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Clinical application of a molecular method based on Real Time RT-PCR for detection of influenza A(H1N1)v virus

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SUMMARY

Given the new diagnostic need following the pandemic caused by the A(H1N1)v virus, we evaluated the performance characteristics of *Xpert® Flu* assay (Cepheid). The overall sensitivity and specificity were 65.6% and 92.8%, respectively. Sensitivity and specificity for A(H1N1)v virus were 85.7% and 94.9%, respectively, and therefore the *Xpert® Flu* assay is suitable for a rapid diagnosis in critically ill patients where diagnosis is crucial for clinical management and for an appropriate public health response.

KEY WORDS: Influenza A(H1N1)v virus, Real-time RT-PCR, Xpert Flu assay.

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Influenza remains a serious health problem in many countries given the epidemic worldwide spread of this disease (Fiore *et al.,* 2008). The basic mechanism underlying the influenza epidemics is correlated with the high rate and quick appearance of mutations within the virus antigenic structure (Gabutti *et al.,* 2004), as clearly illustrated by the recent emergence of A(H1N1)v virus (Babakir-Mina *et al.,* 2009; Conde, 2009; Deem and Pan, 2009). The introduction of influenza A(H1N1)v virus has challenged clinical laboratories to define the most appropriate diagnostic tools for the laboratory diagnosis of this new influenza strain (Welch and Ginocchio, 2010). Currently used molecular methods often proved inadequate to deliver a prompt response to the clinician, and rapid antigen tests are limited by inferior sensitivity (Ginocchio *et al.,* 2009).

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The present study included 60 hospitalized patients with a clinical suspicion of having A(H1N1)v virus infection. From these subjects a nasopharyngeal swab (UTM-RT kit, Copan, Italy) was collected between December 2010 and March 2011 and evaluated for the presence of influenza virus by the Regional Reference Centre for Microbiological Emergencies (CRREM) in Bologna.

Upon arrival of the sample in the laboratory, two different Real-Time RT-PCR (rRT-PCR) were used for each individual swab: a "home brewed" assay based on the method established by the Centers for Disease Control and prevention (CDC) (WHO, 2009) and the commercial kit *Xpert® Flu* (Cepheid, France).

The CDC test was chosen as the reference method given its proven performance (WHO, 2009). This test includes two separated stages: extraction and amplification. 200 µl of the transport medium were extracted using the automated system NucliSENS® easyMAG® (BioMerieux, France), performed according to the manufacturer's instructions.

The amplification step was performed on a StepOne™ Plus (Applied BioSystems, USA) instrument and four different targets are concomi-

tantly detected through the use of a panel of oligonucleotide primers and probes: Influenza A [InfA], swine influenza A [SwInfA], swine H1 [SwH1N1], RNAseP. Particularly, InfA primers and probe set is designed for universal detection of the matrix (M) gene of all influenza A viruses; the SwInfA primers and probe set is designed for universal detection of the NP gene of all swine influenza A viruses; the SwH1N1 primers and probe set is designed to detect the HA gene (H1 subtype) of swine influenza; the RNAseP primers and probe set targets the human RNase P gene and thus serves as an internal positive control for human nucleic acid (Shu *et al.,* 2011).

The *Xpert® Flu* assay (Cepheid, France), on the other hand, is a rapid, random access molecular test capable of detecting and differentiating influenza A, influenza A(H1N1)v and influenza B viruses. The *Xpert® Flu* assay allows extraction, amplification and detection to take place with a single-use disposable cartridge. The assay was performed according to the manufacturer's instructions.

Among the 60 patients evaluated, 47 (78.3%) gave a concordant result when tested with both the methods used. In detail: 3/47 (6.4%) were positive for influenza A, 18/47 (38.3%) were positive both for influenza A and (H1N1)v viruses (and this means that the specimens were positive for the new variant of the virus) and 26/47 (55.3%) resulted negative (Table 1). The methods gave different results for 13 samples: 2 samples were positive both for influenza A and (H1N1)v viruses and 8 samples were positive only for influenza A by using the CDC test and testing negative for *Xpert® Flu* kit; 2 samples were positive for influenza A and A(H1N1)v virus with *Xpert® Flu* kit and negative for the CDC-derived RT-PCR; 1 sample resulted invalid with *Xpert® Flu* kit (the method gave a positive result for A(H1N1)v virus and a negative for influenza A), but was positive with "home brewed" RT-PCR (Table 1). Furthermore, among the 21 concordant positive samples for both influenza A and for (H1N1), one also resulted positive for influenza B by *Xpert® Flu* kit.

According to the data obtained, the new kit tested has a overall specificity and sensitivity of 92.8% and 65.6%, respectively, while the sensitivity and specificity for A(H1N)v virus were 85.7% and 94.9%, respectively.

Although the "home brewed" RT-PCR is more sensitive than the *Xpert® Flu* assay, this first method requires a separate step for the extraction of the RNA and subsequently samples batch processing. This last feature of the test is time-consuming and so this method is poorly applicable in emergency settings, such as all situations in which a rapid response is necessary for the management of the critically ill patient. The *Xpert® Flu* assay allows the rapid identification not only of influenza A and A(H1N1)v viruses but also of influenza B virus and this feature is particularly useful for diagnostic purposes since this assay is able to deliver a full set of epidemiological information within a single run.

Our overall sensitivity results conflict with previous studies comparing the performance of the *Xpert® Flu* assay with other molecular methods

TABLE 1 - *Comparison between the CDC Real Time RT-PCR and the commercially Xpert® Flu assay on nasopharyngeal swabs obtained from hospitalized patients with a clinical suspicion of A(H1N1)v virus infection.*

		Xpert® Flu assay		
		Positive influenza A samples	*Positive influenza A and (H1N1)v samples*	*Negative samples*
CDC Real Time RT-PCR	Positive influenza A samples	3	0	8
	Positive influenza A and (H1N1)v samples	0	18	3*
	Negative samples	0	2	26

1 sample resulted invalid with Xpert® Flu kit (the method gave a positive result for A(H1N1)v virus and a negative for influenza A), but was positive with "home brewed" RT-PCR.

and analyzing a larger number of samples. In fact, the overall sensitivity observed in the comparison between the *Xpert® Flu* assay and the other methods, is more than 90% (Sambol *et al.,* 2010; Popowitch *et al.,* 2011; Novak-Weekley *et al.,* 2012; Salez *et al.,* 2012). However, the sensitivity (85.7%) and specificity (94.9%) for A(H1N1)v virus were similar to other studies (Sambol *et al.,* 2010; Popowitch *et al.,* 2011; Novak-Weekley *et al.,* 2012; Salez *et al.,* 2012).
Although the detection of InfA virus is more expensive when performed by the *Xpert® Flu* assay than by standard PCRs methods, in the light of the findings of this study, the *Xpert® Flu* assay could be used as a screening test for influenza virus infections during the epidemic season in all clinical settings requiring a fast definition of the etiology.
The *Xpert® Flu* assay has a short run time and is simple to perform, allowing considerable time-saving for laboratory personnel who could be involved in additional activities during the run. The assay offers the additional possibility to perform "on demand" testing. The simplicity of the workflow required for this test also allows laboratory personnel not proficient in molecular techniques to perform the test: this is particularly important when the test has to be performed in fast-response laboratories where only a few technicians are involved in many different diagnostic activities. The self-standing equipment required for the *Xpert® Flu* assay is all that is needed in laboratories not equipped with real-time PCR technology.
Therefore this can allow rapid results useful both for rapid treatment and patient management, thus allowing an efficient containment of the spread of the virus in the ICU setting. However, in order to achieve a definitive virological diagnosis, the samples negative with the *Xpert® Flu* assay should be reassessed with more sensitive assays if no alternative diagnosis is made and if influenza diagnosis turns out to be crucial for appropriate clinical decision-making.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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