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Letters to the Editor

Comparison of GeneXpert FluA PCR to Direct Fluorescent Antibody and Respiratory Viral Panel PCR Assays for Detection of 2009 Novel H1N1 Influenza Virus▽

The emergence of 2009 novel H1N1 influenza virus has made accurate detection and subtyping of influenza viruses a priority for many clinical laboratories. The Cepheid GeneXpert instrument is a cartridge-based PCR system for performing nucleic acid extraction, PCR amplification, and real-time detection automatically without intermediate sample-handling steps (2). We performed a study, approved by the UCSF Committee on Human Research, to determine its ability to detect 2009 novel H1N1 influenza virus by using previously tested frozen patient samples.

We analyzed 105 upper respiratory specimens (nasal aspirates/washes or nasopharyngeal flocked swabs in universal transport media). Twenty-six were positive for influenza A virus by use of direct fluorescent antibody (DFA; Millipore/Light Diagnostics, Temecula, CA). Thirteen specimens were DFA negative or indeterminate but positive by respiratory viral panel PCR (RVP; Luminex Corporation, Austin, TX) for the influenza A virus matrix gene and negative for seasonal H1 and H3 genes (presumed 2009 novel H1N1 influenza virus). Forty-nine samples were DFA negative and PCR untested, and 17 samples were DFA and RVP negative. The RVP test has superior sensitivity over the DFA method for detection of 2009 novel H1N1 influenza virus (1).

Excess patient specimens stored at −70°C were thawed and applied to the test cartridge as specified by the manufacturer. Samples with invalid or discordant results were retested with the GeneXpert assay and by the CDC real-time reverse transcription-PCR (RT-PCR) assay which detects the influenza A virus matrix gene, swine influenza A virus matrix gene, and 2009 novel influenza H1 hemagglutinin gene, in addition to a test of specimen integrity by PCR for the RNase P gene (3).

Our GeneXpert results are shown for each sample category in Table 1. Results were classified as true positive, true negative, false positive, or false negative, using the CDC RT-PCR assay as the gold standard for discrepant results. All samples having a detectable 2009 novel H1 gene were also positive for the matrix gene by GeneXpert. All samples with any positive result on the GeneXpert assay were confirmed as positive for 2009 H1N1 influenza virus by the CDC RT-PCR assay. Samples negative by the CDC RT-PCR assay could represent degradation of viral RNA during storage or prior false-positive RVP test and were considered to be true negatives for our analysis.

With the CDC RT-PCR assay as the gold standard, the GeneXpert assay detected the influenza A virus matrix gene in 40 of 44 positives (91%). All of the samples with virus undetected by GeneXpert had matrix gene cycle threshold (C_T) values of >29 cycles with the CDC RT-PCR assay, indicating relatively low viral titers. The GeneXpert assay detected a 2009 novel H1 gene in 31 of 44 positives (70%). Seven of the 13 samples with virus undetected by GeneXpert had hemagglutinin C_T values of >29 cycles with the CDC RT-PCR assay. The GeneXpert test did not detect virus in any specimens that were not confirmed as positive by the CDC RT-PCR assay.

In summary, the GeneXpert FluA panel assay was able to detect influenza virus in all DFA-positive samples and some DFA-negative samples but showed limited ability to detect 2009 novel H1N1 influenza virus in DFA-negative, RVP-pos-

TABLE 1. Comparison of GeneXpert results to prior DFA and PCR test results

GeneXpert result[b]	No. of samples with indicated result[a]				
	DFA positive	RVP positive, DFA negative	DFA negative, PCR untested	DFA negative, RVP negative	Total
Influenza A virus matrix gene detection					
TP	26	8	6	0	40
TN	0	1	43	17	61
FP	0	0	0	0	0
FN	0	4	0	0	4
2009 H1N1 virus hemagglutinin gene detection					
TP	25	5	1	0	31
TN	0	1	43	17	61
FP	0	0	0	0	0
FN	1	7	5	0	13

[a] DFA, direct fluorescent antibody; RVP, respiratory viral panel PCR.
[b] The gold standard test was the CDC RT-PCR assay. TP, true positive; TN, true negative; FP, false positive; FN, false negative.

itive samples. The ease of use and rapid time to results for the GeneXpert assay may make this a useful test to incorporate into diagnostic and infection control algorithms at some institutions.

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