IVD For *in Vitro* Diagnostic Use

CE

ARVI Plus Real-TM

Handbook

Multiplex RT-PCR detection and identification of 16 respiratory viruses: SARS-Cov-2 coronavirus, Influenza A virus, Influenza B virus, Human parainfluenza virus-1-2-3-4 RNA, OC43, 229E, NL63, and HKUI human coronavirus (hCov) RNA, Human bocavirus, Human rhinovirus, hRSV, human Adenovirus, human metapneumovirus

REF **V439-48FRT**

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NAME

ARVI Plus Real-TM

INTRODUCTION

Most acute respiratory diseases are viral infections. Acute respiratory viral infections (ARVI) are the most frequent illnesses experienced by most people globally. Young children have between five and seven of these illnesses per year, with a general decrease in frequency with increasing age. Adults will have about two such illnesses per year.

A wide variety of viruses, containing both RNA and DNA, cause acute respiratory infections. The most important viruses are rhinoviruses, coronaviruses, parainfluenza viruses, respiratory syncytial virus, adenoviruses, metapneumoviruses.

Rhinoviruses are the most frequent cause of the common cold. They circulate all year and are the major cause for the major autumn increase in respiratory illness. Multiple types of virus cause this rhinovirus outbreak, which is different from the typical outbreak of an infectious disease, in which only one viral type is responsible. Coronaviruses are also important agents of the common cold. Scientists have had difficulty in the laboratory working with these viruses, and the usual methods of virus detection have not yielded complete results. There are at least two major types of coronaviruses, each of which appears to occur over a limited period of time, usually in winter and spring.

There are four types of parainfluenza viruses. Parainfluenza type 4 is an agent that is sporadic in occurrence, producing little more than the common cold. As such, its activity is rarely described except when the virus is encountered as part of a comprehensive investigation in which a variety of different agents are sought. In contrast, whenever the severe respiratory illnesses causing hospitalization of young children are studied, parainfluenza types 1, 2, and 3, and RSV, are identified. Both types 1 and 2 most typically cause laryngotracheobronchitis, more commonly called croup. First infection with these viruses is essentially universal by three to four years of age, and, based on the number of infants and young children requiring medical attention, it can be estimated that well over 10 percent of children first encountering these viruses require medical attention specific for this syndrome. Like all respiratory viruses, these agents reinfect repeatedly throughout life, with later infections becoming milder or asymptomatic. Parainfluenza type 3 does not produce this syndrome, but rather one of pneumonia, often with features of obstruction. Again, first infection is universal at an early age, but only occasionally results in the most severe of the potential manifestations of the disease. Reinfection with or without symptoms also recurs throughout life.

INTENDED USE

ARVI Plus Real-TM PCR kit is an in vitro nucleic acid amplification test for multiplex detection and identification of specific nucleic acid fragments of pathogens that cause acute respiratory viral infections – SARS-Cov-2 coronavirus, Influenza A virus, Influenza B virus, Human parainfluenza virus-1-2-3-4 RNA, OC43, 229E, NL63, and HKUI human coronavirus (hCov) RNA, Human bocavirus, Human rhinovirus, hRSV, human Adenovirus, human metapneumovirus– in the clinical material (nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm) by using Real-time PCR detection.

PRINCIPLE OF ASSAY

The implemented method of reverse transcription followed by polymerase chain reaction is based on RNA reverse transcription process and subsequent amplification of cDNA and DNA. The RNA reverse transcription stage and PCR amplification of cDNA/DNA stage are performed in one test tube, that increases the sensitivity of the method, reduces the likelihood of contamination and reduces the time of the study. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **ARVI Plus Real-TM** Kit is based on fluorescent modification of the PCR method. The PCR-mix contains four target-specific probes bearing reporter fluorescent dyes (Fam, Hex, Rox and Cy5) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The **ARVI Plus Real-TM** Kit includes the Internal control RNA-IC “A”, which is intended to assess the quality of the RNA extraction and polymerase chain reaction. DNA probe used for the detection of the specific amplification product includes the fluorescent dyes Fam, Rox or Cy5. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The application of four fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube.

Defined tubes contain additional probe with Rox dye label – “Marker”. It tags the strip orientation. Upon completion of run, software defines actual position of the strip (by means of “marker” position) relative to the position preset by the operator. If it mismatches, the software suggests rearrangement of the tubes by default. In accordance with the operator, order can be rearranged and saved in new file. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

No of tube in a strip	Dye label/detection channel				Color labeling of the buffer
	Fam	**Hex**	**Rox**	**Cy5**	
1	Influenza A virus	RNA-IC	SARS-CoV-2 coronavirus, E, N - genes	Influenza B virus	Blue
2	Human parainfluenza virus type 2	RNA-IC	Human parainfluenza virus type 4	Human coronavirus 229E	Colorless
3	Human bocavirus	RNA-IC	Marker	Human rhinovirus	
4	Human respiratory syncytial virus	RNA-IC	–	Human coronavirus HKU1	
5	Human adenovirus	RNA-IC	–	Human coronavirus NL63	
6	Human coronavirus OC43	RNA-IC	–	Human parainfluenza virus type 3	
7	Human parainfluenza virus type 1	RNA-IC	–	–	
8	Human metapneumovirus	RNA-IC	–	–	

The automatic analysis available on SaCycler-96 instrument.

MATERIALS PROVIDED

- **Strips-ARVI**, 48 8-tube strips (15 µl in each tube), including optical strip caps;
- **RT-PCR-buffer**, 6 x 1,0 ml;
- **Enzyme Taq/RT**, 2 x 0,1 ml;
- **Internal Control RNA-IC "A"**,** 2 x 0,25 ml;
- **Pos Control**, 2 x 0,320 ml;
- **Negative Control***, 1,0 ml*;

Contains reagents for 48 tests.

* *must be used in the isolation procedure as Negative Control of Extraction.*
** *add 10 µl of Internal Control RNA during the RNA purification procedure directly to the sample/lysis mixture*

MATERIALS REQUIRED BUT NOT PROVIDED

- RNA/DNA extraction kit
- Real Time qPCR Thermalcycler instrument
- Workstation
- Pipettes with aerosol barrier
- Tubes and tubes racks

STORAGE INSTRUCTIONS

All components of **ARVI Plus Real-TM Kit**, except the Enzyme Taq/RT, must be stored at temperatures from 2 °C to 8 °C during the storage period. The PCR-mix for amplification must be stored out of light at temperatures from 2 °C to 8 °C during the storage period. The excessive temperature and light can be detrimental to product performance. The Enzyme Taq/RT must be stored at -18 °C/-22 °C during the storage period.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at

temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except the Enzyme Taq/RT, is allowed in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the Enzyme Taq/RT in thermobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit, except the Enzyme Taq/RT, should be stored at temperatures from 2 °C to 8 °C during the storage period; PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- Enzyme Taq/RT should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

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STABILITY

ARVI Plus Real-TM is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

WARNINGS AND PRECAUTIONS

IVD

***In Vitro* Diagnostic Medical Device**

For *In Vitro* Diagnostic Use Only

The user should always pay attention to the following:

- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

- *Sputum, bronchial or tracheal lavage* must be treated with the following procedure:
  - Collect sputum into 50 mL single-use PP tubes with a screw cap.
  - In a biological safety cabinet, homogenize samples after mixing with equal volume of 4% NaOH solution. (*N-acetyl-L-cysteine may be added if required in the amount of 50-70 mg per sample).* Mix intensely with a tube rotator for 5-20 minutes (depending on the density of a sample).
  - Centrifuge samples at 3000 rpm (2800-3000 g) for 15 min and carefully discard the supernatant leaving 500-1000 µl in the tube. Resuspend sediment and transfer it into a 1.5 ml tube.
  - Centrifuge samples at 12000 rpm for 5-10 min, discard the supernatant and use the same 1,5 ml sample tube for DNA isolation from sample sediment.
- *tissue* (~1,0 gr) homogenized with mechanical homogenizer or scalpel, glass sticks, teflon pestles and dissolved in 1,0 ml of saline water or PBS sterile (1 volume of tissue to 1 volumes of saline solution). Vortex vigorously and incubate 30 min at room temperature. Transfer the supernatant into a new 1,5 ml tube;
- *nasopharyngeal and orophayngeal swabs*: swab area and place in “Eppendorf” tube with 0,5 ml of saline water or PBS sterile (Sacace Transport medium is recommended). Agitate vigorously. Repeat the swab and agitate in the same tube. Centrifuge at 1000g/min for 5 min. Discard the supernatant and leave about 100 µl of solution for RNA extraction.

Specimens can be stored at +2-8°C for no longer than 48 hours, or freeze at -20°C to -80°C.
Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

RNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the “SAMPLE COLLECTION, STORAGE AND TRANSPORT” paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

- ⇨ **DNA/RNA-Prep NA** (Sacace, REF K-2-9/2)
- ⇨ **SaMag Viral Nucleic Acids Extraction kit** (Sacace, REF SM003)

Please carry out RNA extraction according to the manufacture’s instruction.

Add 10 µl of Internal Control (**RNA IC)** during isolation procedure directly to the sample/lysis mixture.

RT AND AMPLIFICATION

Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

1.1.1 Mark the required number of strips with paraffin sealed PCR-mix: 1 strip for each sample to be tested, 1 strip for positive control (C+) and 1 strip for negative control (C-).

Example: to test 6 samples, mark 6 strips (one for each sample), one for “C-” and one for “C+”). The resulting number of strips is 8.

1.1.2 Vortex the RT-PCR-buffer and Enzyme Taq/RT thoroughly for 3-5 seconds, then spin for 1-3 seconds.

ATTENTION! Enzyme Taq/RT should be got out from the freezer immediately prior to use

1.1.3 Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT. Add to the one tube:

– 15 x (N+1) µL of RT-PCR-buffer;

– 0.5 x (N+1) µL of Enzyme Taq/RT,

– N is a quantity of tubes in the marked strips.

Example: to test 6 samples, mark 8 strips. Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT for 65 (64+1) tubes. Mix 975 µL of RT-PCR-buffer and 32.5 µL of Enzyme Taq/RT.

ATTENTION! Taking the Enzyme Taq/RT, it is necessary to dip the tip no more than 1.0 mm and observe the rules for dosing viscous liquids. Thoroughly flush the remaining Enzyme Taq/RT from the tip by pipetting at least 5 times.

1.1.4 Vortex the tube with the mixture of RT-PCR-buffer and Enzyme Taq/RT thoroughly for 3-5 seconds, then spin for 1-3 seconds.

ATTENTION! Mixture of RT-PCR-buffer and Enzyme Taq/RT must be prepared immediately prior to use and should be used within one hour after preparation. If it is needed, the prepared mixture can be stored at the temperatures from 2 °C to 8 °C but for no longer than one hour.

1.1.5 Add 15 µL of the RT-PCR-buffer and Enzyme Taq/RT mixture into each tube. Avoid paraffin layer break. Close the strips.

1.1.6 Vortex the tubes with samples, “C-“ and “C+” for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! Open the tube, add NA sample (or control sample), then close the strip before

proceeding to the next RNA sample to prevent contamination. Close the strips tightly. Use filter tips

1.1.7 Add 10 µL of the NA sample into corresponding tubes. Do not add NA into the "C-", "C+" strips. Avoid paraffin layer break.

1.1.8 Add 10 µL of negative control sample (C-), which passed whole NA extraction procedures into corresponding strip. Add 10 µL of positive control sample (C+) into corresponding strip. Avoid paraffin layer break.

1.1.9 Spin down the strips for 3-5 seconds to collect drops.

1.1.10 Set the strips into the Real-time Thermal Cycler.

1.1.11 Launch the RealTime_PCR program.

Table. The PCR program

Step	Temperature, °C	Min.	Sec.	Number of cycles	Fluorescence detection	Type of the step
1	35	20	0	1		Cycle
2	95	5	0	1		Cycle
3	94	0	10	5		Cycle
	64	0	10			
4	94	0	5	45		Cycle
	64	0	10*		X**	
√ - optical measurement						

*[1] For example, SaCycler-96™ (Sacace), CFX-96™*** Deep Well / iQ5™ (BioRad); Mx3005P™/Mx3000P™ (Agilent), ABI® 7500 Real Time PCR (Applied Biosystems);*

** On ABI® 7500 Real Time PCR instrument, please set the fluorescence acquisition time to 30 seconds.*

*** Fluorescence detection on channels FAM/Green, Joe/HEX/Yellow, ROX/Orange, Cy5/Red*

NOTE: FOR CFX-96 and other plate type instruments: it is recommended to use at least two additional empty strips placing them in the last left and right columns of the thermal block to better uniform the thermolid pressure in case of not filling the complete plate.

NOTE: The automatic analysis available on SaCycler-96 instrument.

CONTROLS

The **ARVI Plus Real-TM Kit** contains positive control sample. Positive control is a cloned part of the virus genome. It is produced with genetic engineering techniques and characterized by automatic sequencing. The kit includes the Internal control RNA-IC “A”. RNA-IC “A” is intended to assess the quality of the NA extraction and polymerase chain reaction. To reveal possible contamination a negative control is required.

ATTENTION! A negative control sample should go through all stages of NA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

For **ARVI Plus Real-TM Kit** the test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not taken into account;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

For **ARVI Plus Real-TM Kit** the test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not observed.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

DATA ANALYSIS

Registration of the PCR results is held in automatic mode. Analysis will be performed by Real-Time PCR application. The interpretation should be performed in accordance with Table 5.

Table 5. The interpretation of assay results for control samples

Detection channel				**Interpretation**
Fam	Hex	Rox	Cy5	
Positive control sample				
Cp is specified (Strip test tubes №№1-8)	Cp is not specified	**Cp is specified (Strip test tubes №№1-2)**	**Cp is specified (Strip test tubes №№1-6)**	Positive result The results are valid
Negative control sample				
Cp/Ct is not specified	**Cp is specified**	Cp is not specified	Cp is not specified	Negative result The results are valid

In the samples of human biological material with target viruses NA, the Real-time PCR thermal cycler should register an increase in fluorescence on the corresponding detection channels (Fam, Rox or Cy5), see Table 6. It is necessary to take into account the possibility of the presence in the sample of nucleic acids of several causative agents of acute viral respiratory infections, including those detected in one amplification tube.

In the samples of human biological material free of target viruses NA, the Real-time PCR thermal cycler should register an increase in fluorescence on the Hex (Internal control sample)

detection channel, the increase in fluorescence on the Fam, Rox, and Cy5 channels should be absent.

The results are considered as unreliable (Invalid) if there is no exponential increase in fluorescence on the Fam, Rox, and Cy5 channels (specific product) and on the Hex channel (Internal control sample).

Table 6. The interpretation of assay results for PCR

Detection channel				**Interpretation**
Fam	Hex	Rox	Cy5	
Strip test tube №1				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Influenza A virus RNA is detected**
Cp is not specified	Is not considered	**Cp is specified**	Cp is not specified	**SARS-CoV-2 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Influenza B virus RNA is detected**
Strip test tube №2				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human parainfluenza virus type 2 RNA is detected**
Cp is not specified	Is not considered	**Cp is specified**	Cp is not specified	**Human parainfluenza virus type 4 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus 229E RNA is detected**
Strip test tube №3				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human bocavirus DNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human rhinovirus RNA is detected**
Strip test tube №4				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human respiratory syncytial virus RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus HKU1 RNA is detected**
Strip test tube №5				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human adenovirus DNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus NL63 RNA is detected**
Strip test tube №6				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human coronavirus OC43 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human parainfluenza virus type 3 RNA is detected**
Strip test tube №7				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human parainfluenza virus type 1 RNA is detected**
Strip test tube №8				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human metapneumovirus RNA is detected**
For all test tubes				
Cp is not specified	**Cp is specified**	Cp is not specified	Cp is not specified	Target viruses RNA is not detected
Cp is not specified	Cp is not specified	Cp is not specified	Cp is not specified	Unreliable result

Unreliable results may be caused by the presence of inhibitors in the nucleic acid preparation obtained from the clinical material, errors in the pre-analytical stage, incorrect implementation of the analysis Protocol, non-compliance with the temperature mode of amplification, etc. In this case, either re-staging of reverse transcription and polymerase chain reaction, or re-extracting of the nucleic acid preparation, or re-collect of clinical material (performed sequentially) is required.

When the expressed growing fluorescence (Cp is not specified) through the Fam, Rox, or Cy5 channels is not expressed for positive control (C+), the results of whole series are considered false. It is required to repeat the whole test.

When the expressed growing fluorescence (Cp is specified) through the Fam, Rox, or Cy5 channels is expressed for negative control (C-), the results of whole series are considered false. It is required to eliminate contamination.

ATTENTION! A single negative test result, especially if it is a sample from the upper respiratory tract, does not exclude infection.

ATTENTION! Negative results should not be used as the sole basis for making a decision about the treatment of patients.

If in the samples of human biological material the Real-time PCR thermal cycler registers an increase in fluorescence for the specific product earlier than 25 cycle for Cp, this indicates a high initial NA concentration of the corresponding pathogen. In this case, it is possible to obtain a false negative result during mixed infection for a pathogen whose NA is present in a low concentration. To exclude false negative results, it is recommended to repeat RT-PCR for the extracted NA preparation using the kit for individual detection of the corresponding virus.

SPECIFICATIONS

a. The analytical specificity of the **ARVI Plus Real-TM Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

Since it is impossible to exclude the occurrence of new mutations in the genome of the SARS-CoV-2 coronavirus, two genome sites were selected as targets to improve the reliability of diagnostics: the N and E genes sites.

In the samples of human biological material with target viruses NA, the detecting amplifier should register an increase in fluorescence on the corresponding detection channels.

In the samples of human biological material free of target viruses NA, the detecting amplifier should register an increase in fluorescence on the Hex/Yellow detection channel, the increase in fluorescence on the Fam, Rox, and Cy5 channels should be absent.

There are not cross-nonspecific reactions of each of the oligonucleotide systems included in the kit in relation to viruses determined by other systems.

There are not non-specific positive results of amplification DNA of *Mycoplasma pneumoniae, Streptococcus pneumoniae, Chlamydophila pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Moraxella catarrhalis, Bordetella pertussis, Bordetella parapertussis*, as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

Analytical sensitivity of the **ARVI Plus Real-TM** Kit is 10 copies of DNA per amplification tube ($2,0x10^3$ copies/mL DNA sample). Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

TROUBLESHOOTING

Table 6. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* CFX™ and iQ5™ are registered trademarks of Bio-Rad Laboratories
* MX3005P® is a registered trademark of Agilent Technologies
* ABI® is a registered trademark of Applied Biosystems

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