Of 8 persons who died, none received antiviral treatment and 6 had rapid test results that were falsely negative.

Despite issues with negative test results, RIDTs were available at the point of care to provide quick information in the absence of more sensitive and virus-specific pH1N1 PCR assays. Manufacturers of RIDTs increased production to meet demand, and revenues reportedly increased by 70% [36]. Centers for Medicare and Medicaid Services database records from 2004 through 2009 depicted relatively unchanged use of virus culture and immunofluorescence, whereas antigen detection use, notably RIDTs, doubled during 2008–2009 and increased in use 16-fold since 2004 [8] [Figure 3].

Filling the Gap?

To increase the number of testing options for detecting pH1N1 infection, the FDA approved a total of 16 devices under the EUA [37]. [Figure 1] The first EUA was issued for the CDC pH1N1 PCR test on 27 April 2009. No other EUA was granted until 24 July 2009. Fifteen of these were PCR assays, and 1 was a direct fluorescent antibody assay specific for pH1N1. All but one were

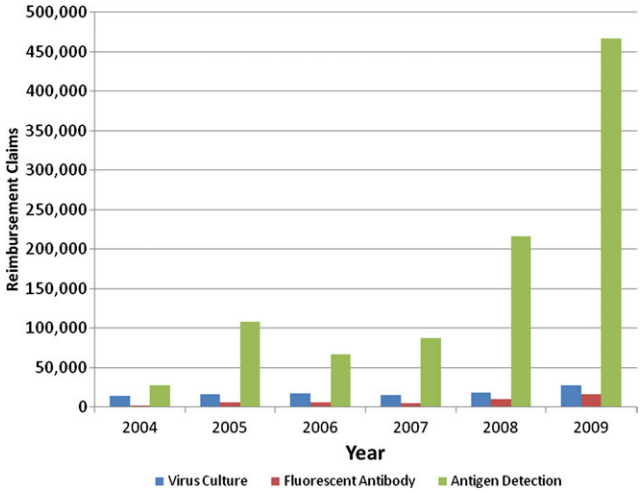

Figure 3. Medicare reimbursement claims during 2004–2009 for virus culture, fluorescent antibody assay, and antigen detection assays. Data compiled according to the Centers for Medicare and Medicaid Service (CMS) Current Procedural Terminology (CPT) codes using the following categories: virus culture (87252, 87253, and 87254), fluorescent antibody (87275 and 87276), and antigen detection (87400, 87449, and 87804). The following codes are specific to influenza testing: 87275, 87276, 87400, and 87804.

limited for use in Clinical Laboratory Improvement Amendments high-complexity laboratories (ie, specialized equipment and processes were needed to perform the test by trained laboratory staff) [38]. None were available for Clinical Laboratory Improvement Amendments–waived testing in outpatient settings or yielded timely results for clinicians. Few of the authorized devices were widely distributed or readily available for use in clinical laboratories.

The majority of additional testing may have been provided through laboratory-developed tests (eg, home brews) generated and validated at clinical laboratories in various settings or using already-existing PCR-based assays for influenza A and B virus strains. Because the CDC rapidly posted the CDC pH1N1 PCR test protocol and pH1N1 gene sequences on public Web sites, molecular diagnostic laboratories in academic medical centers and some community hospitals were able to quickly set up and validate pH1N1-specific PCR assays at their own institutions. In a survey, 62% of ~8000 patients tested in Chicago during the first 4 weeks after recognition of the pandemic virus were first screened for pH1N1 by community molecular diagnostics laboratories; more than half of all cases diagnosed in Illinois were first detected at these laboratories [39].

CONCLUSION

Within 2 weeks after the first recognition of a pandemic influenza virus, a new, FDA-authorized, accurate, sensitive, and virus-specific diagnostic test was manufactured and distributed to laboratories in the United States and abroad. The rapid availability of virus sequence information and the quick translation of these data into diagnostic tools allowed clinicians and public health officials in the United States and worldwide to determine the magnitude of the emerging pandemic, to identify groups at highest risk of infection, and to tailor vaccine and treatment recommendations to have the greatest impact.

The use of PCR molecular tests was central to this rapid response and demonstrated the transforming capability that technology can have for infectious diseases–related emergencies. From the onset of the pH1N1 response, the public health community elected to provide publically available information and tools for response as quickly as they were available. By posting both the genetic sequences and the instructions for performing the CDC pH1N1 PCR assay, the CDC provided the knowledge base for others to quickly develop tests. This open-source approach for rapid sharing of virus sequence and testing information has been recognized as a successful component of the 2009 emergency response efforts and greatly improved clinician access to pH1N1 confirmatory testing [40].

Manufacturers of new tests specific for pH1N1 were able to receive EUA in the United States, enabling manufacture and distribution of the assays during the public health emergency. Although admirable in its offering, the EUA approach may have been able to fill only a small portion of the pH1N1 testing gap during the pandemic. In fact, the majority of molecular testing probably occurred at community and academic hospitals that prepared their own laboratory-developed tests using publicly available sequences and protocols. Although the performance characteristics of these various PCR assays are not known, they represent a large potential resource for rapidly increasing testing for detecting emerging pathogens when no FDA-approved test exists. Incorporation of this potential diagnostic capacity in pandemic planning; improving regulatory approaches, such as EUAs; and better engagement between these institutions and public health laboratories may improve overall laboratory diagnostic response capability in the future.

Perhaps the greatest diagnostic challenge remains at the point of clinical care. In the absence of readily available, rapid, and highly sensitive diagnostic tests, clinicians' decisions regarding infection control and clinical management were difficult. RIDTs provided quick results to clinicians, but the tests were demonstrated to have lower sensitivity and were not able to differentiate pH1N1 from other influenza A subtypes. Greater clinician recognition for appropriate interpretation of negative RIDT results and earlier evaluation of the performance of RIDTs for detecting newly emerging and seasonal influenza strains in the future are needed.

The diagnostic response to pH1N1 benefitted greatly from investments in multiple-use platforms. Beginning in 2008, deployment of PCR devices to public health laboratories through

emergency preparedness and pandemic planning not only provided a testing platform for seasonal influenza, but also served as a ready warm base on which pH1N1 surge testing could be rapidly implemented. Early in the pandemic, the ability to detect an unsubtypable strain with use of the available CDC 5-Target PCR assay, combined with coordinated surveillance efforts and strengthened laboratory capability, facilitated the finding of the pH1N1 needle in a haystack of seasonal influenza, emphasizing the essential role of laboratory-based surveillance. As the pandemic progressed and pH1N1 became the predominant subtype, the same platform, using the added pH1N1-specific PCR component, was able to switch gears to find the seasonal influenza hay in a needlestack of pH1N1.

The increase in demand for testing, along with a concomitant increase in supply of molecular testing reagents, revealed 2 intriguing findings during the early weeks after recognition of pH1N1. The first finding is the impact of the "worried ill." The record increase in specimens submitted for the week ending on 2 May 2009 represented a combination of contributing factors including, (1) strong recommendations from the CDC to collect and refer specimens for testing to identify the geographical spread and clinical spectrum and severity of disease, (2) availability of virus-specific testing only through public health laboratories, and (3) considerable media coverage of pH1N1 activity in Mexico and the United States. On the surface, the increase might be expected to represent the "worried well" (ie, submissions from patients and contacts who did not have illness). Despite the 8-fold increase in testing, the percentage of all tests that were performed for influenza actually doubled, indicating that most patients seen in the clinic were probably the worried ill who do not normally visit the doctor. The second finding is the surprising presence of unrecognized influenza. As the total number of tests increased from 25 April through 2 May 2009, detection of both seasonal and pandemic influenza virus strains also increased [Figure 1]. This does not represent an increase in seasonal influenza circulation but rather demonstrates the effect of increased testing of patients with ILIs, indicating that influenza virus strains may be circulating among persons in a community even at times during the year when influenza is thought to be very uncommon or nonexistent and most influenza testing has stopped. These findings suggest that even during periods when influenza is presumed to have waned significantly, a notable amount of influenza virus strains are circulating and causing disease. The findings also suggest that pH1N1 may have been present at very low levels during the weeks before detection but was unrecognized, because much of the illness was not severe and most persons with influenza are not tested.

Early detection and characterization of the pH1N1 virus and the subsequent prevention and control of infections was made possible because of deliberate and coordinated planning among partners in the public health laboratory community. Broadening and sustaining this laboratory base will support routine influenza surveillance and will serve as a rapid response capacity for increased testing. Future planning efforts should focus on ways to improve availability of reliable testing for clinicians at the point of care and approaches for optimum use of molecular testing for detecting both circulating and emerging influenza virus strains at hospital and commercial laboratories.

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