Respiratory Virus Detection Kit

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1. NOTICES TO CUSTOMERS

1.1 Product Warranty and Liability

Seegene guarantees the performance of all products as described when they are used according to the instructions in this manual. Any problem that occurs for reasons other than misuse should be reported to Seegene immediately. This warranty limits our liability for product replacement.

1.2 Safety Warning and Precautions

This product is limited to research use only.

1.3 Notice to Customers

The PCR process is covered by patents owned by Hoffman-La Roche Inc. No license or immunity under any other patent is either expressed or implied by the sale of any Seegene product.

2. INTRODUCTION

2.1 Seegene's Respiratory Virus Detection Kit

Although the conventional immunofluorescece assay (IFA) has advantages with its speed and easy handling, IFA do not have a good enough sensitivity to detect viruses on the occasions of cross-reactivity with other subtypes and epitope variation resulting from genetic mutation.

To overcome these limitations, viruses can be isolated from the specimen. However, this can be time-consuming and inadequate for clinical test due to the follow up tests with antibody or PCR methods.

Therefore, many of recently developed diagnostic methods for viral diseases employ PCR methods. There are several global companies which have already released diagnostic kits for viruses. However, Seegene's Respiratory Virus Detection Kit is outstanding in terms of sensitivity and specificity due to Seegene's DSO™ technology (patent pending) employed in PCR primer design.

Seegene's Respiratory Virus Detection Kit is designed to detect 11 types of RNA viruses and 1 type of DNA virus, which are responsible for respiratory diseases in human.

11 RNA respiratory viruses include human **metapneumovirus**, human **coronavirus 229E/NL63**, human **coronavirus OC43**, human **rhinovirus**, human **respiratory syncytial virus A**, human **respiratory syncytial virus B**, human **parainfluenzavirus 1**, human **parainfluenzavirus 2**, human **parainfluenzavirus 3**, **influenza A virus** and **influenza B virus**. One DNA respiratory virus is human **adenovirus**.

2.2 Overview of Respiratory Virus Detection

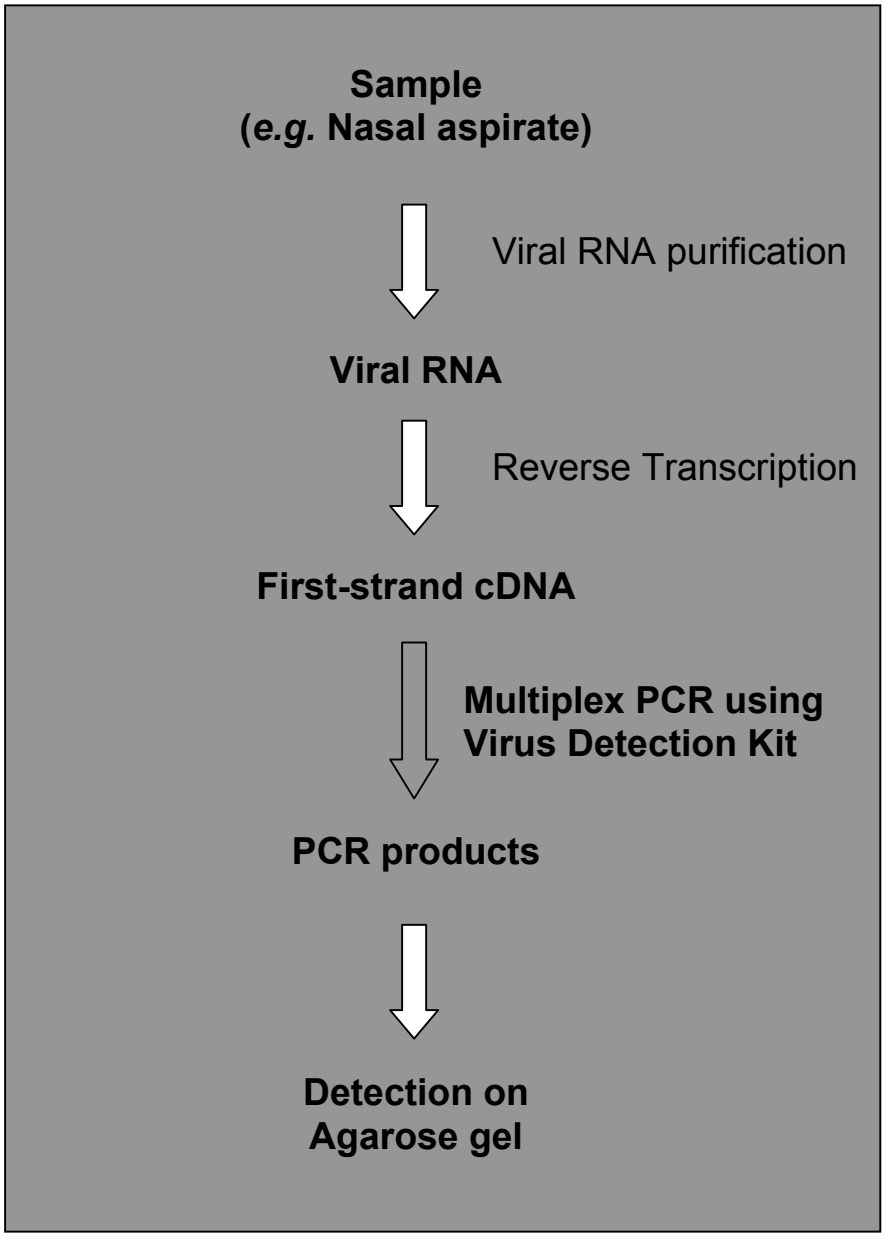

Fig. 1. Overview of respiratory virus detection

2.3 Key Features

(1) High specificity
Seegene's respiratory virus detection kit detected all positive controls which have previously known by other assays and generated only targeted bands in the clinical tests conducted by Seegene and collaborators. This absence of non-specific band in multiplex PCR of viruses, in which consensus primer design is hard to satisfy all factors for primer design and their product sizes, is possible by adopting of DSO™ technology (patent pending) in the primer design.

(2) High sensitivity
In general the sensitivity of multiplex-PCR is much lower than that of single PCR due to primers' interference. The low sensitivity of multiplex PCR sometimes results in false negative. However, Seegene's DSO™ technology enhances the sensitivity of the multiplex PCR resulting in higher sensitivity of Seegene's respiratory virus detection kit than other companies' multiplex respiratory virus detection kits.

(3) Coverage for 12 respiratory viruses
Seegene's Respiratory Virus Detection Kit is designed to detect 12 types of respiratory viruses, some of which are difficult to detect with a commercial IFA kit.

(4) Use of a hot start *Taq* DNA polymerase
Seegene's Respiratory Virus Detection Kit adopted a hot start *Taq* DNA polymerase. By limiting polymerase activity prior to PCR cycling, non-specific amplification is reduced and target yield is increased.

3. COMPONENTS

This kit is made up for **50 samples plus 20 controls** (10 for positive controls and 10 for negative controls). So you can use 1 negative and 1 positive control with every 5 sample.

3.1 List of components

Cap No	Contents	Volume	Comment
1	**5X SRVD-1**	over 280 µl: 200 µl for 50 samples 80 µl for 20 controls	A mixture of 12 primers for 6 viruses of SRVD-1 set
2	**5X SRVD-2**	over 280 µl: 200 µl for 50 samples 80 µl for 20 controls	A mixture of 12 primers for 6 viruses of SRVD-2 set
3	**RV control**	over 120 µl	A mixture of 12 viral clones
	2X RV Master Mix	500 µl X 3 tubes: 1000 µl for 50 samples 400 µl for 20 controls	DNA polymerase and dNTP are included in 2X buffer
	Manual		version 1.0

3.2 Storage Conditions

Store all reagents below –20ºC except the RV Master Mix.
In case of the RV Master Mix, 4ºC for short-term storage and -20ºC for long-term storage are recommended. Avoid repetitive thawing/freezing as it may decrease the activity of the Master Mix.

3.3 Reagents and Equipments to be Supplied by the User

RNase-free H_2O	Reverse transcriptase	2 mM dNTP
RNase inhibitor	Thermal cycler	Random Hexamer

4. PROTOCOL

4.1 Reverse Transcription (not included in this kit)

1. Add the following reagents to an RT tube on ice.

x µl	Total RNA (0.2 µg)
2 µl	10 µM random hexamer
7.5- x µl	DEPC-treated water
9.5 µl	

Note: Mix the reagents by tapping or pipetting.
Note: Although we recommend 0.2 µg of patient RNA, a wide range of patient RNA amount (50 ng – 3 µg) gives out good results. You may ignore the RNA concentration and use the maximum volume of 7.5 µl.

2. Incubate the tube at 80ºC for 3 min.
3. Chill the tube on ice for 2 min and spin the tube briefly.
4. Add the following reagents to the tube from step 3.

4 µl	5X RT buffer
5 µl	2 mM dNTP
0.5 µl	RNase inhibitor (40 u/µl)
1 µl	Reverse Transcriptase (200 u/µl)
20 µl	**Total volume**

5. Incubate the tube at 37ºC for 90 min.
6. Heat the tube at 94ºC for 2 min.
7. Chill the tube on ice for 2 min and spin the tube briefly.

Note: Store all cDNA samples at -20ºC until ready for use.

4.2 Respiratory Virus Detection PCR

1. Add the following reagents to a PCR tube (on ice).

3 µl	First-strand cDNA (~10 ng)
4 µl	5X SRVD-1 or 5X SRVD-2
3 µl	Sterilized water
10 µl	2X RV Master Mix
20 µl	**Total volume**

Note: Depending on the samples, different amounts (1 - 6 µl) of first-strand cDNA can be used as templates for PCR.

2. Place the tube in a preheated (94ºC) thermal cycler (optional).

3. Immediately commence the PCR reaction using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94ºC	15 min
2	40	94ºC	30 sec
		60ºC	1.5 min
		72ºC	1.5 min
3	1	72ºC	10 min

Note: We used the GeneAmp PCR System 9700 of Applied Biosystems that has a heated lid. It will be necessary to determine the optimal temperature and duration for your individual thermocycler.

4. Electrophorese 5 µl of the PCR products on a 2% agarose gel containing EtBr.

Note: If the band intensity of your sample is very weak, the intensity can be increased by raising the amount of first-strand cDNA or SRVD primer in the PCR mixture or by increasing the number of PCR cycles up to 45 cycles.

4.3 Positive Control PCR

Note: RV control contains a mixture of 12 virus control DNAs. For control PCR, use 3 μl of RV control instead of first-strand cDNA as a template.

3 μl	RV control
4 μl	5X SRVD-1 or 5X SRVD-2
3 μl	Sterilized water
10 μl	2X RV Master Mix
20 μl	**Total volume**

Note: For the PCR conditions, refer *4.2 Respiratory Virus Detection PCR part*.
Note: Electrophorese 2 μl of the control PCR products on a 2% agarose gel containing EtBr as a standard size marker and a control for PCR reaction.

SRVD-1 set	Amplicon size (bp)	SRVD-2 set	Amplicon size (bp)
Human adenovirus	534	Influenza A virus*	513
Human metapneumovirus	423	Influenza B virus	455
Human coronavirus 229E / NL63	375	Human respiratory syncytial virus B	391
Human parainfluenzavirus 1	324	Human rhinovirus	337
Human parainfluenzavirus 2	264	Human respiratory syncytial virus A	273
Human parainfluenzavirus 3	219	Human coronavirus OC43	231

Table 1. The amplicon sizes of 12 respiratory virus-detection PCR.

*Influenza A virus primer set can also detect influenza A virus (H5N1) causing highly pathogenic avian influenza.

As the positive control, viral RNA from viral culture soup was used in RT-PCR which is conducted with the SRVD primer mix in accordance with the instructions given in this User Manual. Below are examples of resulting data shown by agarose gel photographs. These agarose gels show the typical results that are generated by such experiments.

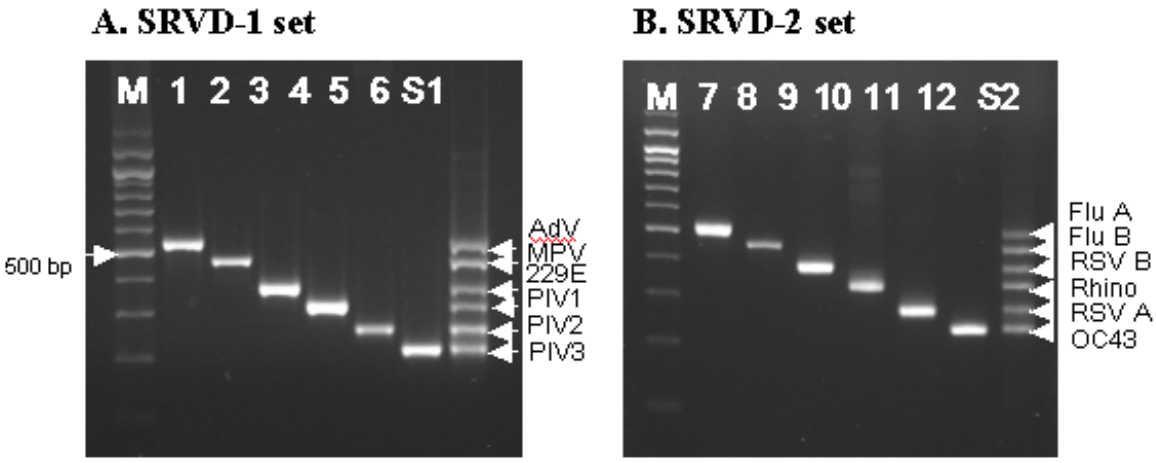

Fig. 2 Multiplex-PCR for the positive control. M;100 bp ladder (Seegene Cat. No. M0100), **1**;Human adenovirus, **2**;Human metapneumovirus, **3**;Human coronavirus 229E, **4**;Human parainfluenza virus 1, **5**;Human parainfluenza virus 2, **6**;Human parainfluenza virus 3, **S1**;SRVD-1 set using RV control as the template, **7**;Influenza A virus, **8**;Influenza B virus, **9**;Human respiratory syncytial virus B, **10**;Human rhinovirus, **11**;Human respiratory syncytial Virus A, **12**;Human coronavirus OC43, **S2**;SRVD-2 set using RV control as the template.

4.4 Negative Control PCR

Use 3 μl of water instead of RV control in the PCR mixture. No PCR band is expected in the negative control.

Note: Please be careful not to contaminate the SRVD primer sets and 2X master mix with PCR products through pipet. To prevent contamination of SRVD primers sets and 2X master mix from PCR products, use of cotton tips is recommended. It is also recommended that exclusive use of pipet for PCR products or pipet for primers.

5. EXPECTED RESULTS

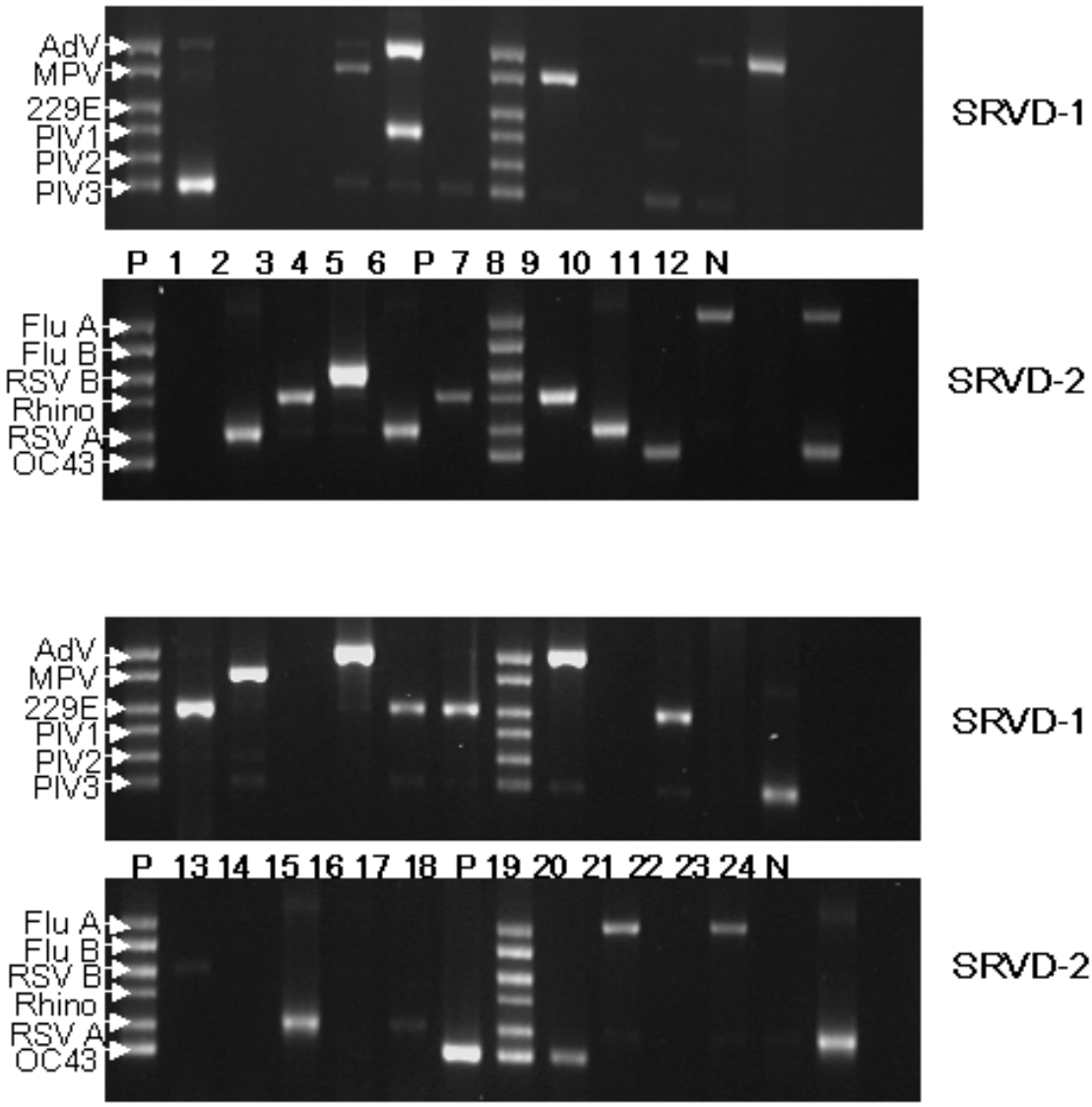

Fig. 3 Detection of respiratory viruses in clinical samples.
P;Positive control, **1~24**;patient 1~24, **N**;Negative control, **AdV**;human adenovirus, **MPV**;human metapneumovirus, **229E**;human coronavirus 229E/NL63, **PIV1**;human parainfluenza virus 1, **PIV2**;human parainfluenza virus 2, **PIV3**;human parainfluenza virus 3, **Flu A**;influenza A virus, **Flu B**;influenza B virus, **RSV B**;human respiratory syncytial virus B, **Rhino**;human rhinovirus, **RSV A**;human respiratory syncytial virus A, **OC43**;human coronavirus OC43.

Patient number	Major infection	Patient number	Major infection
1	PIV3	13	229E
2	RSV A	14	MPV
3	Rhino	15	RSV A
4	MPV, RSV B	16	AdV
5	AdV, PIV1, RSV A	17	229E
6	Rhino	18	229E, OC43
7	MPV, Rhino	19	AdV, OC43
8	RSV A	20	Flu A
9	OC43	21	229E
10	Flu A	22	Flu A
11	AdV	23	PIV3
12	Flu A, OC43	24	RSV A

Table 2. Results of respiratory virus-detection in figure 3.

AdV;human adenovirus, **MPV**;human metapneumovirus, **229E;**;human coronavirus 229E/NL63, **PIV1**;human parainfluenza virus 1, **PIV2**;human parainfluenza virus 2, **PIV3**;human parainfluenza virus 3, **Flu A**;influenza A virus, **Flu B**;influenza B virus, **RSV B**;human respiratory syncytial virus B, **Rhino**;human rhinovirus, **RSV A**;human respiratory syncytial virus A, **OC43**;human coronavirus OC43.

6. TROUBLESHOOTING GUIDE

Problems	Comments and Suggestions
No band	**Seegene's Respiratory Virus Detection Kit doesn't cover all viruses that cause human respiratory disease. Such as enterovirus and parvovirus are omitted in this version. And there may be unknown viruses which cause human respiratory disease.** a. Too small amount of cDNA may cause no or poorly intense bands. If this is the case, add more cDNA and increase the PCR cycles. b. Our kit is only a PCR kit, not an RT-PCR kit. If there is a problem in Reverse transcription or RNA preparation, PCR cannot produce amplicons. c. We tried to cover all isolates which had been reported, but there is a possibility of missing some strains.
Amplicon bands in the negative control	a. Your cDNA or PCR reagents may be contaminated. b. To prevent contamination of SRVD primers sets and 2X master mix from PCR products, use of cotton tips is recommended. It is also recommended that exclusive use of pipet for PCR products or pipet for primers.
Too many bands in patient's sample	a. Check the contamination using the negative control. b. The compromised patient who is hospitalized is susceptible to other viruses from other patient. In many cases, multiple infections were detected in patients who are hospitalized in general hospital.
Bad resolution on agarose gel	We recommend using 2% agarose gel.
DNA band above 534 bp	The problem of by-product which is generated by dimerization of the real PCR products in some cases has been a major remaining homework for current multiplex condition. But it doesn't interfere with the identification of the PCR product because size of the by-product is larger than all true PCR products.

7. ORDERING INFORMATION

Cat. No.	Product	Size
RV1210	Respiratory Virus Detection Kit	50 samples + 20 controls