Evaluation of two newly developed QIAsymphony® SP protocols for efficient isolation of influenza virus RNA from different respiratory samples

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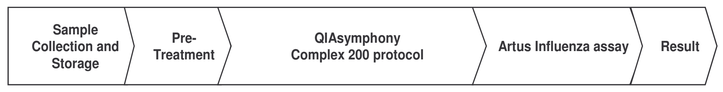
Introduction

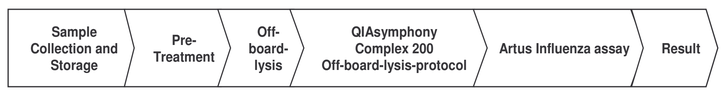
Influenza is one of the most severe respiratory infections. The human population regularly experiences influenza pandemics. Novel influenza A (H1N1) virus, commonly known as swine flu, is a new influenza virus causing illness in humans. This new influenza virus was first detected in April 2009. Detection of influenza virus by real-time PCR is sensitive and fast. The *artus* Infl./H1 LC/RG RT-PCR Kit contains an influenza A (H1N1) specific detection reagent, enabling additional detection of the 2009 pandemic H1N1 virus.

Sensitive detection of influenza RNA by real-time PCR requires purified RNA free of inhibitors. Here, we report the suitability of the QIAsymphony SP for isolation of influenza RNA from different sample materials using the QIAsymphony Virus/Bacteria Mini Kit in combination with the Complex 200 protocol. In addition, this fully automated protocol is compared to a QIAsymphony protocol with manual off-board lysis.

Workflow overview

The QIAsymphony Complex 200 protocol consists of 4 steps: lyse, bind, wash, elute. For some samples it is useful to perform lysis manually, for example, for inactivation of pathogens that must be inactivated in a biosafety cabinet. The Complex 200 protocol with off-board lysis enables a lysis step similar to that included in the Complex 200 protocol to be performed manually. Pretreated samples are transferred to the QIAsymphony SP and processed with the shortened protocol.





Nucleic acids bind to the silica surface of magnetic particles and are washed to remove contaminants. Purified nucleic acids are automatically transferred to collection plates or tubes.

Materials and methods

Different respiratory samples from swine and humans were spiked with influenza-positive material. Viral RNA was extracted either with a manual method (QIAamp® Viral RNA Kit) or with the QIAsymphony Virus/Bacteria Mini Kit using the Complex 200 protocol.

Heads of swabs stored either dried or in Amies agar were transferred to 1 ml Buffer ATL and incubated at 56°C for 15 minutes. After incubation, the buffer containing the biological material was used as sample. Universal transport medium (UTM, Copan, Italy) was used without further treatment. Sputum was liquefied by treating with Sputasol for 30 minutes at 30°C. Broncheoalveolar lavage (BAL) was used without pretreatment.

For off-board lysis, samples were incubated with or without shaking in a lysis mixture similar to the one used in the fully automated procedure. Lysates were transferred to the QIAsymphony SP for automated viral RNA purification.

Purified RNA was analyzed using the *artus*® Influenza LC RT-PCR Kit on a LightCycler® 1.5 or the *artus* Infl./H1 LC/RG RT-PCR Kit on a Rotor-Gene® Q according to the instructions in the kit handbooks.

Detection of swine flu in swabs (I)

Sample number	Storage condition	Complex 200 Target Ct	Complex 200 IC Ct	Complex 200 Off-Board-Lysis Target Ct	Complex 200 Off-Board-Lysis IC Ct	QIAamp Viral RNA Target Ct	QIAamp Viral RNA IC Ct
1	dried swabs	29,5	27,8	28,3	26,3	28,5	26,0
3	dried swabs	26,4	26,6	26,1	26,4	24,9	25,2
4	dried swabs	28,9	27,2	27,3	25,8	28,5	26,2
5	dried swabs	24,4	26,4	23,9	25,8	23,5	25,8
6	dried swabs	34,4	26,8	34,8	27,2	33,7	25,5
7	dried swabs	neg.	26,5	neg.	26,6	37,5	25,5
9	dried swabs	27,5	26,6	27,4	26,6	26,6	25,5
10	dried swabs	26,1	27,5	26,2	26,6	25,4	25,6
12	dried swabs	24,1	27,0	23,8	26,7	23,4	25,8
13	dried swabs	neg.	27,2	38,2	26,5	neg.	26,4
14	dried swabs	31,2	26,6	31,6	26,6	30,5	25,9
24	Amies-Agar	36,7	27,5	37,2	28,8	36,9	26,3
25	Amies-Agar	20,6	26,7	20,2	26,4	22,2	28,6
27	Amies-Agar	23,4	25,8	23,2	26,4	26,6	28,4
28	Amies-Agar	20,7	26,2	21,5	26,9	22,9	28,4
30	Amies-Agar	31,0	26,5	30,0	26,6	36,1	29,9
32	Amies-Agar	24,1	26,4	24,7	27,2	24,6	26,9
33	Amies-Agar	35,2	26,6	36,8	27,7	37,7	27,6
34	Amies-Agar	30,3	26,5	31,0	27,6	31,0	26,7
35	Amies-Agar	32,3	26,1	33,2	27,3	39,6	28,8
pos. C.	Amies-Agar	30,5	26,1	30,6	27,2	32,1	31,5
pos. C.	Amies-Agar	29,5	25,6	30,5	27,5	32,1	33,2
pos. C.	Amies-Agar	31,3	26,1	31,9	27,1	31,0	27,0
pos. C.	Amies-Agar	30,7	26,5	31,5	27,8	30,6	27,7

Influenza RNA was extracted from 46 respiratory swabs (four swabs tested negative were spiked with influenza-positive sample material) using the QIAamp Viral RNA Kit or on the QIAsymphony SP using either the Complex 200 protocol or a modified Complex 200 protocol with off-board lysis. Purified RNA was analyzed using the *artus* Infl./H1 LC/RG RT-PCR Kit on the Rotor-Gene Q.

Detection of swine flu in swabs (II)

Sample number	Storage condition	Complex 200	Complex 200 Off-Board-Lysis	QIAamp Viral RNA
1	dried swabs	26,56	26,31	26,08
3	dried swabs	24,79	24,71	23,88
4	dried swabs	26,10	25,77	26,21
5	dried swabs	22,23	22,13	21,07
6	dried swabs	32,49	32,07	30,20
7	dried swabs	neg.	neg.	35,15
9	dried swabs	25,89	25,84	25,01
10	dried swabs	23,65	23,56	23,08
12	dried swabs	21,58	21,90	20,96
13	dried swabs	34,08	33,00	33,52
14	dried swabs	29,07	29,09	28,01
24	Amies-Agar	22,35	22,86	22,32
25	Amies-Agar	30,86	31,04	32,01
27	Amies-Agar	28,35	28,53	28,54
28	Amies-Agar	28,57	28,54	29,83
30	Amies-Agar	33,77	34,46	33,48
32	Amies-Agar	19,29	19,65	19,70
33	Amies-Agar	22,94	22,36	24,07
34	Amies-Agar	19,82	20,26	21,11
35	Amies-Agar	28,63	28,75	29,89
pos. C.	Amies-Agar	neg.	neg.	neg.
pos. C.	Amies-Agar	neg.	neg.	neg.
pos. C.	Amies-Agar	neg.	neg.	neg.
pos. C.	Amies-Agar	neg.	neg.	neg.

Samples that tested positive for influenza RNA were further analyzed using the *artus* Infl./H1 LC/RG RT-PCR Kit on the Rotor-Gene Q with a 72-well-rotor. The Influenza H1 Master of this assay contains reagents and enzymes for specific amplification of an 80 nt region of influenza virus H1(pandemic H1N1 influenza 2009) genome.

Detection of influenza in different samples

Source	Sample No.	Sample type	QIAsymphony Complex 200	QIAamp Viral RNA	Delta C_T (QS - QA)
swine	1	Nasal swab in UTM	30,5	30,5	0,1
	2	Nasal swab in UTM	29,2	31,1	-1,9
	3	Nasal swab in UTM	29,2	31,9	-2,7
	4	Nasal swab in UTM	29,2	31,6	-2,5
	5	Nasal swab, dried	26,7	30,0	-3,3
	6	Nasal swab, dried	28,6	30,3	-1,7
	7	Nasal swab, dried	29,4	35,7	-6,3
	8	Nasal swab, dried	29,8	34,2	-4,3
human	9	Sputum	29,6	30,2	-0,6
	10	Sputum	23,5	23,6	-0,1
	11	Sputum	27,8	35,9	-8,1
	12	Sputum	30,6	31,5	-0,9
	13	Nasal swab, dried	28,7	30,3	-1,6
	14	Nasal swab, dried	29,2	30,0	-0,8
	15	Nasal swab, Amies-agar	30,0	30,5	-0,5
	16	Nasal swab, Amies-agar	30,1	32,8	-2,7
	17	Pharyngal swab, dried	29,8	29,9	-0,2
	18	Pharyngal swab, dried	29,5	30,0	-0,4
	19	Pharyngal swab, Amies-agar	30,0	32,2	-2,2
	20	Pharyngal swab, Amies-agar	30,9	32,8	-1,9
	21	BAL	30,3	28,8	1,5
	22	BAL	29,7	28,7	0,9
	Control	Water	29,9	29,6	0,3
	Control	Water	28,9	29,6	-0,7

22 respiratory samples were spiked with influenza-positive material. Viral RNA was extracted either with the QIAamp Viral RNA Kit, or with the QIAsymphony Virus/Bacteria Mini Kit in combination with the Complex 200 protocol. Purified RNA was analyzed using the *artus* Influenza LC RT PCR-Kit on a LightCycler 1.5. Sample 10 was positive before being spiked.

Single prep — master mix

To reduce pipetting steps when performing off-board lysis, a master mix of the required buffers, internal control (IC), carrier RNA, and proteinase K can be prepared prior to use. No differences were observed in experiments in which use of a master mix for lysis was compared to individual transfer of components to the sample.

	Sample material	Off-Board-Lysis Mean Ct	Off-Board-Lysis SD	Off-Board-Lysis Mastermix Mean Ct	Off-Board-Lysis Mastermix SD	Complex 200 Mean Ct	Complex 200 SD
target	UTM	33,76	0,21	33,99	0,33	34,06	0,45
	saliva	34,71	0,43	33,08	0,80	33,07	0,51
	ATL	32,77	0,36	32,83	0,37	32,57	0,48
IC	UTM	25,48	0,25	25,44	0,22	25,47	0,35
	saliva	25,79	0,46	25,75	0,27	25,62	0,23
	ATL	25,44	0,56	25,58	0,16	25,48	0,27

Summary of results

On average, the QIAsymphony SP showed lower C_T values compared to the manual QIAamp Viral RNA procedure when using influenza-specific *artus* RT-PCR assays.

During tests with different sample materials spiked with influenza, the standard deviation was lower from the QIAsymphony SP.

C_T values for internal controls (IC) were, in general, lower for eluates generated on the QIAsymphony SP. Standard deviation was also lower compared to the QIAamp Viral RNA Kit.

The Complex 200 protocol with off-board lysis gave similar results to the fully automated Complex 200 protocol.

When using the Complex 200 with off-board lysis, a master mix containing the components required for lysis can be prepared, resulting in reduced total time for preparation of off-board lysis.

Conclusions

The QIAsymphony Virus/Bacteria Kit in combination with the *artus* Influenza RT-PCR Kit or the *artus* Infl./H1 LC/RG RT-PCR Kit is suitable for sensitive analysis of influenza from different respiratory sample types.

Acknowledgment

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The artus kits in combination with the QIAsymphony are currently under development.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.