

SPEED-OLIGO NOVEL INFLUENZA A H1N1

SP015: Oligochromatographic test for the qualitative detection of novel influenza A H1N1 in clinical samples. 40 tests.

INTRODUCTION:

Influenza virus, the causal agent of influenza or flu, is a negative-sense single-strand RNA enveloped virus of the Orthomyxoviridae family. Characteristic symptoms of influenza in adults are: high fever, headache, photophobia, sore throat, cough, malaise and myalgia. Fever usually lasts for three days, while cough persists for longer. Elderly patients suffering from chronic bronchopathy often present tracheobronchitis and bronchiolitis. Infants can present severe respiratory infection together with convulsions and encephalitis. Isolated cases out of the epidemic season are difficult to diagnose clinically. It is also difficult to reach a clinical diagnosis during epidemics, since it can be confused with other respiratory diseases.

There are three types of influenza viruses: A, B and C, the two first causing seasonal epidemics of disease every winter while type C is not thought to cause epidemics. Influenza A viruses are divided into subtypes based on the the hemagglutinin (H) and the neuraminidase (N) surface proteins. There are 16 hemagglutinin and 9 neuraminidase subtypes. The current subtypes of influenza A viruses found in people are A (H1N1) and A (H3N2). Influenza B viruses are not divided into subtypes. Influenza A viruses are found in many different animals (birds, pigs, horses, sea mammals), while Influenza B viruses circulate widely only among humans.

Flu viruses are thought to spread mainly from person to person through coughing or sneezing. Contagion also occurs through contact with objects contaminated with flu viruses. With seasonal flu, infected people may be contagious from one day before symptoms develop to up to 7 days after becoming sick.

Influenza virus has a unique capacity for genetic variation, based on the high variability of its surface proteins and the segmentation of its viral genome (these segments can reassort in mixed infections). Pigs have been hypothesized to act as a mixing vessel for the reassortment of influenza viruses from different origins. Cases and clusters of human infections with swine influenza viruses have been reported sporadically since the 1970s. Triple-reassortant influenza viruses (with genes from human, swine and avian viruses) have been identified in swine in the United States since 1998. On April 2009, a novel H1N1 (nH1N1) reassortant causing human infections in Mexico and USA was identified by the CDC. Continued identification of new cases indicates sustained human-to-human transmission of this novel influenza A virus. Most confirmed cases have been characterized by self-limited, uncomplicated febrile respiratory illness and symptoms similar to those of seasonal influenza; in addition, however, vomiting or diarrhea was common. Some patients required hospitalization due to more severe disease.

The novel H1N1 (nH1N1) virus may be detected by rapid antigen tests and direct fluorescence assays targeting common epitopes of Influenza A viruses, as well as isolated in cell culture. Yet, definite identification can be only carried out through sequencing (only available at reference centers) or by specific RT-PCR.

Most PCR assays have employed either labour-intensive or insensitive detection systems. SPEED-OLIGO NOVEL INFLUENZA A H1N1 is a PCR-based method coupled to a dipstick device that enables a rapid, highly sensitive and specific detection of the nH1N1 influenza A virus in clinical samples. The supplied PCR mix contains a specific oligo pair for the amplification of a fragment in the H gene region. The primers and probes have been designed, after analyzing the sequences presently available in Genebank, so that only the nH1N1 Influenza A virus is detected. The kit has been designed to be easily handled. Depending on the PCR thermocycler used, the amplification step will take between 15 and 75 minutes, whereas only 5-10 minutes are necessary for the dipstick detection. The lyophilized presentation of the PCR mix minimizes handling and pipetting procedures in order to prevent contaminations.

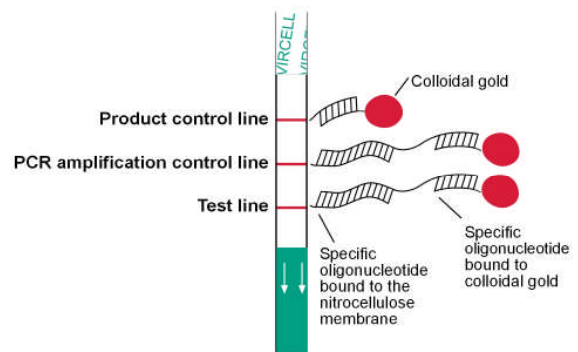
PRINCIPLE OF THE TEST:

It is based on the amplification of a specific fragment gene of nH1N1 influenza A virus, and allows a rapid diagnosis of nH1N1 influenza A virus infections.

An internal amplification control is included in the test to check the absence of carry-over of amplification inhibitors in the sample, and the correct amplification set-up. This control consists of a DNA fragment and a specific oligo pair for its amplification.

The technique is divided into 4 main steps: RNA extraction, reversotranscription, amplification with a specific oligo pair, and detection of the amplified product. The detection is performed by means of a dipstick. When the double amplification is accomplished, the amplification control and the specific test amplicon are denatured and allowed to run through the strip. Those PCR fragments react with specific oligonucleotides coupled to a colloidal suspension of gold particles. Secondly, the complex between PCR products and colloidal gold flows through the membrane up to find the specific probes (test line and PCR amplification control line), where a second hybridization takes place. The product control line appears because of the hybridization of the gold probe excess with a complementary oligonucleotide absorbed on the membrane. Red lines are visible in the positions where gold reacts.

This technique is more sensitive and specific than traditional PCR. The double hybridization enables discrimination between specific and unspecific amplification fragments.



KIT FEATURES:

This kit is based on DNA amplification and hybridization principles. Because of the risk of contamination, it is important to read carefully the "Recommendations and precautions" section.

PCR mix and positive control reagents are lyophilized. It is necessary to reconstitute them before use (see Preliminary preparation of reagents). The rest of the reagents are ready to use.

KIT CONTENTS:

- 1 VIRCELL nH1N1 PCR MIX: 5 vials containing lyophilized PCR buffer, Cl_2Mg , specific primers for nH1N1 influenza A virus, dNTPs, Taq polymerase and a DNA fragment for PCR amplification control together with the specific primers for its amplification.
- 2 VIRCELL nH1N1 POSITIVE CONTROL: 1 vial containing lyophilized non-infectious plasmidic DNA to be used as positive control.
- 3 VIRCELL NEGATIVE CONTROL: 1 vial containing 200 μl of deionized water to be used as negative control.
- 4 VIRCELL nH1N1 STRIPS: 40 strips for specific DNA detection.
- 5 VIRCELL PCR MIX RECONSTITUTION SOLUTION: 1 vial with 1 ml of aqueous solution to reconstitute the PCR mix containing Triton X-100.
- 6 VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION: 1 vial with 500 μl of aqueous solution to reconstitute the positive control, containing Triton X-100.

FOR IN VITRO DIAGNOSTIC USE

Manufacturer: VIRCELL, S.L. Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre. 18320 Santa Fe *GRANADA* SPAIN* Tel.+34.958.441264* Fax+34.958.510712
<http://www.vircell.com>

7 VIRCELL nH1N1 RUNNING SOLUTION: 2 vials containing 1 ml of hybridization solution, containing Proclin.

Store at 2-8°C and check expiration date.

Materials required but not supplied:

RNA extraction kits (see test procedure for recommendations)
 Retrotranscriptase kit (see test procedure for recommendations)
 Thermocycler
 Mineral oil (for thermocyclers without heated lid)
 Precision micropipette (10-200 µl)
 Sterile tips with aerosol barrier
 Disposable gloves
 55°C±2°C heating block
 1.5 ml microcentrifuge tubes
 Microcentrifuge
 PCR cabinet (recommended)
 Vortex

STORAGE REQUIREMENTS:

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are capped and stored at 2-8°C. After VIRCELL PCR MIX and VIRCELL POSITIVE CONTROL reconstitution, store below -20°C and avoid repeated freezing and thawing.

STABILITY OF REAGENTS ONCE OPENED:

REAGENT	STABILITY
Reconstituted VIRCELL PCR MIX and VIRCELL POSITIVE CONTROL	Store below -20°C and use until expiration date
Rest of the components	Store at 2-8°C and use until expiration date

STABILITY AND HANDLING OF REAGENTS:

The kit is stable until the expiration date at 2-8°C. After the reconstitution of the VIRCELL PCR MIX and VIRCELL POSITIVE CONTROL, those reagents are stable until the expiration date at temperature below -20°C. Handle reagents in aseptic conditions to avoid microbial contaminations. Use only the amount of reagents required for the test. Do not return the excess solution into the bottles.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

- For *in vitro* diagnostic use only. For professional use only.
- Use kit components only. VIRCELL PCR MIX RECONSTITUTION SOLUTION, VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION, and VIRCELL NEGATIVE CONTROL can be used with different VIRCELL SPEED-OLIGO kits. Do not exchange VIRCELL PCR MIX, VIRCELL POSITIVE CONTROL, VIRCELL STRIPS and VIRCELL RUNNING SOLUTION between lots and kits.
- Sterile tips with aerosol barrier are essential to prevent contamination during RNA extraction and PCR performance.
- Specimens should be handled as in the case of infectious samples using safety laboratory procedures. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
- Testing of all the samples at the earliest interval following collection will help ensure the most accurate test results. Variation in storage times during specimen shipment has not been assessed.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples.
- Avoid microbial contamination of reagents when removing aliquots from reagent tubes.
- It is essential to have three different areas to perform the test: Pre-Amplification, Amplification, and Post-Amplification or Detection areas. Workflow in the laboratory must proceed in an uni-directional

manner, beginning in the Pre-Amplification and moving to the Post-Amplification areas. Gloves must be worn in each area and disposed before leaving that area. A) Pre-amplification area: It is just for sample collection and RNA extraction. Specific equipment must be dedicated solely to pre-amplification activities (gloves, sterile tubes, barrier tips, micropipettes, microcentrifuge or other required equipment) and not used for other procedures or moved to another area. B) Amplification area: reverse transcription reaction and PCR are performed in this area and reverse transcription and PCR reagents must be handled only within. Specific equipment must be dedicated to amplification activities (gloves, sterile tubes, barrier tips, micropipettes, microcentrifuge, thermocycler, laminar air flow cabinet or other required equipment) and not used for other activities or moved between areas. After finishing the amplification, PCR tubes must never be opened in this area. C) Post-amplification or detection area: Detection is performed in this area. A thermoblock, 1.5 ml microcentrifuge tubes and a micropipette are necessary. Post-Amplification supplies and equipment must remain in the Post-Amplification area at all times.

- Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored to purity. Discard any reagents that may be suspect.
- This product should be limited to personnel who have been trained in the techniques of PCR assays.
- Do not use the kit after expiration date.
- Dispose of unused reagents and waste in accordance with all applicable regulations.
- Reagents in this kit could include genetic material or substances of animal and/or human origin. Although that material is not infectious, it should be handled as potentially infectious. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
- The developed strips must be stored in a place far away from the preamplification area to avoid a PCR contamination risk. Is recommended to cut the edges of the developed strip for a better conservation.

SPECIMEN COLLECTION AND HANDLING:

The following should be collected as soon as possible after illness onset: nasopharyngeal swab, nasal aspirate or a combined nasopharyngeal swab with oropharyngeal swab. If these specimens cannot be collected, a nasal swab or oropharyngeal swab is acceptable. For patients who are intubated, an endotracheal aspirate should also be collected. Bronchoalveolar lavage (BAL) and sputum specimens are also acceptable. Specimens should be placed into sterile viral transport media (VTM) and immediately placed on ice or cold packs or at 2-8°C (refrigerator) for transport to the laboratory. Specimens collected with swabs made of calcium alginate are not acceptable.

Upon receipt, store samples at 2-8°C until testing. Freeze at temperatures below -70°C if the specimen is to be held longer than 72 hours.

PRELIMINARY PREPARATION OF THE REAGENTS:

All reagents supplied are ready to use, except for the VIRCELL PCR MIX and the VIRCELL POSITIVE CONTROL.

4 VIRCELL PCR MIX. Each PCR mix contains enough reagents to perform 8 PCR reactions. Follow the next steps to reconstitute it:

- Add 230 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION 5 to each tube.
- Mix with vortex for 10 seconds.



You have to be sure that the PCR mix is completely homogenized

- Incubate at room temperature during 3 minutes for a complete reconstitution.

FOR IN VITRO DIAGNOSTIC USE

Manufacturer: VIRCELL, S.L. Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre. 18320 Santa Fe *GRANADA* SPAIN* Tel.+34.958.441264* Fax+34.958.510712
<http://www.vircell.com>

2 VIRCELL POSITIVE CONTROL. Follow the next steps to reconstitute it:

- Centrifuge the corresponding tube for 5 seconds at 5000 g.
- Add 200 µl of VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION 6 to the tube.
- Mix with vortex for 10 seconds.
- Centrifuge the tube for 5 seconds at 5000 g.
- Incubate at room temperature during 3 minutes for a complete reconstitution.

After reconstitution, VIRCELL PCR MIX 1 and VIRCELL POSITIVE CONTROL 2 can be frozen at temperature below -20°C to be used in subsequent reactions.

ASSAY PROCEDURE:

The number of samples processed per kit will vary based upon run size strategy.

	ENSAYOS	NÚMERO DE CONTROLES POSITIVOS	NÚMERO DE CONTROLES NEGATIVOS	NÚMERO DE MUESTRAS	TOTAL
40 TIRAS	1	1	1	38	40
	2	2	2	36	40
	4	4	4	32	40
	8	8	8	24	40

TEST PROCEDURE:

1.-RNA extraction: (performed in the Pre-Amplification area).

It is recommended to use commercial extraction kits following manufacturer instructions for RNA extraction. This kit has been tested out with the following RNA extraction kit: QIAamp viral RNA mini kit (QIAGEN). Other commercial kits may be used. Consult with technical service.

2.-Retrotranscription: (performed in the Amplification area):

It has been tested 2 retrotranscriptase kits obtaining good results:

- iScript cDNA Synthesis kit (Biorad), following the manufacturer instructions. This kit includes a mix ready to use, containing all the reagents required to perform the reaction, even the dNTPs and the hexanucleotides.
- AffinityScript Multiple temperature Reverse Transcriptase (Stratagene) using Random Hexamer Primers (Fermentas) to a final concentration in the reaction of 5 µM.

3.-PCR (performed in the Amplification area):

The PCR mix 1 is lyophilized. Each vial contains the components needed for 8 reactions. One or more PCR mix tubes must be reconstituted (see "preliminary preparation of the reagents") depending of the number of samples to be analyzed and the number of controls to be run. Allocate in a rack the number of tubes needed: one tube per sample plus one tube for the positive control and one tube for the negative control. The excess of PCR mix can be frozen at temperature below -20°C to be used in subsequent reactions.

The negative control should be the last sample prepared for a given assay.

- Pipette 23 µl of reconstituted PCR mix per tube.
- Add 2 µl of each RT product or control to each tube. The negative control supplied in the kit is water.
- Insert the PCR tubes in the thermocycler and run the following program*:

1 cycle	92°C	1 minute
40 cycles	92°C	20 seconds
	55°C	20 seconds
1 cycle	72°C	20 seconds
	72°C	1 minute
1 cycle	95°C	1 minute

*(follow the instructions provided by the manufacturer of the thermocycler).

Detection of the PCR products will be achieved by means of a dipstick device. If the hybridization is not immediately performed, the PCR tubes can be frozen at temperature below -20°C. Subsequently, in order to develop the hybridization it is necessary to perform once again the PCR final denaturation step.

Step 1	95°C	1 minute
--------	------	----------

4.-Strip detection: (performed in the Post-Amplification area).

Before performing the strip hybridization, it is necessary to preheat the thermoblock at 55°C.

- Add 35 µl of VIRCELL RUNNING SOLUTION 7 in a 1.5 ml tube* and incubate at 55°C for 2 minutes.
- Denaturalize the sample at 95°C for 1 minute and put it immediately on ice or in an isofreeze rack at 4°C. Do not leave the samples be in a rack at room temperature after the denaturation step at 95°C. In the case you do not have an isofreeze rack or ice, you can pipette the sample without cooling it just after the denaturation step at 95°C and introduce the strip immediately.
- Add 5 µl of denatured PCR product cooled at 4°C. The samples cannot be for more than 1 minute at 4°C without being added to the 1.5 ml tube.



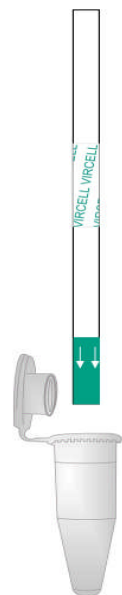
If the product of PCR is not added to the 1.5 ml tube immediately after the final denaturation step of the PCR program, it would be necessary to repeat once more this step (95°C for 1 minute) in order to put it at 4°C on ice or in an isofreeze rack.

In the eventuality of analyzing a lot of samples simultaneously, to avoid more than 1 minute passing from the denaturation step to the strip detection, it is recommended to detect the samples in groups of 3 to 5. In this way, we avoid false negatives to appear.

- Immediately insert the strip in the correct orientation (the arrows pointing towards the sample: see the scheme) and incubate at 55°C for 5 minutes.

FOR IN VITRO DIAGNOSTIC USE

Manufacturer: VIRCELL, S.L. Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre. 18320 Santa Fe *GRANADA* SPAIN* Tel.+34.958.441264* Fax+34.958.510712
<http://www.vircell.com>



- Remove the strip and read the result.
- *The 1.5 ml tube must fit in the thermoblock.



Interpretation of the results must be performed just after removing the strip. Drying may cause changes in the signal intensity and some background may appear in negative samples. The developed strips must be stored in a place far away from the preamplification area to avoid a PCR contamination risk.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control testing before releasing, complying with highly strict specifications. Final quality control results for each particular lot are available.

Use a negative control with each run of the kit, starting from the nucleic acid isolation step to check for PCR product contamination.

INTERPRETATION OF RESULTS AND VALIDATION PROTOCOL FOR USERS:

One negative control must be always included within each test run performed. One positive control should be included within each test run performed. When a new kit is opened the positive control must be run at least one time. The positive control monitors for reagent failures and for correct operation of essential procedure. The negative control will monitor for reagents or environmental contamination.

Place the strip in the position indicated as S on the reading card in order to read the result.

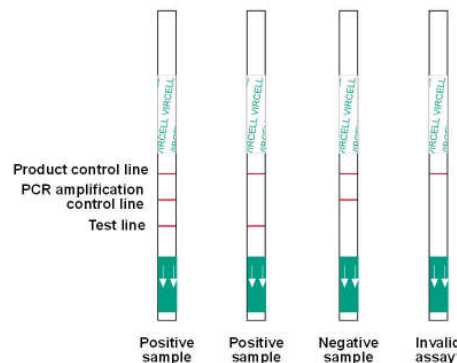
The test includes 3 reading areas:

- Product control line: It must be always positive and legible if the test has been performed correctly. This line indicates that the colloidal gold works accurately, the probe viability is adequate and the running buffer flows properly.
- PCR amplification control line: The absence of a red band in this position indicates the presence of inhibitors in the sample that may have interfered with the amplification reaction. In that case a second aliquot of the specimen or a new specimen should be retested. In strong positive

samples the line could be weak or negative, due to high contents of specific target in the sample but not invalidating the final result.

- Test line: A red band in this position indicates the presence of nH1N1 influenza A virus genetic material in the sample.

The product control line must be always present and readable, otherwise the assay is invalid.



*possible presence of PCR inhibitors

LIMITATIONS:

- 1.-This kit is intended to be used with human clinical samples.
- 2.-The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful handling and timing and temperatures of the incubation steps are essential for accurate results. It is specialty important to perform each assay step in the dedicated areas described in the assay protocol.
- 3.-This test will not indicate the site of infection. It is not intended to replace isolation.
- 4.-Detection of the microorganism depends on the number of organisms present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and/or strain.
- 5.-As with any diagnostic test, results must be interpreted with consideration of all clinical and laboratory findings. The kit results may be used in conjunction with clinical evaluation and other diagnostic procedures.
- 6.-Use of this product should be limited only to personnel trained in the PCR techniques.
- 7.-The test provides qualitative results. No correlation can be drawn between the magnitude of a positive result and the number of microorganisms in the sample.
- 8.-This test has been verified for use with human clinical samples. This test has not been verified with other sample types.
- 9.-Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
- 10.-Performance characteristics have not been determined for all genotypes.
- 11.-The test only works within the limits of the genomic regions from which the probes have been chosen. Due to the high variability of influenza A genome it is possible the appearance of variants that might not be detected. Changes in PCR oligos or probes sequences of oligonucleotides of microorganism may lead to false negative results.
- 12.-A negative test result does not exclude the presence of the target organism at levels below the detection limit of the assay.
- 13.-A positive test does not rule out the possibility that other pathogens may be present.
- 14.-The internal control included in the assay will not eliminate all false negative test results.
- 15.-Detection of influenza A nH1N1 virus in the samples is not necessarily indicative of a causative role of the pathogen in a given episode of the disease. However, the constellation of clinical evidence of a pathology and a positive PCR result points to an etiology by the microorganism detected.

FOR IN VITRO DIAGNOSTIC USE
 Manufacturer: VIRCELL, S.L. Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre. 18320 Santa Fe *GRANADA* SPAIN* Tel.+34.958.441264* Fax+34.958.510712
<http://www.vircell.com>

PERFORMANCE

SENSITIVITY AND SPECIFICITY:

150 samples collected before to the appearance of the nH1N1 influenza A virus and 103 samples collected after to the appearance of the nH1N1 influenza A virus analyzed by qRT-PCR were processed and assayed. The results were as follows:

Sensitivity: 97.5%
Specificity: 100%

Analytical Sensitivity:

Serial dilutions of a purified plasmidic DNA containing a sequence from the H1 gene of nH1N1 influenza A virus were made. They were processed and assayed. The kit was able to detect up to 1 copies of DNA per reaction.

INTRA-ASSAY PRECISION:

2 samples (one positive close to the detection limit and one negative) were amplified 5 times in a single assay performed by the same operator in essentially unchanged conditions. The same results were observed in all the assays.

INTER-ASSAY PRECISION:






2 samples (one positive close to the detection limit and one negative) were individually amplified on 3 consecutive days by 2 different operators. The same results were observed in all the assays.














CROSS REACTIVITY AND INTERFERENCES:

The test was checked with samples spiked with respiratory viruses (Adenovirus, Influenza A (H5N1), Influenza A (H3N2), Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Respiratory Syncytial Virus). No false positive results were obtained.

Virus	Strain	Source
Adenovirus	Adenoid 71	ATCC VR-1
Influenza A (H5N1)	A/Vietnam/1194/2004 NIBRG-14	NIBSC 07/252
Influenza A (H3N2)	A/VICTORIA/3/75	ATCC VR-822
Influenza B	B/HONG KONG/5/72	ATCC VR-823
Parainfluenza 1	Sendai/52	ATCC VR-105
Parainfluenza 2	Greer	ATCC VR-92
Parainfluenza 3	C 243	ATCC VR-93
Respiratory Syncytial Virus	Long	ATCC VR-26

SYMBOLS USED IN LABELS:

	In vitro diagnostic medical device
	Use by (expiration date)
	Store at 2-8°C
	Contains sufficient for <X> tests
	Batch code

	Catalogue number
	Consult instructions for use
	Reconstitute in <X> µl
	Reading card for monoline test
	Interpretation card
	Product Control Line
	PCR Amplification Control Line
	Test Line
	Sample
	Positive Sample
	Negative Sample
	Invalid Assay
	Date

BIBLIOGRAPHY:

1. Belshe., R. B. 2009 Implications of the emergence of a novel H1 Influenza virus. N Engl J Med Editorial:1-2.
2. CDC. H1N1 Flu. Infectious Disease Information by the Centers for Disease Control and Prevention. (<http://www.cdc.gov/h1n1flu/>).
3. Choi, Y.K., Goyal, S.M., Kang, S.W., Farnham, M.W. and Joo, H.S. 2002 Detection and subtyping of swine influenza H1N1, H1N2 and H3N2 viruses in clinical samples using two multiplex RT-PCR assays. J Virol. Methods 102:53-59.
4. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team 2009 Emergence of a novel swine-origin Influenza A (H1N1) virus in humans. N Engl J Med. 361:1-10.
5. Stone, B., Burrows, J., Schepetiuk, S., Higgins, G., Hampson, A., Shaw, R and Kok, T.W. 2004 Rapid detection and simultaneous subtype differentiation of influenza A viruses by real time PCR. J Vir Methods 117:103-112.
6. Taubenberger, J.K., Layne, S.P. 2001 Diagnosis of Influenza virus: Coming to grips with the molecular era. Mol Diag 6(4):291-305.

For any question please contact:
customerservice@vircell.com

REVISED: June-09

FOR IN VITRO DIAGNOSTIC USE

Manufacturer: VIRCELL, S.L. Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre. 18320 Santa Fe *GRANADA* SPAIN* Tel.+34.958.441264* Fax+34.958.510712
<http://www.vircell.com>