Sensitive and reliable detection of Influenza A (H1N1), Influenza A (H3N2) and Influenza B by commercial real-time PCR assays: RIDA®GENE Flu and RIDA®GENE Flu LC2.0 real-time PCR

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Objectives

Worldwide, 3-5 million people are infected with influenza and approximately 250,000-500,000 die from this disease each year.[1] Influenza viruses are divided into the subtypes A, B and C.
Epidemiologically, influenza A viruses play the major role due to diversity: they are responsible for three pandemics in the 20th Century and for the majority of influenza epidemics. The majority of influenza A infections in humans are caused by the H1N1 and H3N2 subtypes. Characteristic for influenza viruses is their high mutational variation (antigenic drift) of the surface antigens, hemagglutinin (HA) and neuraminidase (NA), hence presenting an economic burden and a major annual threat to public health. This was demonstrated in the last Influenza season 2014/2015 with a new Influenza A H3N2 variant emerging, rendering last season's influenza vaccination partially inefficient.

Rapid sensitive and specific diagnosis of influenza is crucial for patient management and appropriate treatment measures, therefore real-time PCR is an essential tool for the reliable detection of influenza viruses.

Picture 1: RIDA®GENE Flu, Art. No. PG0505

Methods

The RIDA®GENE Flu and RIDA®GENE Flu LC2.0 real-time RT-PCR assays simultaneously detect and differentiate Influenza A and Influenza B. In addition, the RIDA®GENE Flu assay also differentiates Influenza A (H1N1v). In a retrospective study, a total of 141 positive and negative clinical respiratory samples (nose/throat swabs) were extracted using the MagNAPure 96 (Roche). Extracted nucleic acids were analysed with the RIDA®GENE Flu assay on the LightCycler® 480II (Roche).
Results were compared to a standard reference in-house real-time PCR assay. The analytical reactivity and analytical specificity of the RIDA®GENE Flu and RIDA®GENE Flu LC2.0 assays was tested using known quality control standards and reference materials. The RIDA®GENE Flu assay was validated on six real-time PCR instruments.

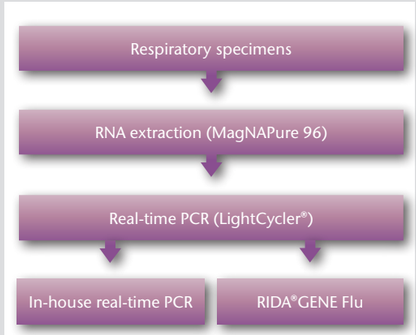

Fig. 1: Workflow clinical study design RIDA®GENE Flu

[1] *World Health Organisation 2009*, Fact Sheet N°211, Influenza (Saisonal) www.who.int/mediacentre/factsheets/fs211/en/index.html. Accessed: 30.05.2012.

Results

Overall, 141 clinical samples were tested, of which 120 were throat swabs and 21 nose swabs. All negative samples were correctly identified by the RIDA®GENE Flu assay (Figure 2, 3, 4). 32 Influenza A positive samples were tested of which two were Influenza A H7N9, two H3N2-positive and 16 H1N1-positive as well as one H5N1-positive (Figure 2, 3). Overall, results of 31 positive Influenza A tested samples concurred with the reference method, indicating a sensitivity and specificity of 96.9 % and 100 %, respectively for Influenza A, and 94.1 % and 100 % for Influenza A (H1N1v) (Figure 2, 3). For Influenza B, 24 out 25 positive samples were detected with the RIDA®GENE Flu assay. This refers to a sensitivity and specificity of 96 % and 100 %, respectively (Figure 4).

The analytical reactivity of the RIDA®GENE Flu and RIDA®GENE Flu LC2.0 assays was tested on currently circulating Influenza A and Influenza B strains, including the new variants of Influenza A (H3N2) from the 2014/2015 season. No cross-reactivity to non-Influenza strains was detected with either assay (Table 1).

An analytical sensitivity of 50 copies/reaction was achieved with the LightCycler® 480II, LightCycler® LC2.0 (RIDA®GENE Flu LC2.0 only), Mx3005P, Rotor-Gene Q, ABI7500, CFX96 and SmartCycler® II real-time PCR instruments (Table 2).

		In-house real-time PCR Influenza A		
		+	-	Total
RIDA®GENE Flu Influenza A	+	31	0	31
	-	1	109	110
	Total	32	109	141

Sensitivity: 96.9 %
Specificity: 100 %

Fig. 2: Clinical performance of the RIDA®GENE Flu real-time RT-PCR assay for influenza A

		In-house real-time PCR H1N1v		
		+	-	Total
RIDA®GENE Flu H1N1v	+	16	0	16
	-	1	99	100
	Total	17	99	116

Sensitivity: 94.1 %
Specificity: 100 %

Fig. 3: Clinical performance of the RIDA®GENE Flu real-time RT-PCR assay for influenza A (H1N1v)

		In-house real-time PCR Influenza B		
		+	-	Total
RIDA®GENE Flu Influenza B	+	24	0	24
	-	1	116	117
	Total	25	116	141

Sensitivity: 96 %
Specificity: 100 %

Fig. 4: Clinical performance of the RIDA®GENE Flu real-time RT-PCR assay for influenza B

Table 1: Analytical reactivity RIDA®GENE Flu and RIDA®GENE Flu LC2.0

Subtype	Strain	Influenza A	H1N1v	Influenza B
H1N1	Influenza A/New Caledonia/20/99	positive	negative	negative
H1N1	Influenza A/Brisbane/59/2007	positive	negative	negative
H1N1v	Influenza A/Bayern/63/2009	positive	positive	negative
H1N1v	Influenza A/California/7/2009	positive	positive	negative
H5N1	Influenza A/Whooper swan/Germany/R65-2/2006	positive	negative	negative
H5N1	Influenza A/Chicken/Germany/R3294/2007	positive	negative	negative
H3N2	Influenza A/Perth/16/2009	positive	negative	negative
H3N2	Influenza A/Victoria/361/2011	positive	negative	negative
H3N2	Influenza A/Bayern/1/2015 (A/H3N2)	positive	negative	negative
H3N2	Influenza A/Sachsen/2/2015 (A/H3N2)	positive	negative	negative
H3N2	Influenza A/Nordrhein-Westfalen/1/2015 (A/H3N2)	positive	negative	negative
H7N9	Influenza A/Anhui/1/2013	positive	negative	negative
	Influenza B/Brisbane/60/2008	negative	negative	positive
	Influenza B/Wisconsin/1/2010	negative	negative	positive
	Influenza B/Massachsetts/2/2012	negative	negative	positive

Table 2: Validated real-time PCR instruments for RIDA®GENE Flu and RIDA®GENE Flu LC2.0

Real-time PCR cycler*	Company
LightCycler® 480/2.0	Roche
Mx3005P	Agilent Technologies
SmartCycler® II	Cepheid
Rotorgene® Q	Quiagen
CFX 96™	Bio-Rad
ABI®7500/FAST	Life Technologies

* LightCycler® is a registered trademark of Roche.
SmartCycler® is a registered trademark of Cepheid.
Rotor-Gene® Q is a registered trademark of Qiagen Group.
CFX 96 Touch TM is a registered trademark of Bio-Rad.
Applied Biosystems® is a registered trademark of Life Technologies.

Conclusion

RIDA®GENE Flu and RIDA®GENE Flu LC2.0 are sensitive and specific real-time RT-PCR assays for the detection and differentiation of Influenza A and Influenza B including all currently circulating strains and avian strains. The RIDA®GENE Flu assay also reliably differentiates Influenza A and Influenza A (H1N1v) strains and can be run on the commonly used real-time PCR instruments including the LightCycler® 480II, Mx3005P, Rotor-Gene® Q, ABI7500, CFX96 and SmartCycler® II. Both assays contain an internal extraction control that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient.

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