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Short report

# Effect of Target Enriched Multiplex-Polymerase Chain Reaction on patient outcomes and costs during the 2013–14 influenza season

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## SUMMARY

The US Centers for Disease Control and Prevention recommends the initial use of rapid antigen influenza diagnostic test (RIDT) for the detection of influenza A (H1N1-09). Nasopharyngeal samples were tested from 246 patients for H1N1-09 using target-enriched multiplex polymerase chain reaction (TEM-PCR), of which 163 were additionally tested via RIDT. RIDTs had a sensitivity of 18.7% compared with TEM-PCR as the reference standard. Patients with false-negative RIDTs were withheld from 111 days of oseltamivir and 65 days of isolation. Patients negative for H1N1 via TEM-PCR had antiviral therapy immediately stopped, thereby evading 408 days of oseltamivir and 315 days of unnecessary isolation. This cost avoidance saved US\$208,982.

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## Introduction

Since its pandemic in 2009, influenza virus A/H1N1 2009 (H1N1-09) has remained a potent pathogen and was the dominant virus detected during the 2013–14 influenza season, causing 28,322 infections throughout the USA [1]. The US

Centers for Disease Control and Prevention (CDC) recognizes polymerase chain reaction (PCR) and viral culture as the gold standard assays for the diagnosis of H1N1, but recommends the rapid antigen influenza diagnostic test (RIDT) as the first-line diagnostic for patients exhibiting influenza symptoms [2]. Despite widespread use, RIDTs have high false-negative rates with sensitivities ranging from 10% to 80% [1,3]. Most RIDT protocols require high virus concentrations, which may not be readily available during sampling. Moreover, since adults tend to shed less influenza virus than children, low sensitivity in adults may be due to sample selection effects [3]. To examine

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the possible limitations of RIDTs, we assessed the clinical and economic impact of the Target Enriched Multiplex-PCR Respiratory Panel (TEM-PCR™; Diatherix Laboratories, LLC, Huntsville, AL, USA) for the diagnosis of H1N1 and its positive effects on isolation practices, on use of neuraminidase inhibitors, and on reduction of antimicrobial coverage.

## Methods

### Patient sample description

Retrospective chart review revealed that 163 symptomatic patients from Huntsville Hospital (Huntsville, AL, USA) were tested for H1N1 by both RIDT and TEM-PCR as detailed below. After comparing test results of these patients, Huntsville Hospital moved away from the RIDT and adopted the TEM-PCR Respiratory Panel for routine use. An additional 83 patients were tested by TEM-PCR only and results were analysed. All 246 patients were inpatients and exhibited severe flu-like symptoms associated with pneumonia, high fever, and respiratory distress. TEM-PCR nasopharyngeal specimens were collected from December 2013 to February 2014 using a nylon flocked swab and transported to a reference laboratory in a tube filled with 1 mL of modified liquid Amies media which are components of the eSwab™ liquid-based collection and transport system (Copan Diagnostics, Murrieta, CA, USA). TEM-PCR results were reported to hospital physicians one day after sample receipt.

### RIDT

BinaxNow® (Alere, Waltham, MA, USA), was used at the point-of-care following the manufacturer's protocol.

This study was approved by the Huntsville Hospital Institutional Review Committee in December 2013.

### Target-enriched multiplex PCR

The TEM-PCR Respiratory Panel contains 35 genetic targets for the simultaneous detection of 27 respiratory pathogens with high diagnostic sensitivity and specificity, including two targets encoding haemagglutinin 1 (H109C) and neuraminidase 1 (N109B) indicative of H1N1-09. Further details on TEM-PCR are available from previous literature [4].

### Calculations of financial savings and clinical impact of TEM-PCR

The CDC recommends that droplet precautions should be implemented for patients with suspected or confirmed influenza for seven days after illness onset or until 24 h after the resolution of fever and respiratory symptoms, whichever is longer, while a patient is in a healthcare facility [2]. In addition, the CDC guidelines state that patients with complicated influenza receive at least five days of antiviral oseltamivir treatment [2]. At Huntsville Hospital, the H1N1 protocol included an average of 14 days of isolation, 10 days of oseltamivir treatment, and  $\pm 8$  days of antibacterial medication [1 g vancomycin/12 h and 4.5 g Zosyn® (piperacillin and tazobactam)/8 h] depending on patient presentation. Patients' charts were reviewed for the date of admission, the date RIDT

was performed, dates of oseltamivir doses, dates and frequency of antimicrobial therapy, isolation days, and the date TEM-PCR results were received. Using these dates, calculations in [Supplementary Table I](#) measured the clinical impact of TEM-PCR in number of days of isolation and medication use.

H1N1 isolation room costs per day from Zarogoulidis *et al.* were applied to this study, euros (€) were converted to US dollars (\$), and 2010 costs were adjusted to 2014 to account for inflation [5]. Cost per day of isolation supplies from Hubben *et al.* were modified from 2007 to reflect inflated costs in 2014 [6]. Inflation calculations were done on the Consumer Price Index (CPI) calculator on the Bureau of Labor Statistics web site. Costs of oseltamivir and RIDTs were taken from pharmacy and billing data at Huntsville Hospital. TEM-PCR cost was taken from billing data at Diatherix.

## Results

Of the 163 patients tested by both RIDT and TEM-PCR, 43 (26.4%) patients tested positive via TEM-PCR and negative via RIDT ([Supplementary Table II](#)). When evaluated with TEM-PCR as the gold standard, the sensitivity of the RIDT was 18.7%. The negative predictive value (NPV) was 71.3% whereas the positive predictive value (PPV) was 76.9%. There was substantial disagreement between RIDT and TEM-PCR results (Cohen's kappa of 0.201,  $P < 0.0001$ , McNemar's test with continuity correction).

Physicians isolated 13 (30.2%) of the 43 patients that initially received false-negative RIDT results, but delayed a total of 83 days of oseltamivir [mean:  $6.4 \text{ days} \pm 5.3$  (standard deviation) per patient] until TEM-PCR results confirmed the presence of H1N1 ([Figure 1A](#)). For five (11.6%) of the 43 patients that received empiric therapy the day of admission, a total of 14 isolation days (mean:  $2.8 \pm 0.8$  days per patient) were withheld until confirmation of H1N1 by TEM-PCR ([Figure 1B](#)). Ten (23.3%) of the 43 patients had both oseltamivir and isolation deferred by 28 days (mean:  $2.8 \pm 1.9$  days per patient) and 51 days (mean:  $5.1 \pm 5.4$  days per patient), respectively ([Figures 1A,B](#)). A total of 111 days of oseltamivir were delayed in 23 patients (mean:  $4.8 \pm 4.5$  days per patient) and 65 days of isolation were withheld in 15 patients (mean:  $4.3 \pm 4.5$  days per patient) who had a false-negative RIDT ([Figures 1A,B](#)). Additionally, after TEM-PCR had verified that H1N1 was the only infection present, antibiotics were stopped in six patients, saving a total of 15 days (mean:  $2.5 \pm 1.6$  days per patient) of antibiotic usage.

Of the 246 patients, 71 (28.9%) were treated as if they had H1N1 upon the date of admission, but were later found to be H1N1 negative via TEM-PCR. Forty-one (57.5%) of the 71 patients presented flu-like symptoms and were started on a 10 day treatment of oseltamivir, but isolation was delayed. After TEM-PCR tests were negative for H1N1, antivirals were stopped and 281 days (mean:  $6.9 \pm 1.7$  days per patient) of oseltamivir therapy were saved ([Figure 1C](#)). TEM-PCR-negative patients experienced an average of 3.2 days of antiviral therapy instead of the suggested 10 days. Of the 71 patients, 11 (15.5%) patients were expected to be isolated for 14 days because of the possibility of H1N1 infection, but oseltamivir medication was withheld until secondary results were received. In all, 115 days of isolation (mean:  $10.5 \pm 2.5$  days per patient) were saved as patients only averaged 3.6 days of excessive isolation instead of the scheduled 14 after TEM-PCR had determined that H1N1

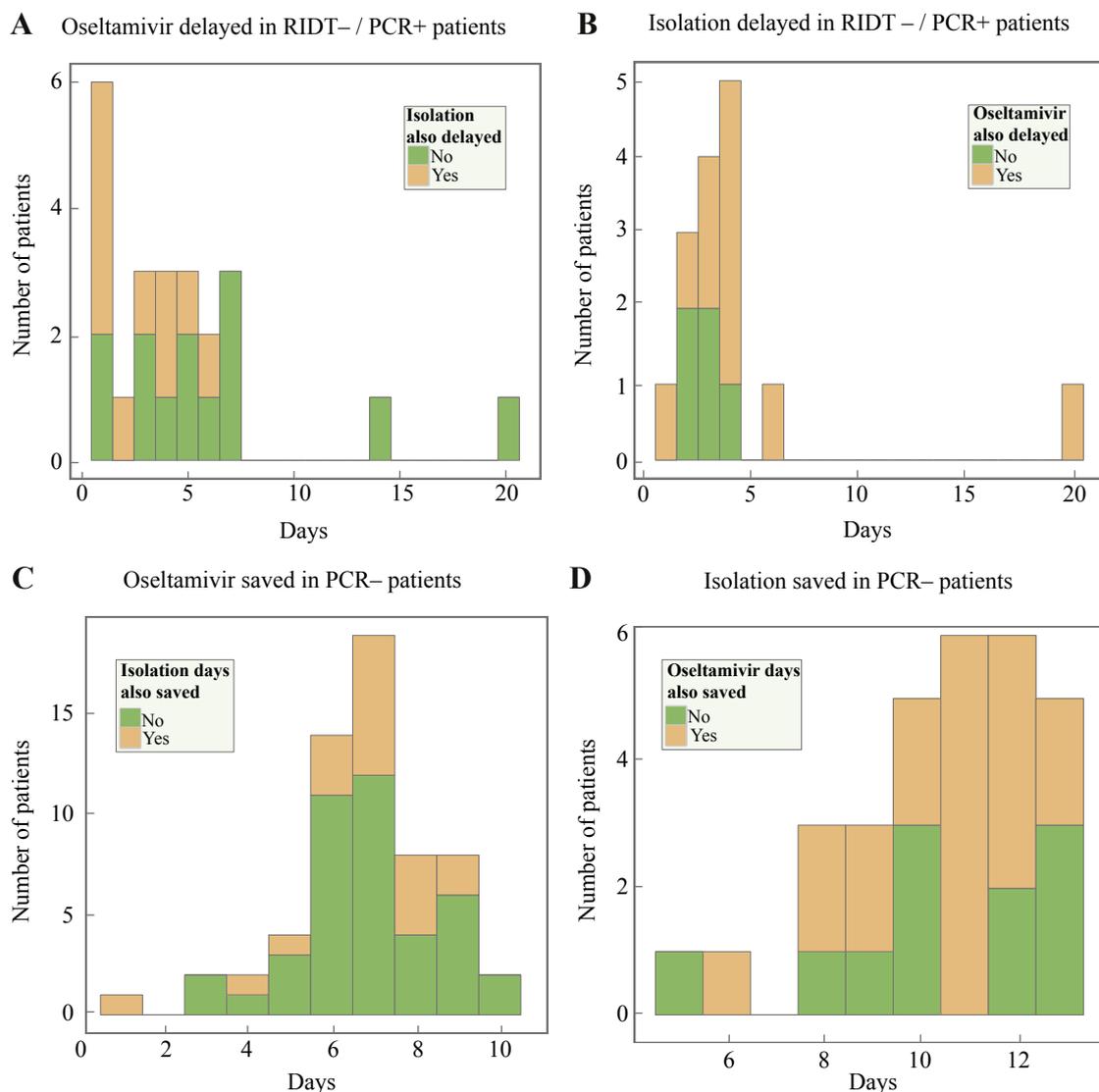
was not present (Figure 1D). Of the 71 patients, 19 (26.8%) were both isolated and provided with empiric therapy upon admission. However, after TEM-PCR results were negative for H1N1, 200 days (mean:  $10.5 \pm 1.8$  days per patient) were saved as patients were removed from isolation after an average 3.5 days and 127 days of oseltamivir were avoided (mean:  $6.7 \pm 1.9$  days per patient; Figures 1C,D orange bars). In all, 408 days of oseltamivir were recalled in 60 patients (mean:  $6.8 \pm 1.7$  days per patient) and 315 days of isolation were suspended in 30 patients (mean:  $10.5 \pm 2.1$  days per patient; Figure 1C,D) which significantly impacted financial savings at Huntsville Hospital.

The 246 patients in this study were severely ill on the date of admission and physicians took precautions by treating 71 patients as if they had H1N1 even if RIDTs were negative. If these 71 patients had received the recommended hospital protocol

therapy of 14 days of isolation and 10 days of oseltamivir, medical costs would have totalled \$393,122. Because TEM-PCR confirmed the absence of H1N1 and therapy was altered, hospital costs totalled only \$184,278, saving \$208,982 during the three-month span of this study. A full cost analysis is shown in Table I.

## Discussion

H1N1 is more aggressive than other influenza subtypes as patients have a higher risk of viral pneumonia and respiratory failure, resulting in a high rate of morbidity and mortality if not rapidly treated. The CDC recommends that decisions about starting antiviral treatment should not be postponed for laboratory confirmation of influenza [2]. However, this



**Figure 1.** Clinical downstream effects on patients. (A) Distribution of oseltamivir delays for patients with false-negative rapid antigen influenza diagnostic test (RIDT) results. Time delays are binned to the nearest integral number of days. In each bin, patients also delayed for isolation are indicated in orange. (B) Total number of patients who had isolation delayed. (C) Number of oseltamivir treatments saved for patients with negative target-enriched multiplex polymerase chain reaction (TEM-PCR) results. Orange bars indicate patients who also had days of isolation saved. (D) Total number of patients who had isolation days saved.

**Table 1**  
Detailed cost savings analysis

Variable	H1N1 suspected <sup>a</sup>	H1N1 (–) by TEM-PCR <sup>b</sup>	Cost difference <sup>c</sup>
Isolation room cost per day <sup>d</sup>	\$835.08	\$835.08	\$835.08
Isolation supplies cost per day <sup>e</sup>	\$55.64	\$55.64	\$55.64
Total days of isolation	420	105	315
Total cost of isolation	\$374,102.40	\$93,524.55	\$280,573.65
Osetamivir cost per day <sup>f</sup>	\$23.50	\$23.50	\$23.50
Total days of osetamivir	600	198	408
Total cost of osetamivir therapy	\$14,100	\$4,653	\$9,588
Assay choice	RIDT	TEM-PCR	Cost difference
Assay cost	\$20 <sup>f</sup>	\$350 <sup>g</sup>	\$330
No. of patients tested	246	246	246
Total cost of testing	\$4,920	\$86,100	–\$81,180
Total cost	\$393,122.40	\$184,277.55	\$208,981.65

TEM-PCR, target-enriched multiplex polymerase chain reaction; RIDT, rapid antigen influenza diagnostic test.

<sup>a</sup> Column describes projected cost of severely symptomatic patients suspected to be H1N1 positive that would have received 14 days of isolation and 10 days of osetamivir if RIDT results were not verified via TEM-PCR.

<sup>b</sup> Column describes the number of days and costs associated with H1N1-negative patients that were initially empirically treated, but had treatment stopped after TEM-PCR results.

<sup>c</sup> Number of days and cost savings from stopping unnecessary antiviral treatment and isolation in H1N1 negative patients.

<sup>d</sup> H1N1 isolation costs per day described in Zarogoulidis et al. converted from € to US\$ and adjusted for inflation to reflect 2014 costs.

<sup>e</sup> Isolation supplies cost per day described in Hubben *et al.* adjusted for inflation to reflect 2014 costs.

<sup>f</sup> Cost data from pharmacy and billing department in Huntsville Hospital.

<sup>g</sup> Cost data from billing department at Diatherix Laboratories LLC.

retrospective study found that some physicians were unaware of the high inaccuracy of RIDTs for the diagnosis of H1N1, and did not confirm negative RIDTs with viral culture or PCR. After the RIDT was negative, these physicians ruled out the likelihood of influenza and did not start the patient on proper treatment. Withholding therapy for patients with H1N1 is problematic as osetamivir must be administered as early as possible for maximum effectiveness [7]. Also, H1N1-positive patients who are misdiagnosed and not isolated may transmit the virus to family members, co-workers, and healthcare staff. Even though the low sensitivity of RIDTs has been published in many previous studies, our data suggest that some physicians are still unaware of these statistics, which negatively impacts patient outcomes.

Treatment guidelines state that if a physician suspects influenza, even after a negative RIDT result, empirical treatment should continue until subsequent testing verifies the initial diagnostic result [2]. For patients with severe respiratory symptoms in whom H1N1 is suspected, recommendations include 10 days of osetamivir,  $\pm 8$  days of antibiotic therapy, and  $\sim 14$  days of patient isolation. Consequently, a majority of severely ill patients were isolated and prescribed osetamivir and antibiotics as precautionary measures. However, empirically treating patients with influenza-like symptoms increases the likelihood of inappropriate medication use, leading to a greater risk of toxicity, antibacterial resistance, and antiviral resistance [8]. During H1N1 outbreaks, antiviral resistance levels may rise, decreasing the efficiency of osetamivir [8]. In addition, patients placed in isolation typically receive 40–50% less contact time with physicians and nurses, as these rooms are often avoided to minimize the risk of viral transmission [9]. Consequently, these patients experience suboptimal care.

A physician can make a prompt and accurate diagnosis of H1N1 in patients exhibiting influenza-like symptoms as TEM-PCR detects the genetic sequences encoding both

haemagglutinin 1 and neuraminidase 1. Typical commercial RIDTs can only detect influenza A and/or B. Most commercially available reverse transcription PCR (RT-PCR) assays test for the presence of H1, but not N1. Thus, a physician may only deduce that the viral subtype is H1Nx ( $x = 1–11$ ) where the type of neuraminidase is unknown. TEM-PCR results are qualitative and H1N1 is only considered a positive if both H1 and N1 targets are detected. The detection of both targets reduces the number of false-positive and false-negative results, and should result in the implementation of more evidence-based and goal-oriented therapies.

Additionally, patients often exhibit flu-like symptoms that may be caused by other pathogens, complicating the correct diagnosis of H1N1. Of the patients in this study, 45.3% were co-detected with at least one bacterial pathogen, 9.3% with at least one additional virus, and 6.6% with both. Of the H1N1-negative patients, 56.1% were detected with at least one bacterial pathogen, 25.1% were detected with at least one virus other than H1N1, and 14.0% were detected with both. The bacterium with the highest detection rate in patients positive or negative for H1N1 was methicillin-resistant *Staphylococcus aureus* (MRSA). Previous literature has shown that H1N1 may prime the respiratory tract for subsequent bacterial infection or co-infection, especially with MRSA, which could result in necrotizing tracheobronchitis, and increased morbidity and mortality [10]. Using multiplex PCR diagnostics might increase the probability of avoiding complicated respiratory co-infections.

This study suggests that TEM-PCR and other nucleic acid assays should be made readily available to improve the diagnosis and treatment of H1N1 during flu seasons. The data add to the numerous publications reporting the inaccuracy of RIDTs for H1N1 detection and indicate that the use of multiplex PCR with a 24 h turnaround time optimized patient care for a hospital system including clinical cost savings of \$208,982 during the 2013–14 influenza season.

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### Conflict of interest statement

A.H. uses TEM-PCR testing assays at Huntsville Hospital. M.D.H. and S.L. are employees of Diatherix.

### Funding source

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jhin.2017.04.010>.

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