AutoGenomics

INFINITI® RVP Plus Assay

Directional Package Insert (DPI)

For *In Vitro* Diagnostic Use

CE

FOR EXPORT ONLY

Manufactured by AutoGenomics, Inc., 1600 Faraday Avenue ,Carlsbad , CA USA 92008

Authorized EU Agent: Medical Device Safety Service GmbH (MDSS)
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INTENDED USE

The INFINITI® RVP Plus Assay is an *in vitro* diagnostic device designed to detect and identify certain respiratory viruses in human nasopharyngeal aspirates (NPA). The INFINITI RVP Plus is used in conjunction with clinical and epidemiological information to identify infected patients.

This test is not indicated for stand-alone diagnostic purposes and results should be used in conjunction with other available laboratory and clinical information.

DESCRIPTION OF TEST

The INFINITI RVP Plus is an *in vitro* diagnostic device designed to detect and identify the following respiratory viruses in human nasopharyngeal aspirates (NPA):

Virus	Viral Type
Influenza	A, A-Swine H1N1and B
Human Parainfluenza Virus (HPIV)	1, 2, 3, 4
Rhinovirus	A and B
Enterovirus	A, B, C, D
Coronavirus	HKU1, OC43, NL63, 229E
Human Metapneumovirus (HMPV)	A and B
Human Respiratory Syncytial (HRSV)	A and B
Adenovirus	A, B, C, E

The INFINITI RVP Plus Assay consists of a multiplex PCR test followed by primer extension and microarray hybridization in an integrated molecular diagnostic device, the INFINITI® Analyzer or INFINITI® PLUS Analyzer. INFINITI Analyzer and INFINITI PLUS Analyzer are CE marked.

The INFINITI RVP Plus Assay consists of the following six major processes:

- Reverse transcription of viral RNA.
- Multiplex PCR amplification of cDNA.
- Fluorescent labeling of the amplified product using specific detection primer extension (DPE).
- Hybridization of the labeled amplified product to a microarray.
- Scanning of the microarray.
- Signal detection and analysis.

Reverse transcription and multiplex PCR amplification is performed off-line with cDNA. The rest of the processes are automated by the INFINITI Analyzer or INFINITI PLUS Analyzer.

WARNINGS AND PRECAUTIONS

Handling Requirements

- **For *in vitro* diagnostic use. To be used by qualified laboratory personnel.**
- This test is to be used only with human nasopharyngeal aspirates.
- All patient specimens are potentially hazardous and care should be taken when handling materials of human origin. **Follow the CLSI Guidelines (Molecular Diagnostics Methods for Infectious Diseases; Approved Guidelines; MM3-A).**
- Upon receipt of samples, visually inspect sample condition. Specifically, look for abnormal signs that indicate that sample integrity has been compromised (e.g., evaporation, decrease in volume, precipitation, spills, discoloration, sedimentation, separation, turbidity, etc.). If you observe or suspect any sample abnormality, do not perform any test.
- Samples should be handled with extreme caution to prevent contamination, spillage, sample mix-up. Sample containers should be labeled clearly to prevent mix-up.
- Store samples at the specified conditions.

- To minimize the risk of cross contamination, sample preparation, PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as CLSI (Molecular Diagnostic Methods for Genetic Diseases: Approved Guideline).
- Do not pool/mix reagents from different lots.
- Do not use a kit or reagent past its expiration date.
- Store kits and reagents according to the product label.

Laboratory Procedures
- Follow normal precautions for handling laboratory reagents.
- Follow safe laboratory procedures: do not pipette by mouth; wear protective clothing (e.g., disposable gloves laboratory coats) and eye protection; do not eat, drink or smoke in the laboratory work areas; wash hands thoroughly after handling samples and reagents.

Waste Handling
- Dispose of unused reagents, specimens and waste according to applicable country, federal, state and local regulations.
- Safety data Sheets (SDS) are available upon request from AutoGenomics Customer Service.

INFINITI Analyzer and INFINITI PLUS Analyzer
- **Read the Operator's Manuals before operating the instruments (**INFINITI Analyzer (EM-34000) or INFINITI PLUS Analyzer. (EM-34041))**.** Pay particular attention to "Notes".
- Follow the Caution and Safety Warning in the Operator's Manual.
- Refer to the Installation Requirements Section when installing the instrument.
- Refer to the Errors Section when errors are encountered while operating the instrument.
- Refer to the Help Section when problems are encountered.

QUALITY CONTROL
- Maintain calibration of thermocycler according to manufacturer's specifications.
- Maintain calibration of INFINITI or INFINITI PLUS Analyzer according to AutoGenomics' specifications.
- Maintain calibration of pipettes according to manufacturer's specifications.

STORAGE / STABILITY

BioFilmChip® Microarray: 24 months Refrigerated (2 to 8°C)

Intellipac® Reagent: 12 months Refrigerated (2 to 8°C)

Note: Remove the Intellipac from the Analyzer and store refrigerated as soon as possible. Do not use after Intellipac has been opened for four weeks.

Amplification Mix: 18 months Frozen (-30 to -15°C)

Note: Specific product expiration date is printed on the product label.

REAGENTS PROVIDED (SUFFICIENT FOR 48 TESTS)
- AutoGenomics Product Number 04-1110-02 INFINITI RVP Plus Assay Magazine - BioFilmChip® Microarray: 4 magazines per box
- AutoGenomics Product Number 04-2110-02 INFINITI RVP Plus Assay Intellipac® Reagent Module: 24 tests per Intellipac which contains:
  - 1.1ml ASP Master Mix:
    - dNTPs
    - Labeled -dCTP
    - Allele Specific Primers
    - Extension reaction buffer

2.6ml Hybridization Buffer
SSC
Hybridization Positive Control
Sodium Azide Preservative 0.08%

- AutoGenomics Product Number 04-3110-02 INFINITI RVP Plus Assay Amplification Mix: Each box contains: 4 x 250µl vials of PCR reaction master mix containing:
dNTPs
Multiplex Primers Mix
$MgCl_2$
Reaction Buffer

REAGENTS REQUIRED BUT NOT PROVIDED BY AUTOGENOMICS

- RNA extraction kit (QIAamp Viral mini kit, Qiagen Catalog Number 52906)
- Armored –RNA: Armored RNA-Hepatitis C Virus (Genotype 1a) in TSM III Buffer, 250 µl (Asuragen Diagnostics, Catalog Number 42004)
- Reverse Transcription kit: SuperScript III Supermix kit for 50 reactions (Invitrogen Catalog Number 18080400).
- Platinum Taq DNA polymerase (Invitrogen, Catalog Number 10966034)
- Titanium Taq DNA polymerase (Clontech Catalog Number 639209)
- SAP (USB Catalog Number 70092Z)
- EXO I (USB Catalog Number 70073Z)
- Distilled water (DNAse and RNAse free)

EQUIPMENT AND CONSUMABLES

The following equipment is required but not provided with the assay reagents

- Pipettors
- Mini Centrifuge
- Pipette tips
- Microfuge tube Racks
- Thermocycler
- Vortex
- 0.2 ml thin wall tubes for PCR
- 1.5 ml micro centrifuge tubes
- 8-well Flat Strip Caps (Genesee Scientific, Catalog No. 22-623)
- AutoGenomics Product Number 11-0060-00: INFINITI Waste tray Stir Bars
- AutoGenomics Product Number 11-0020-00: INFINITI Waste Tray Liners
- AutoGenomics Product Number 11-0080-00: INFINITI Static Free Pipette Tips

- **FOR INFINITI Analyzer***:*
  - AutoGenomics Product Number 10-0010-99: INFINITI Analyzer
  - AutoGenomics Product Number 11-0030-00: 24-Well Plates with Lids
  - AutoGenomics Product Number 11-0050-00: INFINITI Temp Cycle Plate
  - AutoGenomics Product Number 12-0010-02: Wash buffer

- **FOR INFINITI PLUS Analyzer***:*
  - AutoGenomics Product Number 10-0020-99: INFINITI PLUS Analyzer
  - AutoGenomics Product Number 11-0100-00: 48-Well Plates
  - AutoGenomics Product Number 11-0110-00: 48 Well Plate Lid (reusable)
  - AutoGenomics Product Number 12-0330-00: Buffer Solution BF1

SPECIMEN
Nasopharyngeal aspirates (NPA) which should be kept at -80°C until RNA extraction**.** Avoid freeze/thaw.

RNA EXTRACTION
Before nucleic acid extraction, the NPA should be thawed on ice.
It is required to run a known positive control and a negative control.
Positive Control: To control for RNA extraction, 1.0 µl of Armored RNA is added to 200 µl of the thawed specimen before proceeding with RNA extraction. Use 2.0 µl of armored RNA when using an automated extraction method.

Nucleic acid extraction is performed using the QIAamp Viral RNA Mini Kit following the instructions suggested by the manufacturer.

Final elution volume (40 µl) is stored at -80°C. When working with RNA always keep tubes on ice.

cDNA SYNTHESIS USING SUPERSCRIPT III RT SUPERMIX KIT
The SuperScript III Supermix kit provides the random primers, the annealing buffer, the 2X first strand reaction buffer and the enzyme mix required for the reverse transcription process. The reverse transcription is performed in a PCR instrument programmed to meet all the required incubation times and temperatures.

If the Armored RNA control was not added before extraction, it may be added after extraction. Add 0.6 µl of a 1:3 dilution Armored RNA (in RNAse free water) to 5.4 µl of RNA post extraction. [Total volume of extracted DNA with the ARNA is 6.0 µl.]

The reverse transcription reaction mixture is prepared as follows:
1 µl of Random Primers
1 µl of annealing buffer
6 µl extracted RNA containing ARNA
[total volume of the reverse transcription reaction mixture is 8 µl]

The reverse transcription reaction mixture (8 µl) is incubated at 70°C for 5 min, and then the PCR block is cooled to 4°C.

The following reagents are then added to the reverse transcription reaction mixture:
10 µl of 2X first strand reaction buffer
2 µl of enzyme mix
[total volume is 20 µl]

Incubate at 25°C for 5 minutes, then at 50°C for 50 minutes.

Inactivate enzymes at 85°C for 5 minutes. The cDNA is used for PCR.

PCR REACTION
Note:
- Keep Taq DNA polymerase on ice.
- Completely thaw reagents on ice.
- Vortex the amplification mix tube for 2 to 5 seconds then centrifuge briefly to bring the contents to the bottom of the tube.

- To avoid contamination, a separate area is recommended for assembly of the PCR reaction. Decontaminate pipettes and all work surfaces with freshly prepared 10% bleach.
- Filter tips and gloves must be used when handling specimens and controls.
- The PCR product cannot be stored prior to testing. Use immediately.

Note:

- For the INFINTI Analyzer use the 24WP.
- For the INFINTI PLUS Analyzer use the 48WP.

1. Set up the PCR in the well plate as follows:

Amplification Mix	9.9 µl
Platinum Taq	0.1 µl
cDNA sample	10.0 µl
Total volume	20.0 µl

2. Place the well plate in the thermocycler using the following conditions:

Step No.	Temperature °C	Time	No. of Cycles
1	94	2 minutes	1
2	94 55 72	30 seconds 30 seconds 1 minute	39
3	72	3 minutes	1
4	4	Hold	1

Note: When an Eppendorf Mastercycler EP was used, a fixed cooling and heating rate (ramp) of the thermal cycler block should be set such that the total PCR cycling time is about 1 hour and 57 minutes (+/- 5 min).

SAP/EXO PROTOCOL

Post PCR clean- up is a critical step to prevent the remaining PCR substrates from interfering with the signal amplification.

3. Prepare the enzymes mixture as a master mix with excess. **For example**: if there are 10 PCR reactions, prepare a master mix enough for 12 reactions by pipetting 36 µl of SAP, 9 µl of Exo and 6 µl of Titanium Taq.

SAP	3.00 µl / sample
Exo	0.75 µl / sample
Titanium Taq	0.50 µl / sample
Total	4.25 µl / sample

4. Dispense 4.25 µl of the enzyme mix into each reaction well, gently vortex briefly, then place the well plate in a thermal cycler block using the following conditions:

Step No.	Temperature	Time
1	37°C	30 min.
2	94°C	10 min.
3	4°C	hold

5. SAMPLE LOADING FOR THE INFINITI ANALYZER

1) Carefully remove the 8-well flat strip caps to avoid splashing.

2) Load the well plate in the appropriate orientation (with well A1 in the back left corner) into the Analyzer
   - **INFINITI Analyzer:** Load the assembled 24WP with the associated lid (Catalog # 11-0030-00) (see instructions in the INFINITI Analyzer Operator's Manual).
   - **INFINITI PLUS Analyzer:** Load the assembled 48WP with a clean 48WP lid (Catalog # 11-0110-00, reusable) (see instructions in the INFINITI PLUS Analyzer Operator's Manual).

3) Load the following: assay specific magazines, Intellipac, INFINITI Pipette tips, and buffer.

Operation of the Analyzers

Follow the instructions in the Operator's Manuals

INFINITI Analyzer Operator's Manual (Part Number EM-34000)
INFINITI PLUS Analyzer Operator's Manual (Part Number EM-34041)

INTERPRETATION OF RESULTS

The INFINITI RVP Plus Assay is designed to detect and identify certain respiratory viruses. The assay results are reported as "POSITIVE" or "NEGATIVE" for the presence of the specific virus.

If the RNA-D control on the result page is negative, the sample should be repeated.

When the assay is not completed, and no results are reported, the assay should be repeated. If the report displays a message which indicates the reason why no results are reported, then this message is recorded in the Error Log. Please refer to the Trouble Shooting section of the INFINITI Analyzer Operator's Manual where the error may be described, or contact AutoGenomics technical support.

LIMITATIONS

The INFINITI RVP Plus Assay is designed to detect and identify only the respiratory viruses listed in this Package Insert. The test does not report other respiratory viruses and does not claim either the absence or the presence of these viruses. Negative results do not preclude virus infection and should not be used as the sole basis for treatment or other patient management decisions.

PERFORMANCE CHARACTERISTICS

Analytical Specificity

Studies related to specificity were conducted during assay development. There are three levels of specificity required in the assay:

- PCR primers
- ASP – target binding region
- ASP – chip binding region

PCR primer specificity was determined by amplicon size on a gel and sequencing the amplicon. ASP primer specificity was determined by the correct calls made by the assay using known samples (e.g., Coriell). Capture probe specificity is determined by hybridizing different oligos and demonstrating that only the correct oligo hybridizes to the known spot.

Cross-Reactivity

Cross reactivity studies were performed to demonstrate that the presence of the bacterial pathogens and respiratory viruses listed below does not affect the specificity of the INFINITI RVP Plus Assay.

Bacteria	
Streptococcus pneumoniae	Bordetella parapertussis
Bordetella pertussis	Bordetella holmesii
Legionella pneumophila	

Virus	
Adenovirus C	HRSV-A
Adenovirus C	HRSV-A and HMPV-V
Coronavirus HKU1	HRSV-B
Coronavirus HKU1	HRSV-B
Enterovirus A	HRSV-B
Flu A (not S-OIV)	HRSV-B
Flu A (not S-OIV)	Negative
Flu B	Parainfluenza virus 1
HMPV-A	Parainfluenza virus 3
HMPV-B	Parainfluenza virus 3
HRSV-A	Parainfluenza virus 4
HRSV-A	

Limit of Detection (LoD)

The analytical sensitivity studies were conducted using amplicons. The sensitivity of the INFINITI RVP Plus ranged from 10 copies of target DNA for HRSV B and HMPV A to 2,500 copies for parainfluenza viruses type 2 and 3 and coronavirus 229E and 5,000 copies for parainfluenza virus type 1. The analytical sensitivity (LoD) of the INFINITI RVP Plus for the novel H1N1 strain was determined, using serial dilutions of two (2) samples, to be 500 copies.

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Assay Precision and Reproducibility

- A three-site reproducibility study was conducted to demonstrate inter-site, inter-operator, and inter-instrument reproducibility. One lot of reagents was used; each site had one operator and one instrument. Each site received the same twelve (12) contrived nasopharyngeal samples. RNA was extracted from the reproducibility samples as soon as received. For each assay day, the extracted RNA was assayed in duplicate following the instructions in the package insert. A total of 261 tests were run to detect the presence of 6525 viruses (261 tests x 25 viruses per sample). Table 1 provides a summary of the three-site reproducibility study.

Table 1

Virus	# Calls			Correct calls		Incorrect Calls	
	Total	Positive	Negative	# Correct Calls	% Correct Calls	# False Pos	# False Neg
Adeno A	261	0	261	261	100%	0	0
Adeno B	261	0	261	261	100%	0	0
Adeno C	261	22	239	257	98.5%	1	3
Adeno E	261	0	0	261	100%	0	0
CorOC43	261	0	0	261	100%	0	0
CorHKU1	261	0	0	261	100%	0	0
Cor229E	261	0	0	261	100%	0	0
CorNL63	261	22	239	258	98.8%	1	2
EnteroA	261	0	0	261	100%	0	0
EnteroB	261	22	239	258	98.8%	1	2
EnteroC	261	0	0	259	99.2%	1	1
EnteroD	261	0	0	261	100%	0	0
Rhino A	261	22	239	261	100%	0	0
Rhino B	261	0	0	261	100%	0	0
Flu A	261	21	240	257	98.5%	0	4
H1-s	261	20	241	259	99.2%	2	0
Flu B	261	22	239	261	100%	0	0
Hmpv A	261	22	239	251	96.2%	8	2
Hmpv B	261	22	239	261	100%	0	0
HRSV-alpha	261	22	239	256	98.1%	2	3
HRSV-beta	261	22	239	259	99.2%	2	0
Para1	261	0	0	261	100%	0	0
Para2	261	0	0	261	100%	0	0
Para3	261	22	239	255	97.7%	0	6
Para4	261	0	0	261	100%	0	0
TOTAL	6525	261	6264	6484	99.4%	18	23

- A lot-to-lot reproducibility study was conducted at one site using three lots. One operator and one instrument were used in the study. The same 12 reproducibility samples were used in this study. Table 2 provides a summary of the lot-to-lot reproducibility study.

Table 2

Lot	# samples tested	1st time run				
		samples with calls[a]	samples with correct calls[b]	samples with incorrect calls[c]	samples with no calls[d]	correct call rate[e] (%)
Lot 1	118	118	111	7	0	94.1%
Lot 2	24	24	23	1	0	95.8%
Lot 3 [f]	24	20	15	5	4	75.0%
Lot 4 [f]	24	24	24	0	0	100%

a Samples for which a Pos or a Neg call was made.
b Samples for which the call was correct. This does not include samples with no calls.
c Samples for which the was incorrect, e.g., false positive and false wrong negative results. This does not include samples with no calls.
d Samples which had no results
e Samples with correct calls/samples with calls. This does not include samples with no calls.
f Lot 3 had operator sample errors, therefore Lot 4 was added to the studies

Clinical Performance – Method Comparison

- Two-hundred twenty-one (221) nasopharyngeal aspirates (banked) were tested using the INFINITI RVP Plus Assay and the results compared to Real-Time PCR. The results with both methods were compared for each specimen and a concordance in the diagnosis was observed for 94.1% of the specimens. When specific diagnosis for each virus was considered, 100% concordance between the two methods was observed for HRSV type A and B, parainfluenza viruses, HMPV type B and coronavirus OC43.

  Of the 5.9% (13/221) specimens with discordant results (all positive with qRT-PCR only), there were 7 adenoviruses, 2 coronaviruses NL63, 1 coronavirus HKU1, 1 HMPV A, 1 influenza A and 1 picornavirus. Discordant specimens were confirmed by DNA sequencing. Seven discordant specimens were confirmed by DNA sequencing. Five (5) specimens positive for adenovirus with the qRT-PCR assay were not confirmed. One specimen, positive for coronavirus NL63 with the qRT-PCR assay and giving equivocal coronavirus 229E signal with the INFINITI RVP assay, was shown to be positive for 229E by DNA sequencing, suggesting possible specificity issues with the qRT-PCR assay.

  There were no false positives with the INFINITI RVP assay when compared to the qRT-PCR assay and to DNA sequencing.

  The results of the comparison study were published in the Journal of Clinical Microbiology, March 2009.

- Ninety-five (95) nasopharyngeal specimens were prospectively assayed using the INFINITI RVP Plus Assay. All 95 assay results from the INFINITI Assay were 100% confirmed by Real-Time PCR (Canadian approved protocol). The following patient samples were tested.

Specimen Type	# of Patient Samples
Adeno B	1
Adeno E	4
Coro43	1
RhinoA	1
FluA-sH1N1	22
FluB	1
HmpvA	5
HmpvB	1
Para1	1
Para3	2
Negative	56

- In another prospective comparison study, sixty-six (66) patient samples were tested using the INFINITI RVP Plus Assay by a US hospital. Results were compared with the laboratory developed/validated PCR method. During the initial INFINITI RVP Plus assay, 58 of the 66 samples (88%) gave the correct call and were confirmed by the comparison method. Eight (12%) had no calls. There were no incorrect calls. Of the eight no calls, only six were repeated; there was no repeat testing available for two samples for unknown reasons. Of the six repeats, three gave calls which were confirmed by the comparison method and three still had no calls. Only one repeat was allowed.

  During the comparison study, all calls made by the INFINITI RVP Plus Assay (61 patients) were confirmed by the PCR method (100% agreement). There were no incorrect calls. The following patient samples were tested.

Specimen Type	# of Patient Samples
Adeno C	1
Coro 43	1
Entero A	1
Rhino A	3
Flu A	1
FluA-sH1N1	2
Flu B	1
Para 1	1
Para 3	2
Negative	48