The information previously contained on these webpages were authorized under the 2009 H1N1 Influenza Emergency Use Authorizations (EUAs). As of June 23, 2010, the EUAs have been terminated and this information is no longer current.

artus® Inf. A H1N1 2009 LC RT-PCR Kit Protocol

Σ 96

IVD

Emergency Use Authorization

For in vitro diagnostic use

Qualitative detection of 2009 H1N1 influenza virus RNA

For use with the LightCycler® 2.0 Instrument

REF 4524095

HB 1058052

QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden

MAT 1058052

QIAGEN®

Sample & Assay Technologies

Contents

Intended Use

The *artus* Inf. A H1N1 2009 LC RT-PCR Kit is intended for the *in vitro* qualitative detection and differentiation of 2009 H1N1 influenza viral RNA in nasopharyngeal swabs (NPS) from patients with signs and symptoms of respiratory infection. This test is to be used in CLIA High Complexity Laboratories using the LightCycler 2.0 Real-Time PCR System and the EZ1 DSP Virus System.

Testing with the *artus* Inf. A H1N1 2009 LC RT-PCR Kit should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens. The identification of 2009 H1N1 influenza should be made in conjunction with clinical and epidemiological assessment.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

Summary and Explanation

Influenza is an acute respiratory disease caused by influenza viruses A, B, and C. These viruses are enveloped single-stranded RNA viruses that belong to the Orthomyxoviridae family. Influenza type A is usually the most common of the circulating influenza types. A number of different subtypes are classified within the influenza A type based on two viral surface proteins: hemagglutinin (HA) and neuraminidase (NA). Yearly epidemics occur in part due to antigenic drift within these proteins. Influenza subtypes are named according to serological differences of these proteins and the most common include H1, H2, or H3 in combination with N1 or N2. A novel influenza A H1N1 strain emerged in March 2009 and is the causative agent of the current public health emergency.

Symptoms of influenza caused by the 2009 H1N1 influenza virus are similar to those of seasonal influenza strains, including cough, nasal congestion, rhinorrhea, sore throat, fever, headache, and myalgia. Knowledge of the infecting virus can impact patient management decisions.

Principle of the Procedure

The *artus* Inf. A H1N1 2009 LC RT-PCR Kit contains reagents and instructions for the detection of Influenza A viral RNA and the detection and differentiation of 2009 H1N1 influenza virus RNA in nasopharyngeal swabs of symptomatic patients.

The assay utilizes the EZ1 Advanced instrument with the EZ1 DSP Virus Card v. 2.0 (QIAGEN) and the EZ1 DSP Virus Kit (QIAGEN) for viral nucleic acid extraction. The Light Cycler 2.0 instrument with software v. 4.1 (Roche) is used for amplification and detection.

The kit includes two reaction Masters:

The **Influenza A Master** contains primers/probe, enzymes, and other reaction components (except Mg solution) needed for the specific amplification of a 143 bp region of the matrix (M) gene of the Influenza A genome, and for the direct detection of the specific amplicon in fluorescence channel 530 of the LightCycler.

The **Influenza H1N1 Master** contains primers/probes, enzymes, and other reaction components (except Mg solution) needed for the specific amplification of two regions (71 and 81 bp, respectively) unique to the hemagglutinin (HA) gene of the 2009 H1N1 influenza virus. Probes specific to each amplicon are labeled with the same fluorophore for the direct detection in fluorescence channel 530 of the Light Cycler. Amplifying two regions of the gene provides increased assurance that the assay will still detect the 2009 H1N1 virus in the event of a mutation occurring in either of the targeted regions.

In addition, both Master Mixes contain a second heterologous primer/probe set to detect the Influenza Internal Control (IC). The IC result identifies possible failure of RNA extraction or the presence of PCR inhibition. The Internal Control is detected in fluorescence channel 610.

A Positive Control is provided for each Master Mix. PCR grade water is provided as a negative (no-template) control.

Table 1. Target genes and detection channels

Component	Primer / Probe Set Name	Target Virus or Control	Target Gene	Detection channel (nm)

Influenza A Master	InfA	Influenza A	Matrix	530
	IC	Influenza Internal Control	synthetic sequence	610
Influenza H1N1 Master	H1_F and H1_C	2009 H1N1	Hem-agglutinin	530
	IC	Influenza Internal Control	synthetic sequence	610

nm: nanometers

Materials Provided

Kit Contents

artus Inf. A H1N1 2009 LC RT-PCR Kit			(96)
Catalog no.			4524095
Number of reactions			96
Blue	Influenza A Master		8 x 138 µl (8 x 12 reactions)
Violet	Influenza H1N1 Master		8 x 144 µl (8 x 12 reactions)
Yellow	Influenza Mg-Sol*	Mg-Sol	800 µl
Red	Influenza Control		200 µl
Brown	Influenza H1 Control		200 µl
Green	Influenza IC†	IC	1000 µl
White	Water (PCR grade)		1000 µl
	Protocol	HB	1

* Magnesium solution.

† Internal control.

Materials Required But Not Provided

- EZ1 DSP Virus Kit (QIAGEN, cat. no. 62724)
- EZ1 Advanced DSP Virus Card (cat. no. 9018306)
- EZ1 Advanced instrument (cat. no. 9001410)*
- Pipets (adjustable, for volumes between 1 µl and 1000 µl)*

- Sterile, RNase-free pipet tips with filters for use with the pipets
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes capable of >2000 rpm
- Freezer* at –15 to –30°C (for kit component storage)
- Freezer* at –70°C or lower (for sample or eluate frozen storage)
- Soft paper tissue
- LightCycler 2.0 (Software Version 4.1) Instrument,* Roche Diagnostics
- LightCycler Multicolor Demo Set (Roche Applied Science, cat. no. 03 624 854 001) for the LightCycler 2.0 Instrument
- LightCycler Capillaries, 20 µl (Roche Applied Science, cat. no. 04 929 292 001)
- LightCycler Cooling Block and centrifuge adapters (Roche Applied Science, cat. no. 11 909 312 001)
- LightCycler Capping Tool (Roche Applied Science, cat. no. 03 357 317 001)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and precautions

- Strict compliance with this protocol as well as with the *EZ1 DSP Virus Kit Handbook* and the *EZ1 Advanced User Manual* is required for optimal results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Follow universal precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- Diagnostic laboratory work on clinical samples from patients who are suspected cases of 2009 H1N1 influenza virus infection should be conducted in a BSL2+ laboratory. All sample manipulation should be performed inside a biosafety cabinet.
- Do not pipet by mouth.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs).

- Use sterile pipet tips with filters.
- Workflow in the laboratory should proceed in a unidirectional manner, beginning in the Pre-amplification Areas (I, II, and III) and moving to the Amplification/Detection Area. The following assay steps should be performed in separate areas with dedicated supplies and equipment.
  - RNA extraction — Pre-amplification Area I
  - Reagent preparation — Pre-amplification Area II
  - PCR setup — Pre-amplification Area III
  - LightCycler setup/run — Amplification/Detection Area
- Dispose of amplified samples without opening reaction tubes.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly.
- Work quickly and keep components on ice or in the cooling block.

Reagent Storage and Handling

The *artus* Inf. A H1N1 2009 LC RT-PCR Kit is shipped frozen. The components of the *artus* Inf. A H1N1 2009 LC RT-PCR Kit should be stored at –15 to –30°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>1 x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

Specimen Handling and Storage

The *artus* Inf. A H1N1 2009 LC RT-PCR Kit can be used with nasopharyngeal specimens (dacron, nylon, or rayon swabs) placed in virus transport medium. For specimen transport and storage, please follow the recommendations of the WHO[1]. Eluates may be stored at –70 °C or lower for up to 4 weeks.

Assay Procedure

Overview

The procedure consists of 4 consecutive steps:

- Sample collection: Collect nasopharyngeal swab specimens from symptomatic patients using a polyester, nylon, or rayon swab and place it into virus transport medium.
- Nucleic acid extraction: Add the Influenza IC to the carrier RNA before starting the extraction procedure. Extract viral RNA, using the EZ1 DSP Virus Kit in combination with the EZ1 Advanced instrument.
- Real-time RT-PCR: Add the extracted RNA and positive and negative control material to Influenza A Master and separately to Influenza H1N1 Master. Perform real time RT-PCR using the LightCycler 2.0 instrument.
- Result interpretation: Evaluate the results of the positive and negative controls to determine if the run is valid. If the run is valid, the internal control and target-specific results of each specimen are evaluated. Samples positive for influenza A and at the same time positive for H1 can be considered to contain RNA of the 2009 H1N1 influenza virus.

RNA extraction

(Pre-amplification Area I)

Make sure to familiarize yourself with the EZ1 Advanced instrument before beginning the RNA extraction procedure. See the *EZ1 Advanced User Manual*.

Viral RNA is extracted from 0.4 ml of virus transport medium of the patient sample on the EZ1 Advanced instrument using the Virus DSP protocol version 1.0 as described in the *EZ1 DSP Virus Kit Handbook*. Elution volume is 60 µl.

Internal control

An internal control (Influenza IC) is supplied. This allows the user both to control the RNA isolation procedure and to check for possible PCR inhibition. For this application, add the internal control to the Carrier RNA Solution before starting the extraction, as described in the *EZ1 DSP Virus Kit Handbook*, at a ratio of 0.1 µl per 1 µl elution volume. For elution in 60 µl, 6 µl of the internal control should be added initially.

Note: The internal control should be added only to the carrier RNA solution as described in the *EZ1 DSP Virus Kit Handbook*.

Note: Do not add the internal control and the carrier RNA to the sample material directly.

PCR and Data Analysis

Important points before starting

- Take time to familiarize yourself with the LightCycler 2.0 Instrument before starting the protocol. See the instrument user manual.
- Make sure that the appropriate positive controls (Influenza Control and Influenza H1 Control) as well as one no template control (Water, PCR grade) are included per PCR run.

Things to do before starting

- Make sure that the cooling block and the capillary adapters (accessories of the LightCycler 2.0 Instrument) are precooled to 2–8°C.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Reagent preparation

(Pre-amplification Area II)

1. Place the desired number of LightCycler capillaries into the adapters of the cooling block.

   Up to 15 reactions can be processed in each LightCycler run. Remember to account for controls.

2. Prepare the influenza A master mixes according to Tables 2 and 3.

   The reaction mix contains all of the components needed for PCR except the sample.

   Note: Once the master mix has been made, the run must be started within one hour.

Table 2. Preparation of influenza A master mix without Influenza IC (for use with extracted sample RNA)

Number of samples	1	12
Influenza A Master	11.5 µl	138 µl
Influenza Mg-Sol	3.5 µl	42 µl
Influenza IC	0 µl	0 µl
Total volume	15 µl	180 µl

Table 3. Preparation of influenza A master mix including Influenza IC (for use with controls)

Number of samples	1	12
Influenza A Master	11.5 µl	138 µl
Influenza Mg-Sol	3.5 µl	42 µl
Influenza IC	0.5 µl	6 µl
Total volume	15.5 µl*	186 µl*

* The volume increase caused by adding the internal control is negligible. The sensitivity of the detection system is not impaired.

3. Prepare the influenza H1N1 master mixes according to Tables 4 and 5.

   The reaction mix contains all of the components needed for PCR except the sample.

   Note: Once the master mix has been made, the run must be started within one hour.

Table 4. Preparation of influenza H1N1 master mix without Influenza IC (for use with extracted sample RNA)

Number of samples	1	12
Influenza H1N1 Master	12 µl	144 µl
Influenza Mg-Sol	3 µl	36 µl
Influenza IC	0 µl	0 µl
Total volume	15 µl	180 µl

Table 5. Preparation of influenza H1N1 master mix including Influenza IC (for use with controls)

Number of samples	1	12
Influenza H1N1 Master	12 µl	144 µl
Influenza Mg-Sol	3 µl	36 µl
Influenza IC	0.5 µl	6 µl
Total volume	15.5 µl*	186 µl*

* The volume increase caused by adding the internal control is negligible. The sensitivity of the detection system is not impaired.

PCR setup

(Pre-amplification Area III)

4. Pipet 15 µl of the master mix into the plastic reservoir of each capillary (see Table 6). Add 5 µl of the appropriate extracted sample RNA or control (Influenza Control, Influenza H1 Control, or PCR grade water) to the appropriate capillary.

Table 6. Preparation of PCR assay

Number of samples	1	12
Master mix	15 µl	15 µl each
Extracted RNA or control	5 µl	5 µl each
Total volume	20 µl	20 µl each

5. Close the capillaries. To transfer the mixture from the plastic reservoir into the capillary, centrifuge the adapters containing the capillaries in a bench top centrifuge for 10 s at a maximum of 400 x *g* (2000 rpm).

LightCycler setup/run

(Amplification/Detection Area)

6. For the detection of influenza virus RNA, create a temperature profile according to the following 4 steps (see Figures 1–4 and Table 7).

Reverse transcription of the RNA	Figure 1
Initial activation of the hot-start enzyme	Figure 2
Amplification of the cDNA	Figure 3
Cooling	Figure 4

Note: Pay particular attention to the settings for Analysis Mode, Cycle Program Data, and Temperature Targets. In the illustrations these settings are framed in bold black. The numbers outside of the boxes indicate the order of the programming steps.

All specifications refer to the LightCycler 2.0 Instrument. Please find further information on programming the LightCycler 2.0 Instrument in the instrument operator's manual.

Table 7. Cycling profile

Step	Time	Temperature	Ramp rate
Reverse transcription	10 min	50°C	20°C/min
Initial PCR activation step	10 s	95°C	20°C/min
Amplification of the cDNA 3-step cycling:			
Denaturation	2 s	95°C	20°C/min
Annealing and acquisition	12 s	60 °C	20°C/min
Extension	10 s	72°C	10°C/min
Number of cycles	45		
Cooling	30 s	40°C	20°C/min

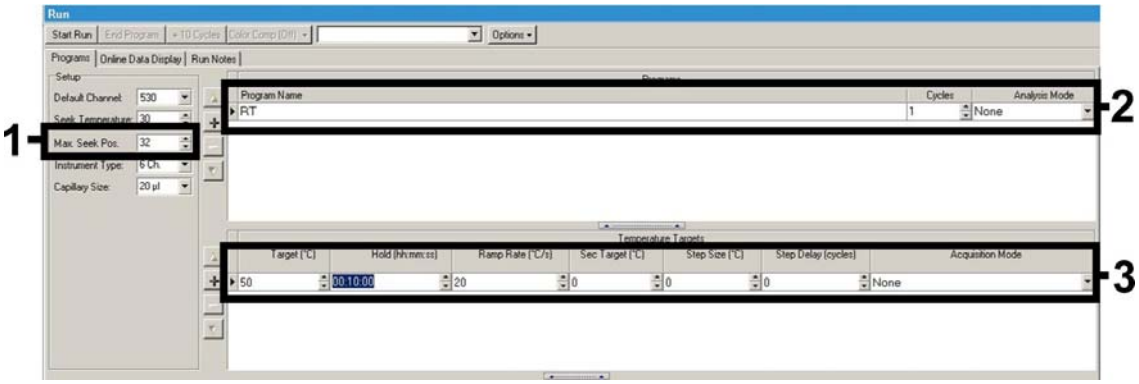

Figure 1. Reverse transcription of the RNA.

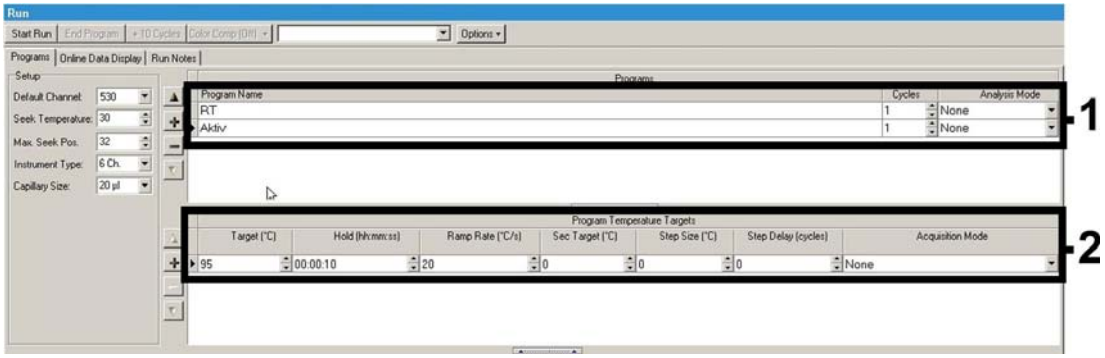

Figure 2. Initial activation of the hot-start enzyme.

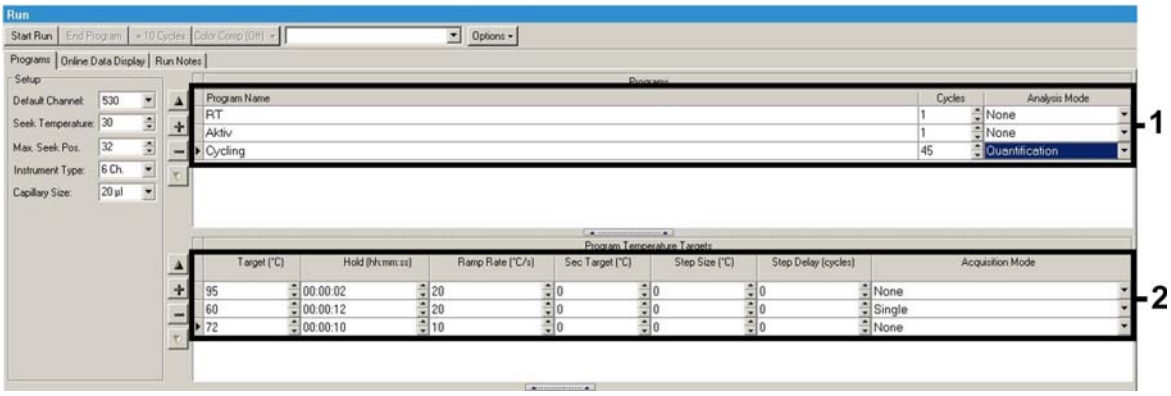

Figure 3. Amplification of the cDNA.

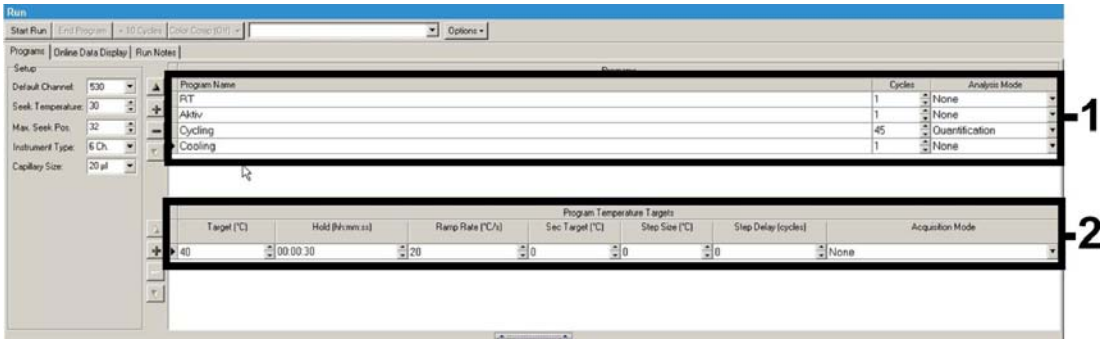

Figure 4. Cooling.

7. Place the capillaries in the LightCycler 2.0 Instrument and start the cycling program
8. In multicolor analyses, interferences occur between fluorimeter channels. The LightCycler 2.0 Instrument's software contains a file termed "Color Compensation File", which compensates for these interferences. Open this file before, during, or after the PCR run by clicking the "Choose CCC File" or the "Select CC Data" buttons.

Note: If no Color Compensation File is installed, generate the file using the LightCycler Multicolor Demo Set for the LightCycler 2.0 Instrument according to the instructions in the instrument operator's manual.

After the Color Compensation File has been activated, separate signals appear in fluorescence channels 530 and 610.

Interpretation of Control Results

Results for each control should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Positive and Internal Controls for the *artus* Inf. A H1N1 2009 LC RT-PCR Kit

For a run to be considered valid, the following criteria must be met.

1. **Influenza Control** (with Influenza A Master)
   - Ct ≤ 30 in Channel 530

2. **Influenza H1 Control** (with Influenza H1N1 Master)
   - Ct ≤ 30 in Channel 530

3. **No-Template (negative) Control**

   If the No-Template Control is negative (Ct > 45 in Channel 530) and the Internal Control Ct value (channel 610) is < 34 with Influenza A Master or < 30 with the Influenza H1N1 Master, then this control is valid and results may be reported given that all other assay run criteria are met.

   If the no-template control is positive (Ct ≤ 45 in Channel 530), then this control is invalid. This indicates possible contamination of prepared samples. Positive patient results cannot be reported. Positive specimens on this run must be repeated.

   Note: Negative specimens may be reported given that all other assay run criteria are met.

Interpretation of specimen results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be acceptable.

In a valid run, Ct values of the target specific channel and the IC channel are evaluated for each sample as follows:

- Target specific channel (530):
  - Ct ≤ 45 is Positive
  - No Ct or Ct > 45 is Negative
- Internal Control Channel (610):
  - Ct ≤ 45 is Positive
  - No Ct or Ct > 45 is Negative

Note: Detection of the Internal Control (channel 610) is not required for a positive result. High viral load can lead to reduced or absent Internal Control signal.

The Internal Control and target specific responses for each specimen are interpreted as follows:

Influenza A Master		**Influenza H1N1 Master**		
Influenza A (channel 530)	**Internal control (channel 610)**	**Influenza 2009 H1N1 (channel 530)**	**Internal control (channel 610)**	**Interpretation**
Positive	Positive or negative*	Positive	Positive or negative*	Influenza A RNA: detected 2009 H1N1 RNA: detected
Positive	Positive or negative*	Negative	Positive	Influenza A RNA: detected 2009 H1N1 RNA: not detected
Negative	Positive	Negative	Positive	Influenza A RNA: not detected 2009 H1N1 RNA: not detected
Negative	Positive	Negative	Negative	Influenza A RNA: not detected 2009 H1N1 RNA: Invalid†
Negative	Negative	Positive or negative	Positive or negative	Invalid†

* Detection of the Influenza Internal Control (channel 610) is not required for positive results. High viral load can lead to a reduced or absent internal control signal.

† For invalid specimen results, the sample must be re-extracted and retested. If the retest result is not resolved, the result is considered indeterminate.

Note: A Ct value reported by the software in brackets [] indicates that the slope of the response curve was unusual. The curve should be manually reviewed to determine if the response is positive or negative. The RT-PCR should be repeated if necessary, in accordance with the Interpretation of Specimen Results table above.

Examples of positive and negative PCR reactions are given for influenza A virus in Figures 5 and 6, and for 2009 H1N1influenza virus in Figures 7 and 8.

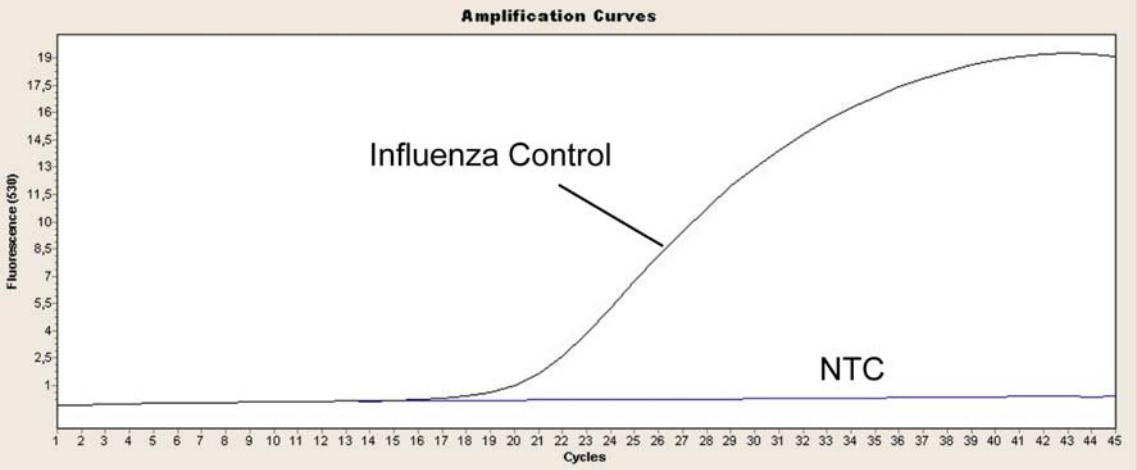

Figure 5. Example curves for the Influenza Control and the NTC in channel 530. NTC: no template control (negative control).

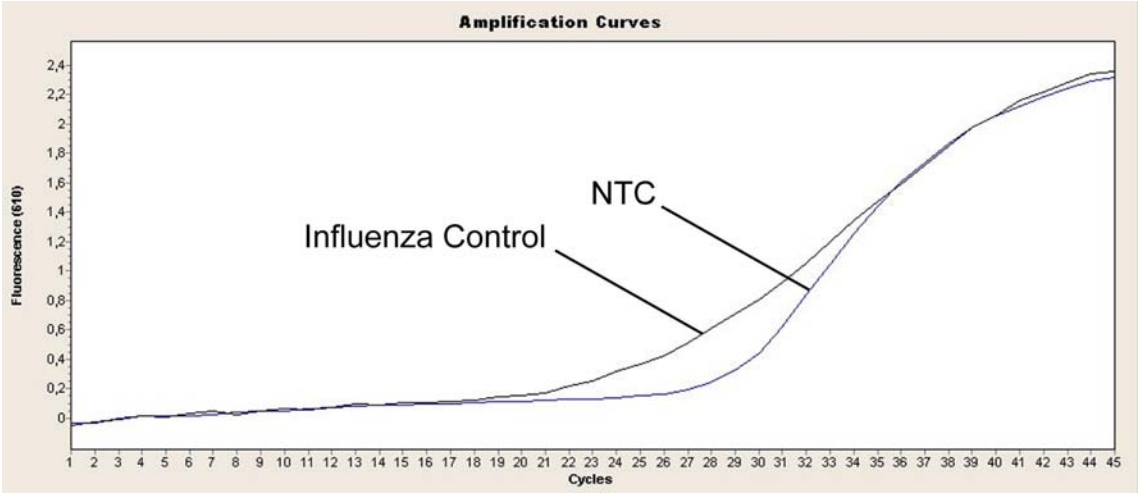

Figure 6. Detection of the Internal Control (IC) in channel 610 for the NTC reaction and the Influenza Control reaction. NTC: No template control (negative control).

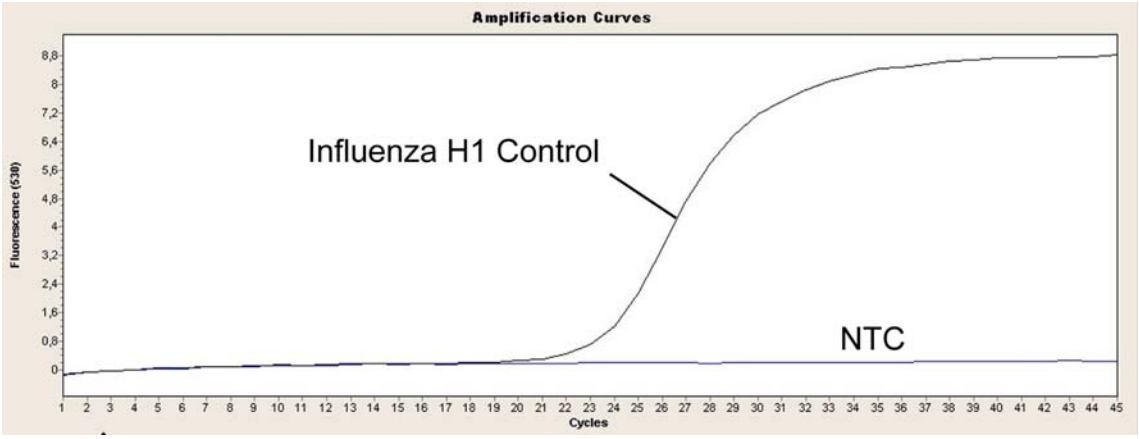

Figure 7. Detection of the Influenza H1 Control and the NTC in channel 530. NTC: No template control (negative control).

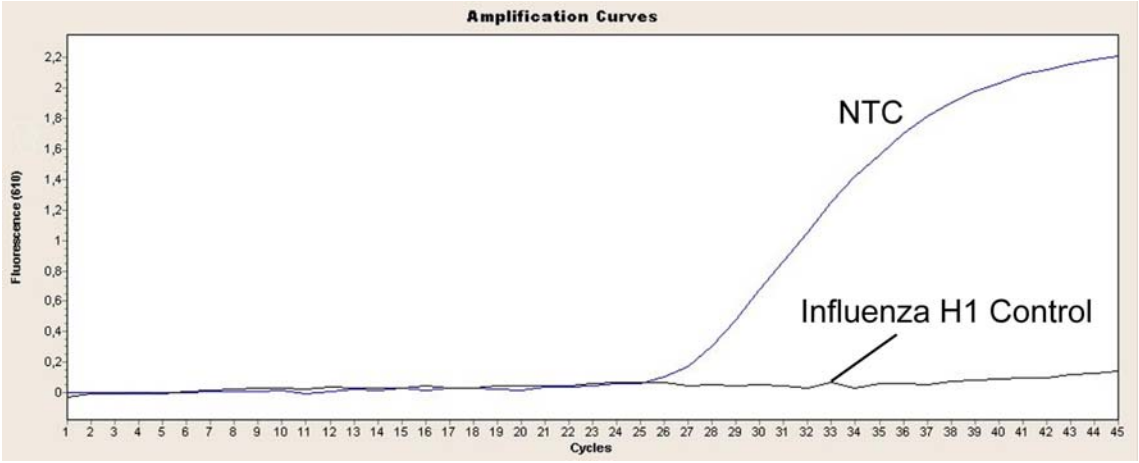

Figure 8. Detection of the internal control (IC) in channel 610 for the NTC reaction and in the Influenza Control reaction. NTC: No template control (negative control).

Quality Control

The Influenza Control, Influenza H1 Control, and no template control (PCR grade water) are provided with the *artus* Inf. A H1N1 2009 LC RT-PCR Kit and should be included in each run of the LightCycler instrument. Control results are evaluated to determine if the run is valid. Acceptance criteria for the controls are provided in the "Interpretation of Control Results" section above. If the run is invalid, the samples should be retested.

A known positive (for influenza A or 2009 H1N1 influenza) specimen should be included as an additional positive control in each run of the LightCycler. If the positive control does not meet the acceptance criteria established by the laboratory, the samples should be retested.

Each laboratory should ensure compliance with applicable local, state, and federal regulations as well as the laboratory's quality control procedures.

Limitations

- Results should be interpreted in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Specimens negative for 2009 H1N1 virus RNA do not rule out influenza A or 2009 H1N1 influenza infection, and may contain other influenza or respiratory viruses. Additional testing for seasonal influenza A or B or other respiratory specimens may be required.

- The prevalence of infection will affect the test's predictive value.
- False negative results due to loss of nucleic acids may occur if a specimen is improperly collected, transported, or handled. The internal control does not indicate whether or not nucleic acids have been lost due to inadequate collection, transport, or storage of specimens.
- False negative results may occur if inadequate numbers of organisms are present in the specimen.
- False negative results may occur due to the presence of sequence variants in the assay's target regions, and to genomic mutations, insertions, deletions, or emerging genetic rearrangements in the infecting organism.
- This test has not been evaluated for screening of blood or blood products.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 26.

Performance Characteristics

Analytical sensitivity (LoD)

The analytical sensitivity of the *artus* Inf. A H1N1 2009 LC RT-PCR Kit was determined by limiting dilution studies using quantified viral stocks of seasonal Influenza A/Brisbane/57/07 (seasonal H1N1) and 2009 H1N1 Influenza A/Hamburg/05/09.

For the initial LoD determination, six half-logarithmic dilutions of the characterized influenza viruses described above were tested to identify an endpoint for detection with each primer and probe set. For each dilution, 12 aliquots of seasonal Influenza A H1N1: A/Brisbane/57/07 or 24 aliquots of 2009 H1N1 influenza virus: A/Hamburg/05/09 were processed for RNA extraction using the EZ1 Advanced Instrument. Each extracted RNA was tested with the *artus* Inf. A H1N1 2009 LC RT-PCR Kit. The LoD for the Influenza A Master and the Influenza H1N1 Master were determined by Probit analysis. A summary of the results is presented in Table 8.

Table 8: Summary of initial LoD results for the seasonal H1N1 and 2009 H1N1 strains tested with the Influenza A Master, and the 2009 H1N1 strain tested with the Influenza H1N1 Master:

	Virus Strain Tested	Stock Virus Titer	Serial 10-fold dilution factor	Conc. Tested*	Call Rate	Ct range	Avg. Ct (for Ct < 40)	Limit of Detection** (LoD) per Virus Strain
Influenza A Master	A/Brisbane/57/07	1.6×10^8 TCID50/mL	10^6	160	12/12	34.3 - 37.3	35.42	57.0 $TCID_{50}$/mL (95% CI: 30.2 – 188.4 TCID50/mL)
			$10^{6.5}$	50.60	11/12	35.7 - >45	36.72	
			10^7	16.00	9/12	35.7 - >45	36.51	
			$10^{7.5}$	5.06	4/12	35.4 - >45	36.20	
			10^8	1.60	2/12	36.6 - >45	36.64	
			$10^{8.5}$	0.51	0/12	>45	>45	
			10^9	0.16	0/12	>45	>45	
			$10^{9.5}$	0.05	0/12	>45	>45	
	A/Hamburg/05/09	8×10^6 PFU/mL	10^6	8	24/24	33.5 - 36.6	34.67	4.2 PFU/mL (95% CI: 2.3 – 11.1 PFU/mL)
			$10^{6.5}$	2.53	23/24	34.2 - >45	35.88	
			10^7	0.8	15/24	36.0 - >45	36.78	
			$10^{7.5}$	0.25	6/24	36.2 - >45	36.64	
			10^8	0.08	4/24	37.5 - >45	n/a	
			$10^{8.5}$	0.03	2/24	>40 - >45	>40	
Influenza H1N1 Master	A/Hamburg/05/09	8×10^6 PFU/mL	10^6	8	24/24	33.0 - 34.2	33.62	1.01 PFU/mL (95% CI: 0.52-2.3 PFU/mL)
			$10^{6.5}$	2.53	24/24	34.6 - 37.2	35.48	
			10^7	0.8	23/24	35.3 - >45	36.91	
			$10^{7.5}$	0.25	14/24	36.1 - >45	37.40	
			10^8	0.08	9/24	37 - >45	37.74	
			$10^{8.5}$	0.03	3/24	39.3 - >45	39.25	

* unit is same as that of the stock virus titer.
** as determined by Probit analysis.

The LOD for influenza, A/Brisbane/57/07 (seasonal H1N1), tested with the Influenza A Master is 57.0 TCID50/mL (95% CI: 30.2 – 188.4).

The LOD for 2009 H1N1 influenza, A/Hamburg/05/09, tested with Influenza A Master is 4.217 PFU/mL (95% CI 2.3 – 11.12.249 – 11.104 PFU/mL).

The LOD for 2009 H1N1 influenza, A/Hamburg/05/09, tested with, Influenza H1N1 Master is 1.01 PFU/mL (95% CI 0.58 - 2.57 PFU/mL).

Confirmation of the initial LoD determination for each Master was achieved by testing 9 replicates of the same seasonal Influenza A strain and 24 replicates of the same 2009 H1N1 influenza strain described above, with concentrations at the respective upper and lower bounds of the 95% confidence interval of the initial LoD determination. The initial LoD determination is confirmed by the results demonstrating a > 95% hit rate for the upper bound concentration and a < 95% hit rate for the lower bound concentration. A summary of the results is presented in Table 9.

Table 9: Confirmation of LoD results for the seasonal H1N1 and 2009 H1N1 strains tested with the Influenza A Master, and the 2009 H1N1 strain tested with the Influenza H1N1 Master:

	Virus Strain Tested	Concentration Tested *	*artus* Inf. A H1N1 2009 LC RT-PCR Kit			
			Ct range	Avg. Ct**	Observed Call Rate (%)	Expected Call Rate %
Influenza A Master	A/Brisbane /57/07	188.4 $TCID_{50}$/mL	33.35 - 35.82	34.66	9/9 (100)	>95
		30.2 $TCID_{50}$/mL	35.82 - >45	36.92	7/9 (77.8)	< 95
	A/Hamburg /05/09	11.1 PFU/mL	33.16 - 38.65	35.73	24/24 (100)	>95
		2.3 PFU/mL	35.68 - >45	37.45	22/24 (91.7)	< 95
Influenza H1N1 Master	A/Hamburg /05/09	2.3 PFU/mL	33.16 - 38.65	35.73	23/23*** (100)	>95
		0.51 PFU/mL	35.68 - >45	37.45	22/24 (91.7)	< 95

* Corresponds to the upper and lower bounds of the 95% CI for the initial LoD concentration.
** Average of Ct values <40.
*** One result was invalid due to operator error.

Analytical Reactivity

Reactivity (inclusivity) was evaluated for five seasonal influenza A strains (two seasonal H3N2, three seasonal H1N1). Each virus strain was tested in triplicate. Results of the reactivity testing are presented in Table 10.

Table 10. Reactivity (inclusivity) results for the *artus* Inf. A H1N1 2009 LC RT-PCR Kit with five seasonal influenza A strains.

		# detected by *artus* Inf. A H1N1 2009 LC RT-PCR Kit	
Virus Strain Tested	Titer	Influenza A Master # detected/# tested	Influenza H1N1 Master # detected/# tested
H3N2 A/Hong Kong/8/68	5.93E+03 *	3/3	0/3
H3N2 A/Victoria/3/75	5.93E+02 *	3/3	0/3
H1N1 A/WS/33	5.93E+03 **	3/3	0/3
H1N1 A/PR/8/34	1.05E+04 **	3/3	0/3
H1N1 A/Denver/1/57	1.87E+03*	3/3	0/3

* $CEID_{50}$/mL (chicken embryo infectious dose)

** $TCID_{50}$/mL (tissue culture infectious dose)

The strains listed above all showed the expected reactivity with the Influenza A Master.

Analytical Specificity (cross-reactivity)

Cross-reactivity of the *artus* Inf. A H1N1 2009 LC RT-PCR Kit was evaluated by testing DNA or RNA from other human respiratory pathogens and seasonal influenza viruses. For some pathogens, concentration was estimated using a quantitative (copies/µL result) or qualitative (Ct result) real-time PCR test. To be included in the study, each of these pathogens was required to show a robust signal in a pathogen-specific, PCR based assay.
Results of the cross-reactivity study are presented in Table 11.

Table 11. Summary of cross-reactivity results.

			# detected/# tested:	
Pathogen	**Source**	**Titer**	**Influenza A Master**	**Influenza H1N1 Master**
Adenovirus B	ATCC VR-848	1.87E+04 $TCID_{50}$/mL	0/3	0/3
Adenovirus E	ATCC VR-1572	1.05E+04 $TCID_{50}$/mL	0/3	0/3
Coxsackievirus (CVA21)	ATCC VR-850	1.87E+04 $TCID_{50}$/mL	0/3	0/3
Coxsackievirus (CVB4)	ATCC VR-184	1.87E+05 $TCID_{50}$/mL	0/3	0/3
Echovirus	ATCC VR-41	5.93E+04 $TCID_{50}$/mL	0/3	0/3
Enterovirus 71	Robert Koch Institute, Berlin	10,000 c/µl	0/3	0/3
Human coronavirus 229E	ATCC VR-740	1.05E+03 $TCID_{50}$/mL	0/3	0/3
Human coronavirus OC43	ATCC VR-1558	1.05E+04 $TCID_{50}$/mL	0/3	0/3
Human parainfluenza type 1	ATCC VR-94	1.05E+03 $TCID_{50}$/mL	0/3	0/3
Human parainfluenza type 2	ATCC VR-92	1.87E+04 $TCID_{50}$/mL	0/3	0/3
Human parainfluenza type 3	ATCC VR-93	1.05E+06 $TCID_{50}$/mL	0/3	0/3
Human parainfluenza type 4	ATCC VR-1377	5.93E+02 $TCID_{50}$/mL	0/3	0/3
Inf A, A/Hong Kong/8/68	ATCC VR544	5.93E+07 $CEID_{50}$/mL	N/A	0/3
Inf A, A/Victoria/3/75	ATCC VR822	5.93E+06 $CEID_{50}$/mL	N/A	0/3
Inf A, A/WS/33	ATCC VR-1520	1.87E+05 $TCID_{50}$/mL	N/A	0/3
Inf A, A/PR/8/34	ATCC VR1469	1.05E+06 $TCID_{50}$/mL	N/A	0/3
Inf A, A/Denver/1/57	ATCC VR-546	5.93E+07 $CEID_{50}$/mL	N/A	0/3

			# detected/# tested:	
Pathogen	**Source**	**Titer**	**Influenza A Master**	**Influenza H1N1 Master**
Inf B, B/Lee/40	ATCC VR-101	5.93E+04 $CEID_{50}$/mL	0/3**	0/3
Inf B, B/Taiwan/2/62	ATCC VR-295	2.10E+04 $CEID_{50}$/mL	0/3	0/3
Inf B, B/Maryland/1/59	ATCC VR-296	5.93E+03 $CEID_{50}$/mL	0/3	0/3
Mumps virus	ATCC VR-106	1.05E+05 $TCID_{50}$/mL	0/3**	0/3
Respiratory syncytial virus A (RSV-A)	ATCC VR-1540	5.93E+04 $TCID_{50}$/mL	0/3	0/3
Respiratory syncytial virus B (RSV-B)	ATCC VR-1580	1.05E+03 $TCID_{50}$/mL	0/3	0/3
Rhinovirus – Type 85	ATCC 50-525CV54,	3.34E+04 $TCID_{50}$/mL	1/6***	0/3
Bordetella parapertussis	DMSZ* (Nr. 13415)	Ct 13	0/3	0/3
Bordetella pertussis	DMSZ (Nr. 5571)	Ct 10	1/6***	0/3
Chlamydia pneumoniae	ATCC VR-1356	1.05E+05 $TCID_{50}$/mL	1/6***	0/3
Legionella. pneumophila	University Hamburg	1,200 c/µl	0/3	0/3
Mycoplasma pneumoniae	TU Dresden, Reference Lab	4,600 c/µl	0/3	0/3
Mycobacterium tuberculosis	QCMD ring trial material, 02-08	Ct 26	0/3	0/3
methicillin-resistant *Staphylococcus aureus* (MRSA)	DSMZ 11729	Ct 30	1/6**	0/3
methicillin-sensitive *Staphylococcus aureus (MSSA)*	DSMZ 1104	Ct 16	0/3	0/3

*DSMZ: German Collection of Microorganisms and Cell Cultures

** This result was reported by the software in brackets, indicating that the slope of the response curve was unusual and that it should be manually reviewed. Visual inspection of the curve indicated that the result was negative. The sample was re-tested in triplicate and showed a negative result for each replicate.

***After an initial weak positive the sample was re-tested with three additional replicates and showed a negative result for each replicate.

Clinical Performance:

To establish the performance of the *artus* Inf. A H1N1 2009 LC RT-PCR Kit with clinical specimens, 104 residual de-identified nasopharyngeal swab specimens from symptomatic patients (including seasonal flu A positive, 2009 H1N1 positive, and influenza A negative specimens) were evaluated in a blinded fashion. Each specimen was tested at Focus Diagnostics using the EUA authorized Influenza A H1N1 (2009) Real-Time RT-PCR test and at QIAGEN with the *artus* Inf. A H1N1 2009 LC RT-PCR Kit.

Agreement of results between the *artus* Inf. A H1N1 2009 LC RT-PCR Kit and the Focus test are presented in Table 12.

Table 12. A comparison of the *artus* Inf. A H1N1 2009 LC RT-PCR Kit with the Focus Influenza A H1N1 (2009) Real-Time RT-PCR test for detection of 2009 H1N1 influenza virus in NPS specimens.

		Focus H1N1 (2009) Real-Time RT-PCR			
		Influenza A Positive	**2009 H1N1 Positive**	**Negative**	**Total**
***artus* Inf. A H1N1 2009 LC RT-PCR Kit**	**Influenza A Positive**	5**	0	2	7
	2009 H1N1 Positive	0	27	1	28
	Invalid/ Indeterminate*	0	1	1	2
	Negative	0	0	67	67
	Total	5	28	71	104

2009 H1N1	**Results (%, n/N)**	**95% Confidence Interval**
Positive Agreement	96.4% (27/28)	81.6% - 99.9%
Negative Agreement	94.7% (72/76)***	87.1% – 98.6%

* Two invalid results (retested as per the assay instructions) that did not resolve in a valid result were considered indeterminate and tallied against the performance of the *artus* test.
** Specimens corresponding to seasonal influenza A virus.
*** Seasonal flu A positives, negative for 2009 H1N1, were included in the analysis.

Symbols

 <N>	Contains reagents sufficient for <N> tests
	Use by
	Catalog number
	Lot number
	Material number
	Components
	Contains
	Number
	Temperature limitations
	Legal manufacturer
	Consult instructions for use

References

[1]http://www.who.int/csr/resources/publications/swineflu/storage_transport/en/index.html

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Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
No signal with positive controls (Influenza Control or Influenza H1 Control) in fluorescence channel 530	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis select the fluorescence channel 530 for the Influenza A and Influenza H1N1 PCR. Select the fluorescence channel 610 for the internal control RT-PCR.
b) Incorrect programming of the temperature profile of the instrument	Compare the temperature profile with the protocol. See page 13.
c) Incorrect configuration of the PCR	Check your work steps by means of the pipetting scheme, and repeat the PCR, if necessary. See page 11.
d) The storage conditions for one or more kit components did not comply with the instructions given in Reagent Storage and Handling	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* Inf. A H1N1 2009 LC RT-PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Weak or no signal of the Influenza IC in fluorescence channel 610 with simultaneous absence of a signal in channel 530

a) The PCR conditions do not comply with the protocol	Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
b) The PCR was inhibited	Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
c) RNA was lost during extraction	If the internal control was added to the extraction, an absent signal of the internal control can indicate the loss of RNA during the extraction. Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
d) The storage conditions for one or more kit components did not comply with the instructions given in Reagent Storage and Handling	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* Inf. A H1N1 2009 LC RT-PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Positive signal in channel 530 of the no-template (negative) control.

a) Contamination occurred during preparation of the PCR	Repeat the PCR with new reagents in replicates. If possible, close the PCR capillaries directly after addition of the sample to be tested. Make sure to pipet the positive controls last. Make sure that work space and instruments are decontaminated at regular intervals.

	Comments and suggestions
b) Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that work space and instruments are decontaminated at regular intervals.

Ordering Information

Product	Contents	Cat. no.
artus Inf. A H1N1 2009 LC RT-PCR Kit (96)	For 96 reactions: 2 Masters (Influenza A and Influenza H1N1), Mg Solution, 2 Positive Controls (Influenza and Influenza H1), Internal Control, Water (PCR grade)	4524095
EZ1 DSP Virus Kit — for automated, simultaneous purification of viral DNA and RNA from 1–6 human plasma, serum, or CSF samples using the EZ1 Advanced		
EZ1 DSP Virus Kit (48)	For 48 viral nucleic acid preps: Prefilled Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers, Carrier RNA	62724
EZ1 Advanced DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol	9018306
EZ1 Advanced	Robotic workstation for automated purification of nucleic acids using EZ1 DSP Kits, 1-year warranty on parts and labor	9001411

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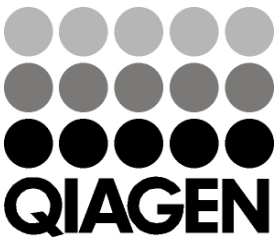

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