Initial characteristics of respiratory viruses in symptomatic subjects during the pandemic, a cohort study

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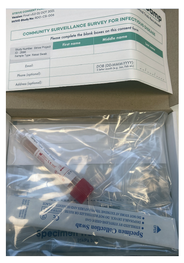
Background

During the most intense waves of the recent pandemic, we have witnessed an clear and almost complete wipe-out of many respiratory viruses routinely circulating in the community over the north-hemisphere winter. As such a situation has never occurred before, the hVIVO team, based in London, United Kingdom, launched an active surveillance with the intent to assess the circulation and co-circulation of viruses over the 2021-2022 winter period.

Methods

hVIVO employees, their relatives and friends were provided with collection kits and asked to contribute to this research. When becoming symptomatic for respiratory virus-like infection, subjects were swabbing themselves and posting their sample by mail. Kits were then processed and tested by multiplex PCR (BioFire multiplex PCR respiratory panel 2.1). Adenovirus, Coronavirus 229E, HKU1, NL63, OC43, MERS-CoV, SARS-CoV-2, HMPV, Human Rhinovirus / Enterovirus, Flu A, Flu B, Parainfluenza Virus 1, 2, 3, 4, RSV, *Bordetella Parapertussis, Bordetella Pertussis, chlamydia pneumonia and Mycoplasma pneumonia* were tested for. Symptomatic subjects recorded their clinical signs of infection and also signed a consent form for the testing of their sample.

- Sampling kit content
  - 1 x 3mL Copan Universal Transport Media,
  - 1 x Nasal swab,
  - 1 x Shipping bag,
  - 1 x Security label,
  - 1 x Instruction leaflet,
  - 1 x Informed Consent form,
  - 1 x Stamped return envelope,
  - 1 x UN3373 shipping box



- BioFire multiplex PCR Respiratory Panel & Self reporting syndrome questionnaire

BioFire® Respiratory Panel 2.1 *plus* — BIOFIRE® A BIOMERIEUX COMPANY — www.BioFireDx.com

Run Summary

Sample ID:	000001_1406	Run Date:	17 Mar 2022 2:17 PM
Detected:	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	Controls:	Passed
Equivocal:	None		

Result Summary

	Viruses
Not Detected	Adenovirus
Not Detected	Coronavirus 229E
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Coronavirus OC43
Not Detected	Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
✓ Detected	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
Not Detected	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
Not Detected	Respiratory Syncytial Virus
	Bacteria
Not Detected	*Bordetella parapertussis* (IS*1001*)
Not Detected	*Bordetella pertussis* (*ptxP*)
Not Detected	*Chlamydia pneumoniae*
Not Detected	*Mycoplasma pneumoniae*

Run Details

Pouch:	RP2.1*plus* v1.0	Protocol:	NPS2 v3.2
Run Status:	Completed	Operator:	[redacted]
Serial No.:	53509959	Instrument:	TM078F2
Lot No.:	26B221		

RECORD YOUR SYMPTOMS

Once you have signed the consent form as above please tick boxes relating to any symptoms associated with your illness. Please tick as many as you think you have.

Dry cough	Phlegmy cough	Fever
Loss of smell and/or taste	Runny nose	Sneezing
Sore throat	Shortness of breath	Headache
Body aches	Exhaustion	Diarrhoea
Earache	Sinus pain	

Thank you for participating

Dry Cough	Phlegmy Cough	Runny Nose	Sneezing	Earache	Sore throat	Sinus Pain
Fever	Loss of smell/ taste	Shortness of breath	Headache	Body Aches	Exhaustion	Diarrhoea

Upper Respiratory Symptoms Systemic Symptoms

Results

352 participants entered the survey (with signed Informed Consent Form, completed questionnaire and a valid BioFire result). 225 were positive for at least 1 of the 16 viruses tested, with 14 of these participants co-infected with 2 viruses and 1 with 3 viruses. 127 participants were found negative for all viruses (Figure 1). None of the 4 bacteria tested were detected. Mostly Enterovirus, SARS-CoV-2 and Coronavirus OC43 were recovered. Co-infection were detected at a non-negligeable level with OC43 and Rhinovirus/Enterovirus the most present in co-virus infection. In one subject, 3 different viruses were identified.

Figure 2 shows that in our study, there is no clear evidence in predicting the contracted virus- type based on reported symptoms. Fever, sneezing and sore throat could be among the main possible differentiators between infected and non-infected patients. Interestingly, we detected 15 different types/sub-types of viruses in total.

Figure 3 also shows that of the 3 main viruses detected (Enterovirus, OC43 and SARS-CoV-2), there are no clear differences in the symptoms reported. Anosmia and ageusia were reported in only 10% of SARS-CoV-2 confirmed infections (6% in Enterovirus confirmed subjects).

Figure 4 focuses on two of the most common symptoms triggering actions, such as consulting their General Practitioner or self-isolation, in the general population. Interestingly, fever was reported by almost as many subjects with Enterovirus as by those with SARS-CoV-2 infection.

In the supplementary Figure we report the co-circulation of several sub-variants of SARS-CoV-2 Omicron. We successfully isolated these on VERO cells.

Conclusions

Very few RSV and Influenza viruses were recovered, pointing out once more the abnormality of the past winter season due to the pandemic and its impact on circulation of other viruses and hygiene behaviours. Interestingly we observed several SARS-CoV-2 variants co-circulating and also 3 cases of co-infection with SARS-CoV-2 (2 with Enterovirus and 1 with Adenovirus).

Our results confirm the general observation that, over the past two winter seasons, the usual pattern of circulating respiratory viruses and their intensity were almost completely disrupted. Such testing program and analysis may contribute to predicting which family of virus is most relevant for efficient public health preparedness.

Acknowledgements

We would like to acknowledge the generous contributions of our colleagues and relatives who entered this cohort study as well as the hard work of hVIVO clinical, laboratory, and the operational staff (especially Zoe Cagnoni) involved in the studies.

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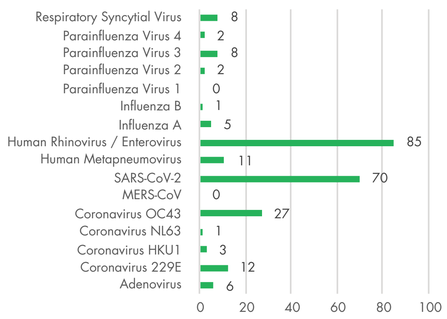
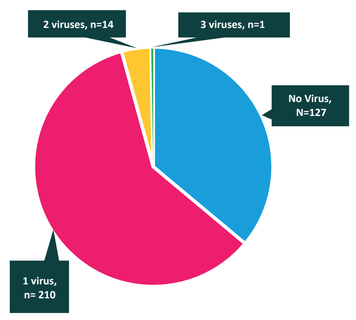
Type and number of viruses recovered overall (N=352)



Number of viruses per sample (N=352)

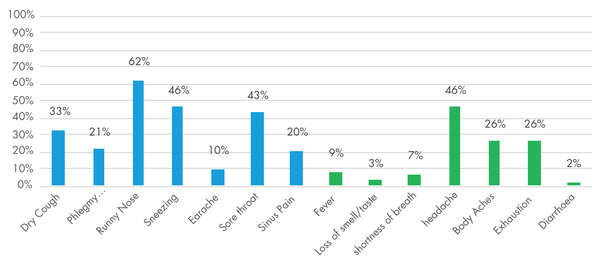


Detected Combinations for Co-infections

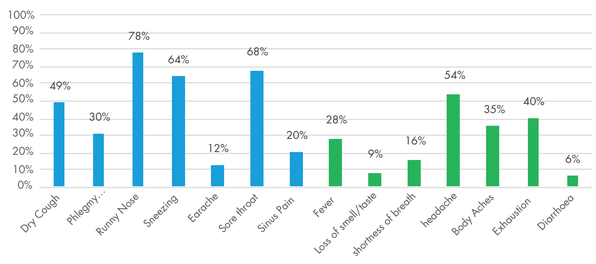
ID	Adenovirus	Coronavirus 229E	Coronavirus OC43	SARS-CoV-2	Human Rhinovirus/ Enterovirus	Parainfluenza Virus 3	Parainfluenza Virus 4	Respiratory Syncytial Virus
Subject 1			X		X			
Subject 2			X		X			
Subject 3			X		X			
Subject 4			X		X			
Subject 5			X		X			
Subject 6	X		X		X			
Subject 7	X				X			
Subject 8				X	X			
Subject 9				X	X			
Subject 10		X			X			
Subject 11					X			X
Subject 12	X			X				
Subject 13	X					X		
Subject 14		X				X		
Subject 15			X				X	

Figure 1: Identification of viruses in nasal samples from community acquired respiratory infections (as identified by BioFire multiplex PCR respiratory pathogen panel analysis). Left, distribution and frequency of each virus type identified, each count is one sample from one patient. Middle, graphical representation of the proportion of samples that tested positive for one, two or three pathogens or for which no pathogen was detected. Right, List of the 15 samples and the associated viruses for which co-infections were identified.

Symptoms reported when No virus were detected (N=127)



Symptoms reported when 1 virus was detected (N=210)



Average number of symptoms reported per type of virus detected

	# single Virus	Upper Respiratory Tract	Systemic	Total # Symptoms Reported
Adenovirus	2	1.0	1.0	2.0
Coronavirus 229E	10	2.9	1.8	4.7
Coronavirus HKU1	3	2.3	2.0	4.3
Coronavirus NL63	1	3.0	1.0	4.0
Coronavirus OC43	20	3.9	1.5	5.3
SARS-CoV-2	67	2.9	2.4	5.2
hMPV	11	4.1	2.3	6.4
Human Rhinovirus / Enterovirus	74	3.5	1.5	4.9
Influenza A	5	3.0	2.4	5.4
Influenza B	1	2.0	1.0	3.0
Parainfluenza Virus 1	0			
Parainfluenza Virus 2	2	2.0	3.0	5.0
Parainfluenza Virus 3	6	3.2	2.0	5.2
Parainfluenza Virus 4	1	1.0	1.0	2.0
RSV	7	3.1	1.7	4.9

Total number of confirmed single infections	210

Figure 2: Analysis of the type and distribution of the specific symptoms from self-reported subject's symptom diary cards. Left, frequency of each symptom reported as a percentage of the total number of responding subjects, illustrated separately for samples that tested positive for a specific viral pathogen by BioFire PCR (lower chart) or tested negative for any specific pathogen (upper chart). Right, average number of symptom reported per virus detected, separated by upper respiratory tract symptoms, Systemic symptoms or total symptoms reported

Distribution of reported symptoms for the 3 most recovered viruses (single infection)

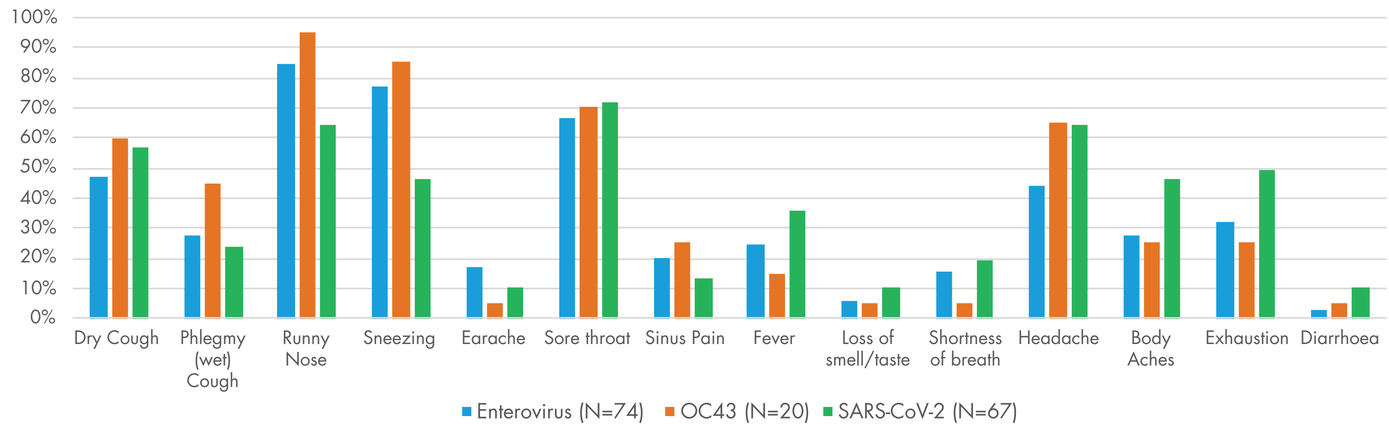
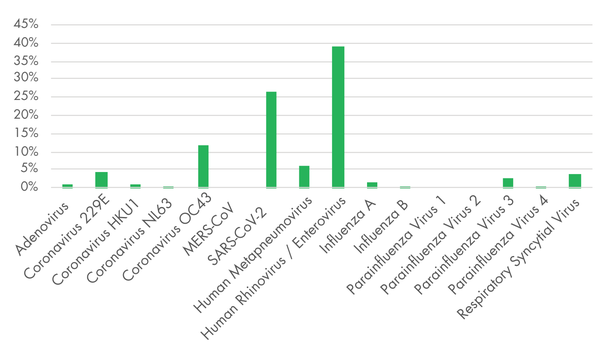


Figure 3: Frequency of each symptom reported for the three most commonly detected respiratory viruses: Enterovirus (blue), OC43 coronavirus (orange), SARS-CoV-2 (grey). Each bar shows the proportion of subjects infected with that virus that reported the specific symptom (co-infected subject data excluded from the analysis).

Runny Nose



Fever

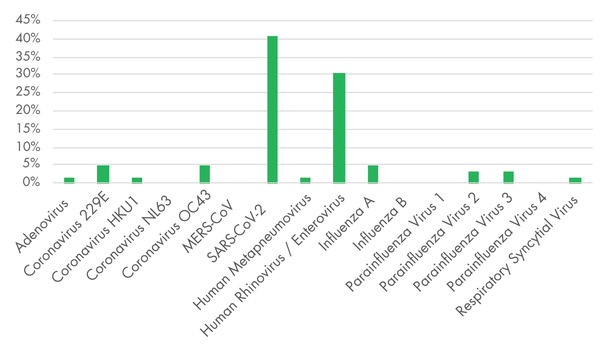
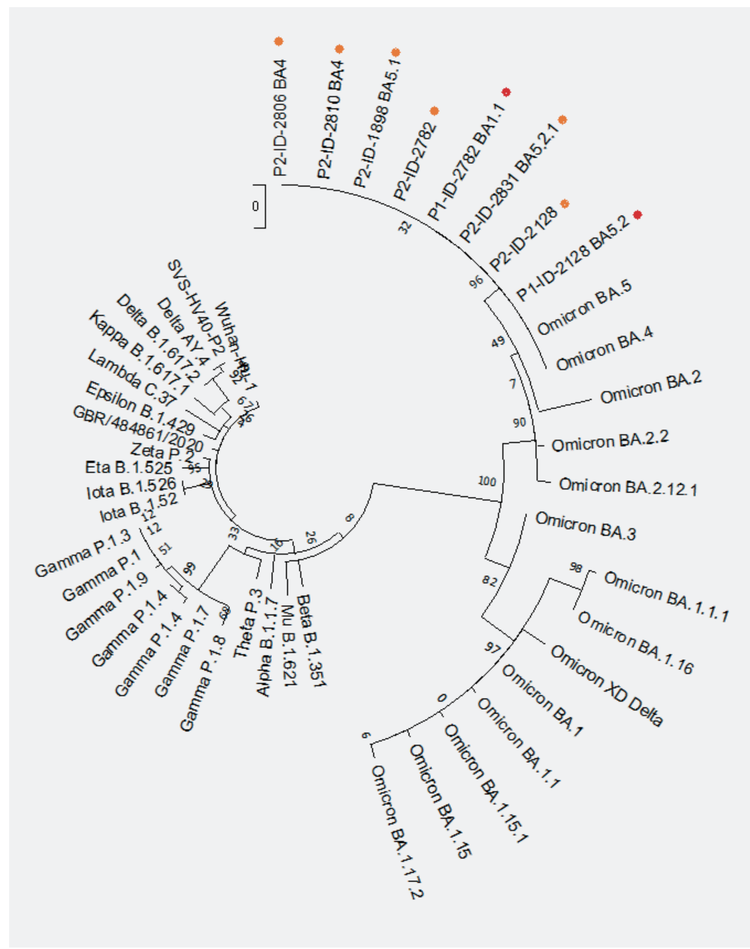


Figure 4: Proportion of subjects reporting two key symptoms of interest, Fever (lower chart) and Runny nose (upper chart), separated by virus-type.



Supplementary Figure: Graphical representation of the phylogenetic tree of SARS-CoV-2 samples recovered in July 2022. Clinical isolates propagated on VERO cells for 1 and 2 passages are marked in Red and Orange respectively.