

**IDENTIFICATION OF RESPIRATORY VIRUSES IN ADULTS:  
NASOPHARYNGEAL VS. OROPHARYNGEAL SAMPLING**

**<sup>1,2</sup>David Lieberman MD, <sup>2,3</sup>Devora Lieberman MD PhD, <sup>2</sup>Avi Shimoni MD**

**<sup>4</sup>Ayelet Keren-Naus PhD, <sup>4</sup>Rachel Steinberg PhD and <sup>4</sup>Yonat Shemer-Avni**

<sup>1</sup>Pulmonary Unit

<sup>2</sup>Division of Internal Medicine

<sup>3</sup>Department of Geriatric Medicine

<sup>4</sup>Laboratory of Clinical Virology

Soroka Medical Center and the Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

**Running title:** Nasopharyngeal vs. oropharyngeal virus sampling

**Keywords:** influenza viruses, rhinovirus, coronaviruses, RSV, oropharyngeal, nasopharyngeal, sampling, adults, respiratory viruses

**Address for correspondence and reprints:**

David Lieberman, MD

Pulmonary Unit

Soroka Medical Center

Beer-Sheva, Israel 84101

Tel. 972-7-6400411

Fax. 972-7-6403022

Email. Lieberma@bgu.ac.il

## **Abstract**

The optimal method for identifying respiratory viruses in adults has not been established. The objective of the study was to compare the sensitivity of three sampling methods for this purpose. One thousand participants were included (mean age  $63.1 \pm 17.8$  years). Of these 550 were patients hospitalized for acute febrile lower respiratory tract infections and 450 were controls. Oropharyngeal swabs (OPS), nasopharyngeal swabs (NPS) and nasopharyngeal washings (NPW) were obtained from each participant and were tested for 12 respiratory viruses by multiplex based real time PCR (mqRT-PCR). Patients were defined as positive for a specific virus if the virus was identified by at least one sampling method. In all, 251 viruses were identified in 244 participants. For the detection of any virus sensitivity rates for OPS, NPS and NPW were 54.2%, 73.3%, and 84.9%, respectively (OPS vs. NPS and NPW  $P < 0.00001$ ; NPS vs. NPW  $P < 0.003$ ). Maximal sensitivity was obtained only with sampling by all three methods. The same gradation of sensitivity for the three sampling methods was found when influenza viruses, coronaviruses and rhinoviruses were analyzed separately. The three sampling methods yielded equal sensitivity rates for RSV. We conclude that nasopharyngeal sampling has a higher sensitivity rate than oropharyngeal sampling and NPW has a higher sensitivity than NPS with rigid cotton swab for the identification of respiratory viruses in adults. Sampling by all three methods is required for maximal detection of respiratory viruses.

## **Introduction**

The oropharynx and the nasopharynx are the most common pathways for the introduction of airborne microorganism into the respiratory tract. For this reasons several methods have been developed over recent decades for the identification of viruses that cause respiratory viral infections at these sites. Although these infections are common in all age groups the vast majority of studies that have assessed and/or compared the various sampling methods has been conducted in the pediatric age group [1].

The paucity of this type of study in the adult population is striking in the light of data indicating that the same sampling methods have lower sensitivity rates in adults than among children and adolescents [2, 3]. Furthermore, different sampling methods can affect the results of laboratory testing. The prevailing view today is that the preferred laboratory technique for viral detection is the nucleic acid amplification test (NAAT) [1]. Another important variable is the specific viruses that are compared using the different sampling methods. Most published studies have compared these methods for single viruses and only a minority has looked at all the common respiratory viruses [4-6].

To address these methodological problems we designed a NAAT-based study in a large adult population with the aim of comparing the sensitivity of samples from the oropharynx and the nasopharynx for identification of all respiratory viruses. We also aimed to compare the sensitivity of nasopharynx sampling by swabs as opposed to washings for the same purpose.

## **Materials and methods**

### **The study population**

The study population was comprised of two groups of subjects, patients hospitalized with lower respiratory tract infection and controls. Recruitment of patients and controls was conducted over three winter periods, the first between January 1, 2004 and March 31, 2004,

the second between November 1, 2004 and March 15, 2005, and the third between November 1, 2005 and April 15, 2006. The study was approved by the Helsinki Committee for research on human beings of the Soroka Medical Center, and all participants gave signed informed consent to participate.

The patient groups included patients over 18 years of age who were hospitalized from the community in one of the internal medicine departments of the Soroka Medical Center and fulfilled the following three criteria over the week prior to hospitalization: (1) had an acute febrile illness, (2) had a cough that appeared or worsened, and (3) had at least one of (a) appearance or worsening of shortness of breath, (b) sputum production, (c) wheezing, and (d) chest pain or discomfort. None of the patients was recruited from a nursing home. In accordance with accepted criteria the patients were sub-classified into three groups: community acquired pneumonia, non-pneumonic lower respiratory tract infection, or acute exacerbation of COPD.

The control group was comprised of ambulatory patients over 18 years of age who came to one of the out-patient clinics of the Soroka Medical Center, agreed to participate in the study, and fulfilled the following two conditions: (1) by medical documentation and in response to a direct question there was no evidence of a known chronic lung disease or a state of immunosuppression, (2) by response to a direct question there was no evidence that in the month prior to inclusion the patient (a) had a febrile illness, (b) had a cough, (c) had a throat ache, (d) had hoarseness, (e) had a running nose, (f) had taken antibiotic medications, or (g) was definitely or possibly pregnant (in the case of women). For each of the participants in both groups we collected data concerning age, sex, smoking habit and vaccination status.

#### Sampling

Three physicians, who were trained specifically for the task, took all the samples from the patients and controls. In all hospitalized patients the samples were taken as close as possible

to the time of admission to the hospital, and in no case more than 24 hours later. Three consecutive samples were taken from each participant in the following order: oropharyngeal swab (OPS), nasopharyngeal swab (NPS), and nasopharyngeal washing (NPW). The OPS was taken under direct observation of the posterior throat and tonsil area using a commercial rigid cotton-tipped swab applicator (Virocult<sup>®</sup>, green cap, MW950, Medical Wire & Equipment Co. [Bath] Ltd, Corsham, Wiltshire, England). The NPS was taken using the same type of rigid swab applicator, which was introduced directly into the depth of the inferior meatus of one of the nostrils until resistance was felt. After sampling, both swab applicators were cut and placed separately into two tubes containing RPMI solution (Biological Industries, Beit Haemek, Israel). The NPW was obtained by instilling 2.5 ml of a sterile physiological saline solution into one of the patient's nostrils with the patient lying down. The instilled water was then gently suctioned out through a delicate tube that was introduced deep into the nostril and emptied into a special collection container (Mucous trap, Unomedical A/S, Lyngø, Denmark) that was connected to the portable suction equipment (Easy Go Vac Aspirator, Precision Medical, Northampton, PA, USA). The two test tubes with the swabs were shaken in a Vortex for five minutes after which the head of the applicator was drained against the sides of the test tubes and then removed. The raw washing matter was also added to the test tube containing RPMI solution that was also shaken. The contents of the three test tubes was frozen within an hour and kept at  $-80^{\circ}\text{C}$  until processed.

#### Detection of respiratory viruses

Nucleic acid extraction was performed using NucliSense EasyMag (Biomérieux, Marcy l'Etoile, France), according to the manufacturer's instruction. 400  $\mu\text{l}$  of aspirate were extracted into 50  $\mu\text{l}$  of elution solution. The sets of primers and probes used to detect 12 viruses by multiplex hydrolysis probes-based real time PCR (mqRT-PCR) are described in Table 1. Each sample was tested in parallel, in three test tubes, for the following viruses:

influenza A and B, parainfluenza 2 and 3, human respiratory syncytial virus (RSV), human metapneumovirus (hMPV), rhinovirus, adenovirus, and corona viruses 229E, HKU1, OC43 and NL63. Amplification was carried out in a final volume of 10  $\mu$ l, using the RNA ultrasense one-step qRT-PCR system (Invitrogen, Carlsbad, California) with 4  $\mu$ l of nucleic acid and four sets of primers and probes to detect four viruses, and an internal control (IC) set (see Table 1 for details of concentrations of primers and probe sets and for virus testing combinations).

#### Statistical analysis

Sample size calculations were based on data that was collected in a preliminary phase of the study that involved 100 subjects (50 patients and 50 controls). In that population 11, 16, and 21 viruses were identified by OPS, NPS, and NPW, respectively. The calculated sample size on the basis of these data using standard methods, with an alpha level of 0.05, a power of 80% and a patient:control ratio of 1:1, was 985 subjects. To adjust for the possibility that the study period might have a lower rate of viral activity, 50 patients were added to the study population at the expense of the control group.

Data were recorded and analyzed using the Epi Info version 3.3.2 software. Rates between samples were compared using the  $\chi^2$  test with Yates correction or Fisher's exact test, as appropriate. Statistical significance was set at  $P < 0.05$  throughout.

## Results

The study population consisted of 1000 subjects, 550 hospitalized patients and 450 controls. Two hundred twenty eight of the patients were diagnosed with community-acquired pneumonia, 250 with non-pneumonic lower respiratory tract infection, and 72 with acute exacerbation of COPD. Table 2 shows age and gender data for the two study groups and the total study population.

In all, 251 respiratory viruses were identified in 244 subjects (seven subjects had a dual infection with two different viruses). These numbers refer to the identification of at least one virus by at least one of the sampling methods in one subject. This index served, for this study, to define positivity for a specific virus and as the gold standard for the determination of the sensitivity of each of the three sampling methods. Table 3 shows the frequency distribution of the 12 different viruses in the total study population and by study group.

Table 4 depicts the distribution of all 251 viruses identified by the three methods, the three combinations of these methods and the calculated sensitivity for each method or combination of methods. The sensitivity for sampling from the oropharynx was only 54.2%, which was significantly lower than the two sampling methods from the nasopharynx. The NPW technique had a significantly higher sensitivity than the NPS (84.9% vs. 73.3%, respectively). A combination of two of the three methods raised the sensitivity rate compared to each method alone. NPW, combined with OPS or NPS, had a sensitivity of more than 94%. None of the three methods or the three combinations yielded the maximal sensitivity, which was attained only when all three methods were combined. The same trend was seen when sensitivity was calculated separately for the two study groups, i.e., an advantage for nasopharyngeal sampling over oropharyngeal sampling and an advantage for NPW over NPS.

To analyze the study results in terms of the various respiratory viruses we grouped the viruses into four main groups: influenza viruses, rhinovirus, RSV, and coronaviruses. Table 5 shows the frequency distributions for each of the four principal virus groups in the same format used for all 251 viruses. In the three groups with the highest frequencies, influenza A and B viruses, coronaviruses, and rhinovirus, the same trend was seen as in the analysis for all the viruses, i.e., an advantage for nasopharyngeal sampling over oropharyngeal sampling and an advantage for NPW over NPS. Although the study was not powered to compare the sensitivity of the sampling methods for specific virus groups, some of the

differences described above were statistically significant. The results for RSV were different from the others with an identical sensitivity for the three sampling methods that reached 84%. However, as with the other viruses, samples from both the oropharynx and nasopharynx were required to reach the maximal sensitivity.

## **Discussion**

The present study compared three accepted sampling methods for identification of respiratory viruses. This study is unique and important in that it combines a large adult study population (with a broad age spectrum including a majority in the elderly age range) with a sophisticated molecular biological method for identifying all main respiratory virus groups. In terms of clinical characteristics the study population included patients with a broad spectrum of acute respiratory diseases and a control group with a similar age distribution to the patient group. The subjects in the patient group were all hospitalized, but the sampling was done close to the time of their admission with the aim of averting the effect of hospital-based colonization. The study objectives focused exclusively on the technical/methodological aspect of viral identification, purposely ignoring the clinical significance of this issue, which would require a different study design.

The three sampling methods used in our study sample the upper respiratory tract, in effect, while the subjects included in the study had clinical manifestations of lower respiratory tract infections. It is possible that sampling of the lower respiratory tract by more invasive procedures such as bronchoalveolar lavage (BAL) or protected brush swab would have provided more information, but this is an assumption that is not yet confirmed by the literature. The accepted method for identification of viral etiologies, for lower respiratory tract infections as well as upper ones, is by naso/oropharyngeal sampling. Moreover, the high rate of respiratory viruses identified in this study of patients with lower respiratory tract infections, compared to healthy controls, supports the possibility that the viral population in

the naso/oropharynx reflect, at least in part, the presence of these same pathogens in the lower respiratory tract.

Some methodological issues related to the study require clarification. The first is the merging of the two study groups, patients and controls, for the purpose of data analyses. This could be problematic, at first glance, in light of the differences in the rates of respiratory viruses that were identified in the two groups. However, in this study we purposely ignored the question of the identification rates in the study groups and focused only on the methodological issue of the relative sensitivity of the three sampling methods for identification of respiratory viruses. The sensitivity of each of the three methods was compared separately between the two study groups and found to be similar. In light of this we believe that merging the two groups for further data analyses was justified. A second issue has to do with the swab type used in our study. Samples were obtained using conventional cotton tipped swabs. In many clinical settings these are now being replaced by flocked swabs, based explicitly on studies that have indicated that the flocked polyester material has less adsorption and markedly better recovery for respiratory pathogens in OPS and NPS than do the older cotton swabs [7, 8]. This weakens the observations and conclusions of our study in relation to the inferiority of OPS and NPS compared to NPW, in as much as both of these sample types are likely to have performed better with the newer swab material. This does not impact on the comparison between OPS and NPS however, as the same swab type was used in both and the improved recovery in NPS as opposed to OPS should be independent of this.

Another issue is the two specific methods that were used for nasopharyngeal sampling. To swab the nasopharynx a rigid swab applicator was used and not a flexible one. At the preliminary stage of this study the investigators tested both types of applicators for nasopharyngeal swabbing. Their impression was that adult patients are much more tolerant of the rigid applicator than the flexible one, so the rigid applicator was used for all

nasopharyngeal samplings in this study. This choice would not necessarily be the case in the pediatric population in which sampling is usually conducted while the child is held by the parents or the staff. The second method for nasopharyngeal sampling was washing. This method, which is commonly used to identify viruses in children, is not used routinely in adults. Gooskens et al. found it equally sensitive to nasopharyngeal swabs by PCR. However, they felt that it would be impractical due to functional limitations of nursing homes residents [9]. In contrast, our impression from washings conducted in our very large number of adult subjects was that this method is well tolerated by all adults including the low functional capacity elderly adults who were included in the study. Furthermore, the sensitivity rate with this method in our study was higher than that of nasopharyngeal swabs.

Nasopharyngeal sampling was shown to have advantages over oropharyngeal sampling for the identification of influenza viruses in previous studies of adult and mixed adult-pediatric populations [10]. A similar advantage was found in a study that tested for all viruses in a pediatric population [4]. A comprehensive review of the literature did not reveal any corresponding studies on all viruses in adults. Only the two studies cited above compared nasopharyngeal sampling by NPS and NPW in adults, and in those cases only for the influenza viruses. A similar sensitivity rate was found for the two methods in nursing home residents by PCR [9]. In another study of a mixed adult and pediatric population, NPW was found to have an advantage over NPS using two non-PCR detection methods [10].

The paucity of publications on studies conducted in adults lends greater importance to the results of the present study. In relation to all respiratory viruses we found a clear and significant advantage to nasopharyngeal compared to oropharyngeal sampling and an advantage for NPW over NPS. These differences did not change when we conducted separate analyses for the three common groups of respiratory viruses, influenza viruses, coronaviruses, and rhinovirus, although there was a mild variation among these groups in the

degree of differences found among the three sampling methods. Combining two of the three sampling methods significantly raised the sensitivity rate for identification of all viruses, reaching 94-95% when one of the sampling methods was NPW. The same trend was found when the sensitivity achieved by combinations of the three methods for the common virus groups was analyzed. To reach maximal sensitivity for all viruses tested it was necessary to use all three sampling methods. We do not think that there is a clear-cut answer to the question as to whether it is important to attain the maximal sensitivity rate, compared to a rate that is close to this rate, in routine clinical work. We believe that this depends on the clinical circumstances in which the test is conducted, on the exact degree of differences in sensitivity rates, as well as on the outlook of the clinician who faces the question. In contrast to the results seen for the three common virus groups the sensitivity rates were identical for the three sampling methods for RSV. In two studies limited to pediatric populations in which NPS and NPW were compared, a similar sensitivity rate for the diagnosis of viral respiratory disease was found for both methods [11, 12].

We conclude that the nasopharyngeal sampling is superior to oropharyngeal sampling and that NPW is superior to NPS with rigid cotton swab for the identification of respiratory viruses in adults. To obtain a complete picture of respiratory virus infection the three methods need to be combined.

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Table 1. Primers and probes used in pentaplex real-time RT-PCR assays

Tube	Viral set	Primers and probe sequence	Concentration nM	Target gene	Reference
<b>I</b>	influenza B	ATCGGATCCTCAACTCACTCTT	500	NS	
		TGACCAAATTGGGATAAGACTC	500		
		FAM/YAK-CTCGAATTGGCTTTGRATGTCCTTCAT-BBQ	<b>250</b>		
<b>I</b>	parainfluenza 2	ATCCAATCGATACTCGGAGGT	250	N	
		TCTGGTTGTTTGGTTGTCCA	500		
		Cyan500-TGATGGTGAGGACAGAATTGACAAC-BBQ	<b>125</b>		
<b>I</b>	parainfluenza 3	AAGATCTACAAGTTGGCAYAGCAA	500	HN	
		AATGTCCCATGGACATTCAT	500		
		ROX-TTCCTGGTCTTGATAGCACATTATGCCA -BBQ	<b>250</b>		
<b>I</b>	rhinovirus	TGGACAGGGTGTGAAGAGC	500	5' UTR	[13]
		CAAAGTAGTCGGTCCCATCC	500		
		FAM-TCCTCCGGCCCTGAATG-BHQ1	<b>150</b>		
<b>II</b>	influenza A	GGACCTCCACTTACTCCAAAACAGAAAC	100	NS	Modified from [14]
		GTAAGGCTTGCATGAATGTTATTGCTC	200		
		YAK-AA+GTTT+GAA+GARATMA+GAT+GGCT-BBQ	<b>50</b>		
<b>II</b>	hMPV	AACCGTGTACTAAGTGATGCACTC	500	Np	[15]
		CATTGTTGACCGGCCCATAA	500		
		FAM-CTTTGCCATACTCAATGAACAAACT-BBQ	<b>250</b>		
<b>II</b>	RSV	GCCAAAAAATTGTTCCACAATA	250	L	Modified from [14]
		TCTTCATCACCATACTTTTCTGTTA	500		
		ROX-TCAGTAGTAGACCATGTGAATTCCTGCA-BBQ	<b>125</b>		
<b>II</b>	adenovirus set I	ATGACTTTTGAGGTGGATCCCATGGA	100	H	
		GCCGAGAAGGGCGTGCGCAGGTA	100		
		Cyan500-AGCCCAACCTKC+T+T+TA+T-BBQ	<b>50</b>		
<b>II</b>	adenovirus set II	GCCCCAGTGGTCTTACATGCACATC	100	H	[16]
		GCCACGGTGGGGTTTCTAAACTT	100		
		Cyan500-TCGGAGTACCTGAGCCCGGTCTGGTGCA-BBQ	<b>50</b>		

Tube	Viral set	Primers and probe sequence	Concentration nM	Target gene	Reference
III	coronavirus HKU1	TTTTCAGATGGTCAAGGAGTTC	250	(NP)	
		CCGGCTGTGTCTATAACCAATATCC	250		
		Cyan500-TCGGAGTACCCCTTCTGAAGCAAAAG-BBQ	125		
III	coronavirus NL63	ACGTACTTCTATTATGAAGCATGATATTA	1000	POL	[13]
		AGCAGATCTAATGTTATACTTAAACTACG	1000		
		YAK-ATTGCCAAGGCTCCTAAACGTACAGGTGTT-BHQ1	300		
III	coronavirus 229E	CAGTCAAATGGGCTGATGCA	1000	Np	[13]
		AAAGGGCTATAAAGAGAATAAGGTATTCT	1000		
		FAM-CCCTGACGACCACGTTGTGGTTCA-BHQ1	300		
III	coronavirus OC43	CGATGAGGCTATTCCGACTAGGT	125	N	[13]
		CCTCCTGAGCCTTCAATATAGTAACC	1000		
		ROX-TCCGCCTGGCACGGTACTCCCT-BHQ2	300		
	Human endogenous retrovirus ERV3 (IC)	CATGGGAAGCAAGGGAACATAATG	233	Human ERV-3	Modified from [14]
		CCCAGCGAGCAATACAGAAATT	233		
		CY5-TCTTCCCTCGAACCTGCACCATCAAT-BBQ	116		

(+) LNA addition

(HN) hemagglutinin-neuraminidase

(NS) non-structural protein

(UTR) untranslated region

(H) hexon protein

(N) nucleocapsid protein

(Np) nucleocapsid phosphoprotein

(1a)(POL) RNA polymerase

Table 2. Gender and age data by study group and for the entire study population

	<b>Patients</b> (N=550)	<b>Controls</b> (N=450)	<b>Merged data</b> (N=1000)
<b>Age (years)</b>			
mean $\pm$ SD	63.9 $\pm$ 19.4	62.2 $\pm$ 15.6	63.1 $\pm$ 17.8
Range	19-99	19-93	19-99
> 65 years [N (%)]	329 (59.8)	238 (52.9)	567 (56.7)
<b>Gender</b>			
Females (%)	255 (46.4)	243 (54.0)	498 (49.8)

Table 3. Frequency distribution of identified viruses by study group and for the entire study population [N (%)\*]

<b>Virus</b>	<b>Patients (N=550)</b>	<b>Controls (N=450)</b>	<b>Merged data (N=1000)</b>
<b>influenza A</b>	75 (13.6)	2 (0.4)	77 (7.7)
<b>influenza B</b>	3 (0.5)	0	3 (0.3)
<b>rhinovirus</b>	41 (7.5)	9 (2.0)	50 (5.0)
<b>RSV</b>	27 (4.9)	4 (0.9)	31 (3.1)
<b>hMPV</b>	5 (0.9)	0	5 (0.5)
<b>adenovirus</b>	4 (0.7)	0	4 (0.4)
<b>parainfluenza virus 3</b>	6 (1.1)	0	6 (0.6)
<b>parainfluenza virus 2</b>	0	0	0
<b>coronaviruses</b>			
<b>NL63</b>	6 (1.1)	6 (1.3)	12 (1.2)
<b>229E</b>	11 (2.0)	2 (0.4)	13 (1.3)
<b>OC43</b>	36 (6.5)	8 (1.8)	44 (4.4)
<b>HKU1</b>	5 (0.9)	1 (0.2)	6 (0.6)
<b>Total</b>	219 (39.8)	32 (7.1)	251 (25.1)

\*% = The percentage subjects positive for the specific virus of all subjects in that population

RSV - respiratory syncytial virus

hMPV -human metapneumovirus

Table 4. Comparison of the frequency distribution and sensitivity for all viruses, identified by the three sampling methods separately and in combination

<b>Sampling method</b>	<b>Number of viruses identified</b>	<b><sup>1</sup>Sensitivity (95% CI)</b>
<b>OPS</b>	136	0.542 (0.478-0.600) <sup>3</sup>
<b>NPS</b>	184	0.733 (0.673-0.786) <sup>4</sup>
<b>NPW</b>	213	0.849 (0.797-0.889)
<b>OPS and/or NPS</b>	212	0.845 (0.792-0.886)
<b>OPS and/or NPW</b>	236	0.940 (0.901-0.965)
<b>NPS and/or NPW</b>	239	0.952 (0.916-0.974)
<b>OPS and/or NPS and/or NPW <sup>2</sup></b>	251	

OPS – oropharyngeal swab

NPS – nasopharyngeal swab

NPW – nasopharyngeal washing

<sup>1</sup>The sensitivity of the method/combination of methods was calculated as the number of viruses identified by this method/combination of methods of the number of viruses identified by at least one of the three sampling methods (gold standard), which appears on the bottom line of the table.

<sup>2</sup>In addition to the corresponding numbers in Tables 5-8, the number of viruses in this group includes these 15 viruses: parainfluenza virus 3 (identified in 6 subjects), hMPV (5), and adenovirus (4).

<sup>3</sup>P<0.00001 vs. NPS and vs. NPW

<sup>4</sup>P<0.003 vs. NPW

Table 5. Comparison of the frequency distribution and sensitivity for the study viruses, identified by the three sampling methods separately and in combination.

Sampling method	Influenza A and B		Coronaviruses		Rhinoviruses		RSV	
	N identified	<sup>1</sup> Sensitivity (95% CI)	N identified	<sup>1</sup> Sensitivity (95% CI)	N identified	<sup>1</sup> Sensitivity (95% CI)	N identified	<sup>1</sup> Sensitivity (95% CI)
OPS	45	0.56 (0.45-0.67) <sup>2,3</sup>	43	0.57 (0.45-0.68) <sup>5,6</sup>	17	0.34 (0.22-0.49) <sup>8,9</sup>	26	0.84 (0.65-0.94) <sup>11,12</sup>
NPS	61	0.76 (0.65-0.85) <sup>4</sup>	55	0.73 (0.62-0.83) <sup>7</sup>	32	0.64 (0.49-0.80) <sup>10</sup>	26	0.84 (0.65-0.94) <sup>13</sup>
NPW	77	0.96 (0.89-0.99)	57	0.76 (0.64-0.85)	40	0.80 (0.66-0.89)	26	0.84 (0.65-0.94)
OPS and/or NPS	67	0.84 (0.73-0.90)	64	0.85 (0.78-0.92)	38	0.76 (0.61-0.86)	30	0.97 (0.81-0.998)
OPS and/or NPW	80	1.00 (0.94-1.00)	67	0.89 (0.79-0.95)	44	0.88 (0.75-0.95)	31	1.00 (0.86-1.00)
NPS and/or NPW	78	0.98 (0.90-0.996)	72	0.96 (0.88-0.99)	47	0.94 (0.82-0.98)	28	0.90 (0.73-0.97)
OPS and/or NPS and/or NPW	80	---	75	---	50	---	31	---
<b>P</b>	<sup>2</sup> P<0.02 vs. NPS <sup>3</sup> P<0.00001 vs. NPW <sup>4</sup> P<0.0006 vs. NPW		<sup>5</sup> NS vs. NPS <sup>6</sup> P<0.03 vs. NPW <sup>7</sup> NS vs. NPW		<sup>8</sup> P<0.005 vs. NPS <sup>9</sup> P<0.00001 vs. NPW <sup>10</sup> NS vs. NPW		<sup>11</sup> NS vs. NPS <sup>12</sup> NS vs. NPW <sup>13</sup> NS vs. NPW	

<sup>1</sup>The sensitivity of the method/combination of methods was calculated as the number of viruses identified by this method/combination of methods of the number of viruses identified by at least one of the three sampling methods (gold standard), which appears on the bottom line of the table.

OPS – oropharyngeal swab

NPS – nasopharyngeal swab

NPW – nasopharyngeal washing