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FUJIFILM

Determine the presence of virus influenza A & B in samples from patients with suspected influenza using the FDC IMMUNO AG1 as a diagnostic tool.

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1/ Retrospective/Prospective study : Clinical Samples

All the samples used for the study came from the surveillance in the general population. Patients presenting with ILI were sampled. The nasopharyngeal swabs were collected in a transport medium (Sigma-virocult[®]) and sent by post to the NIC. Upon arrival, samples are tested for influenza A&B par PCR (Duplex A&B) and seeded on MDCK for isolation of the viruses. The remaining volume is stored frozen at -20°C then -80°C for long storage.

The samples from 2014/15 winter surveillance were stored frozen at -80°C. The selected samples from the current season were stored at +4°C overnight or stored at -20°C for a few days before testing. Altogether, for this part of the study 41 samples have been tested on Fujifilm device.

RT-PCR

The samples are extracted on the easyMAG[®] according to the bioMérieux procedure. Then samples are analyzed for Influenza by RT-PCR on a daily basis within the NIC protocol. For Influenza A, the “in house” technique targets the M gene and for Influenza B the RT-PCR targets the NS gene in accordance to the CDC protocol.

Cell Culture

The viral growth is confirmed by ELISA after 3-5 days of cell culture.

FUJIFILM Test

An aliquot of 200µl per sample was used for the testing.

RESULTS

Practicability :

The device is very easy to use. The presentation of the cartridge allows a secure handling and the scan of the labelling printed on the result ticket presents a real added value. We perform all samples with a similar volume : 200µl instead of 4 drops described in the notice. The only comment, in our laboratory conditions, concerns dust on the lens and the necessity to test the controls each day.

The **negative samples** were selected from the 2014/15 and the 2015/16 season. We also included **positive specimens for other viruses** (Rhinoviruses, RSV, Metapneumoviruses & Parainfluenza viruses) selected on the current season. All the 21 samples were **negative** on the Fujifilm device. The result for the transport medium was also negative.

No false positive were observed either after long storage at -80°C or on « fresh » testing conditions.

The **influenza positives samples** have been selected among the circulating viruses at the beginning of the winter. The samples were stored at +4°C overnight or at -20°C for a few days. We tested 20 influenza samples : 8 influenza A and 12 influenza B.

The results are available in the enclosed table. The preliminary results gave an indication of the detection threshold according to the RT-PCR Ct value. The detection limit depends on the type of the virus. So it was decided (4/02/2016) to check again some samples. The initial results, positive or negative, have always been confirmed. Enclosed table. **The threshold value is Ct=20 -22 for influenza B and Ct=26 for influenza A.**

During the meeting on the 4/02 it was decided to continue the evaluation of the device on the samples of the current season. The dominant virus this winter belongs the B-Victoria lineage but some influenza A (H1N1)pdm09 are also circulating. So we will test the samples presenting a RT-PCR Ct value close to the threshold observed in the preliminary testing. The proposal is to test 50 samples for each virus.

Regarding the A H3N2 and the B-Yamagata lineage if the viruses are not available during the surveillance we will use frozen samples from last winter surveillance to test 50 specimens for each virus. The general purpose of this second part of the study is to test 200 influenza virus positive specimens.

2/ Dilution studies to detect sensitivity level using titrated prototype strains produced on MDCK

The selected prototypes are the strains selected for the previous evaluation of 22 TROD available in France. (Feuillets de biologie N°324- Mai 2015). The strains were kindly provided by WHOcc London and grown on MDCK cells.

- A(H1N1)pdm09 : A/California/7/2009
- A H3N2 : A/Texas/50/2012
- B-Victoria lineage : B/Brisbane/60/2008
- B-Yamagata lineage : B/Massachusetts/20/2012

All viruses were adjusted to 10^8 TCID₅₀/ml. Dilutions were prepared in EMEM and tested twice.

The results are presented in the enclosed documents.

For both **A viruses** the sensitivity level is **10^4 TCID₅₀** identical to Quidel/Sofia. About the B viruses similar results are observed for the **Yamagata lineage : 10^5 TCID₅₀** but the sensitivity is slightly lower for the **Victoria lineage : 10^6 TCID₅₀**.

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Retrospective/Prospective Study

Selection of negative samples for Influenza.

Specimens from the last and the current winter surveillance.

Winter	N°	Diagnostic	Fujifilm	Storage	
2014/15	2015/1658	negative	negative	-80°C	
	2015/1661	negative	negative		
	2015/1665	negative	negative		
	2015/1751	negative	negative		
	2015/1756	negative	negative		
	2015/1757	negative	negative		
	2015/1758	negative	negative		
	2015/1759	negative	negative		
	2015/1760	negative	negative		
	2015/1964	negative	negative		
Winter 2015/16	TM*	negative	negative	-20°C	
	2015/2597	Rhinovirus	negative		
	2015/2662	Rhinovirus	negative		
	2015/2666	RSV	negative		+4°C
	2015/2668	Rhinovirus	negative		
	2015/2669	Metapneumovirus	negative		
	2015/2659	Parainfluenza	negative		
	2015/2681	Parainfluenza	negative		
	2016/401	negative	negative		
	2016/403	negative	negative		+4°C
2016/406	negative	negative			
2016/415	negative	negative			

TM*: Transport medium

Prospective Study

Selection of 20 Influenza positive samples (PCR) from the 2015/16 winter season.

Sample N°	Diagnostic PCR NIC				Rapid Test		Storage
	PCR	Ct	Sub-type/ Lineage	Cell Culture	Fujifilm		Samples
76	FLU B	21,9	Victoria	+	négatif		-20°C
119	FLU B	20,1	Victoria	+	GB		
121	FLU B	20,3	Victoria	+	GB	<15'	
135	FLU B	17	Victoria	+	GB		
142	FLU B	16,1	Yamagata	+	GB	<15'	
164	FLU A	26,6	H1N1pdm09	+	GA		
166	FLU B	20,2	Victoria	+	negative		
169	FLU B	21,3	Victoria	+	negative		
172	FLU A	19,9	H3N2	+	GA		
173	FLU B	14,8	Victoria	+	GB	<15'	
176	FLU B	18,2	Victoria	+	GB	<15'	
182	FLU B	22,1	Victoria	-	GB		
231	FLU A	20,4	H1N1pdm09	+	GA	<15'	
232	FLU A	28,6	H1N1pdm09	+	negative		
251	FLU B	22,4	Victoria	-	negative		
255	FLU A	26,13	H1N1pdm09	+	GA	<15'	
259	FLU A	21,24	H1N1pdm09	+	GA	<15'	
264	FLU A	25,84	H1N1pdm09	+	negative		
265	FLU A	17,37	H1N1pdm09	+	GA	<15'	
269	FLU B	27,16	Victoria	+	negative		



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Prospective Study

Reproducibility of the results

Diagnostic PCR NIC				Fujifilm		
N°	PCR	Ct	Sub-type/ Lineage	1rst Test	2nd test	3rd test
76	FLU B	21,9	Victoria	negative	negative	/
166	FLU B	20,2	Victoria	negative	negative	/
169	FLU B	21,3	Victoria	negative	negative	/
182	FLU B	22,1	Victoria	FLU B	FLU B	FLU B
251	FLU B	22,4	Victoria	negative	negative	/
164	FLU A	26,6	H1N1pdm09	FLU A	FLU A	FLU A
264	FLU A	25,84	H1N1pdm09	negative	negative	/



FUJIFILM Sensitivity Level

Influenza Reference strains were produced in MDCK Cells.

Dilutions tested in duplicate to determine the detection threshold.

Type/Sub-type	A H1N1pdm09	A H3N2	B-Victoria	B-Yamagata
Strains	A/California/7/2009	A/Texas/50/2012	B/Brisbane/60/2008	B/Massachusetts/2/2012
TCID ₅₀ /ml	10 ⁴ / 10 ⁴	10 ⁴ / 10 ⁴	10 ⁶ / 10 ⁶	10 ⁵ / 10 ⁵

Results observed for other rapid tests (Feuillets de Biologie/ N°324- Mai 2015)

	A H1N1pdm09	A H3N2	B-Victoria	B-Yamagata
	A/California/7/2009	A/Texas/50/2012	B/Brisbane/60/2008	B/Massachusetts/2/2012
QUIDEL SOFIA	10 ⁴	10 ⁴	10 ⁵	10 ⁵
BD Dignostics	10 ⁵	10 ⁴	10 ⁵	10 ⁵

