



University of  
Zurich<sup>UZH</sup>

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# **Etiology of upper respiratory tract infection in outpatients before and during the SARS-CoV-2 pandemic**

Michael Huber

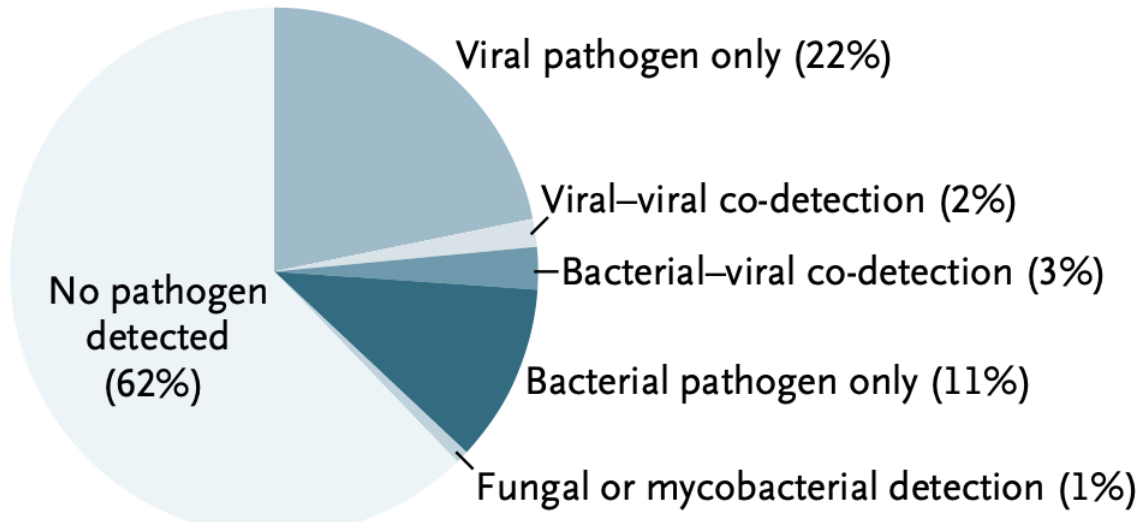
# Virus diagnostics is challenging

## Viral pathogens

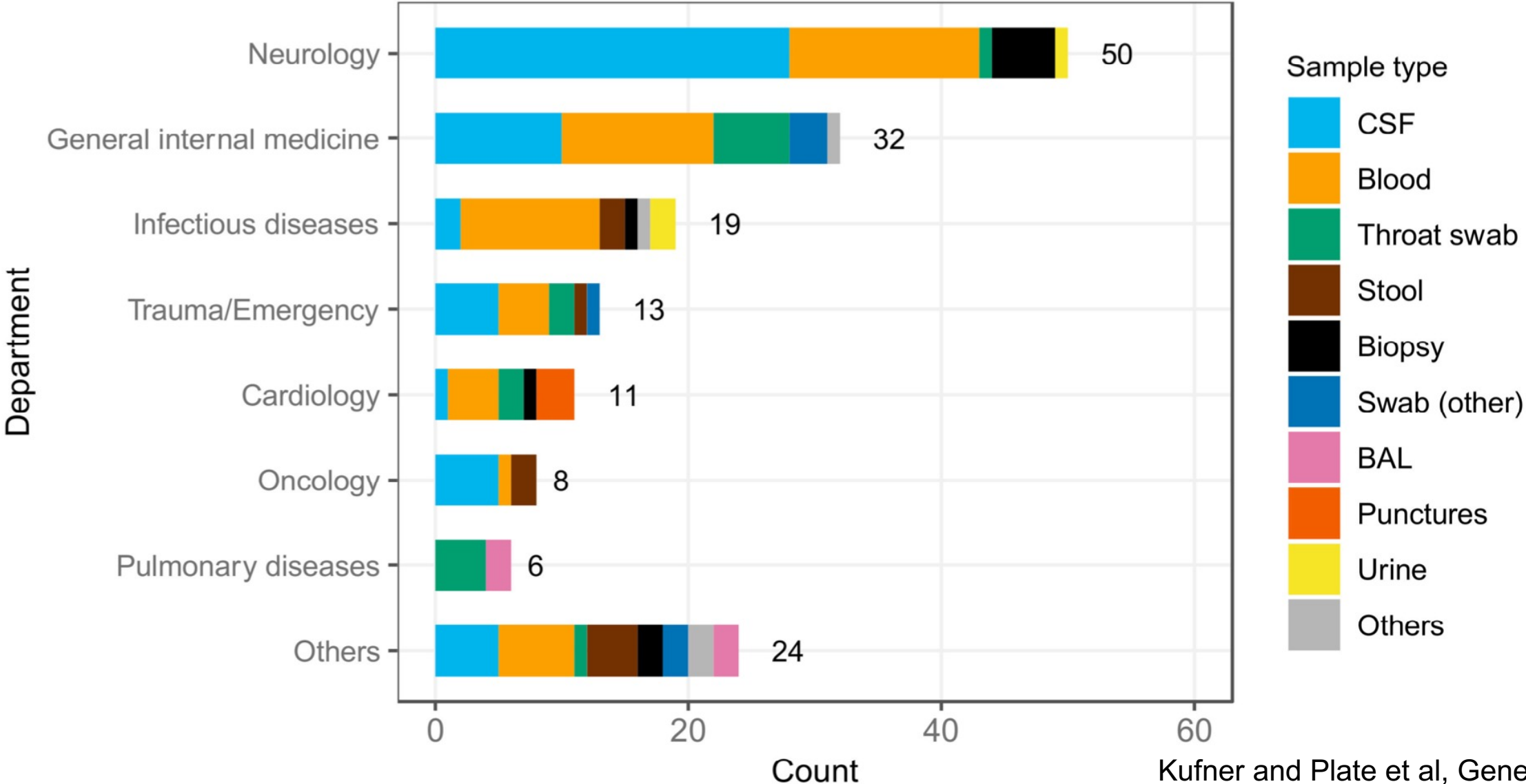
- more than 200 viruses pathogenic for humans
- rare, emerging, new viruses (SARS-CoV-2)
- frequent co-infections

## Routine virus diagnostics

- molecular methods
- highly sensitive
- rapid
- cost effective
- pathogen/sequence-specific
- multiple tests necessary



# Two years of viral metagenomics in a tertiary diagnostics unit: evaluation of the first 105 cases



# Large range of different viruses detected

- 34 out of 105 cases positive (32%)
- 27 distinct virus species belonging to 13 virus families
- Anelloviruses, Flaviviruses and Herpesviruses found most frequently
- Decisive diagnosis in a few cases

Tschumi et al. *BMC Infectious Diseases* (2019) 19:591  
<https://doi.org/10.1186/s12879-019-4231-9>

BMC Infectious Diseases

## CASE REPORT

Open Access

Meningitis and epididymitis caused by Toscana virus infection imported to Switzerland diagnosed by metagenomic sequencing: a case report

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# **Viral metagenomic sequencing as a diagnostic tool in primary care for patients with respiratory tract infection**

## **Acute upper respiratory tract infections**

- Common reasons for GP consultation
- Etiology is predominantly viral (Rhinovirus, Coronavirus, Adenovirus)
- Diagnosis based on a clinical assessment
- Specific testing only in selected situations (immunocompromised, outbreak)
- Antiviral and antibiotic treatment is rarely indicated.
- Inappropriate antibiotics prescription frequent and a problem

# 277 patients were recruited by 21 GPs between October 2019 and December 2020

## Study Design

- Prospective cross-sectional study
- October 2019 – November 2020
- 21 general practitioners (cantons ZH, BL)

## Inclusion criteria

- Clinically suspicion of upper respiratory tract infection
- Immunocompetent
- Age > 18 years

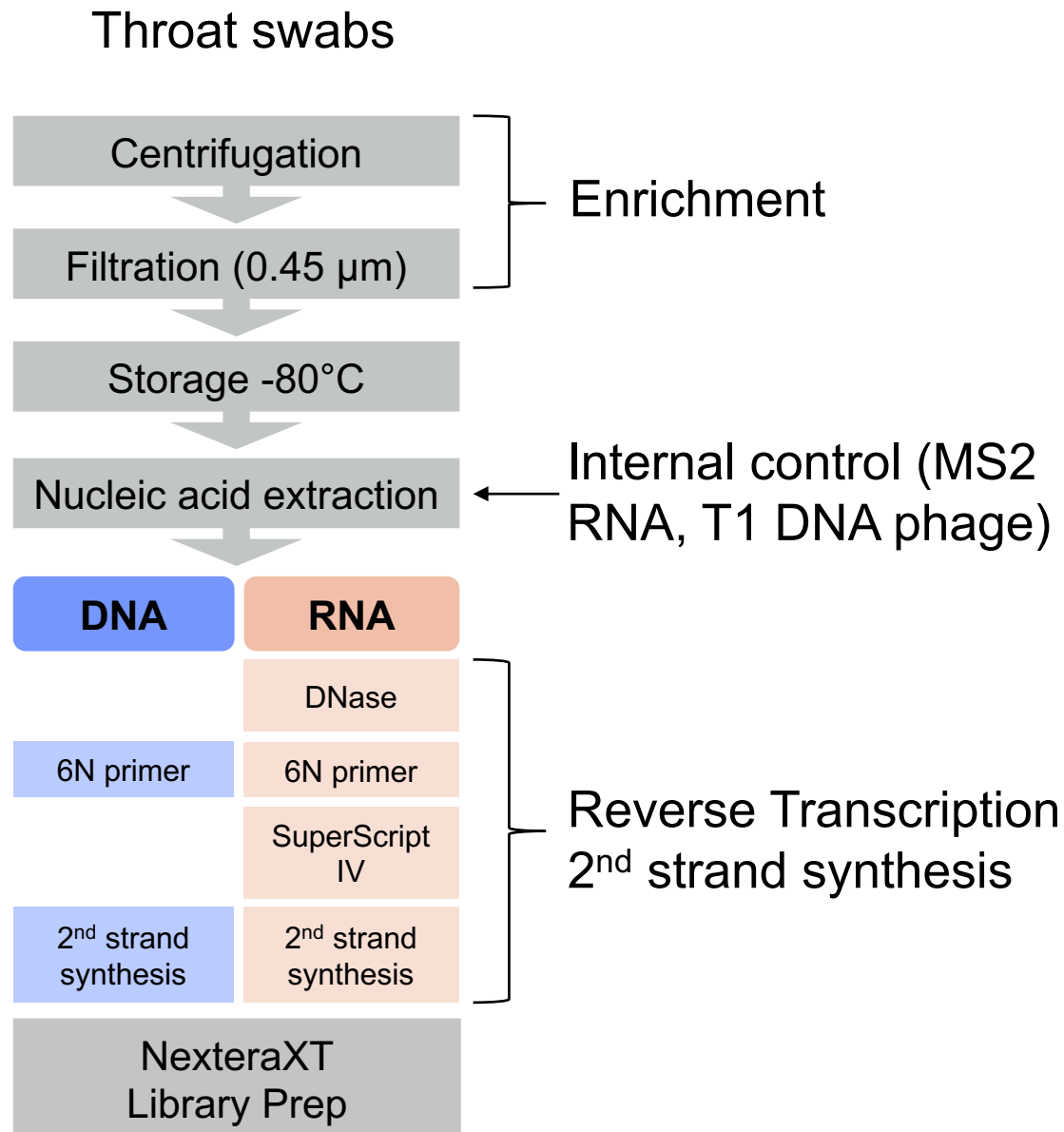
## Baseline

- Median duration of symptoms 5 days
- Sore throat (68%)
- Rhinitis (58%)
- Dry cough (49%)

## Diagnosis

- Common cold/rhinitis (49%)
- Pharyngitis/tonsillitis, non-streptococcal (16%)
- Suspected cause: viral 252, bacterial 21

# Metagenomic virus sequencing workflow at IMV



## Illumina MiSeq

- 5 samples per run
- 7.5 M reads per sample
- 150 nucleotides read-length



# Bioinformatic pipeline “VirMet”



```
>read_1
ATCGTACGTGATCGTACGGGACATACGGCTGGTACGTAGCATCG...
>read_2
CGTGATCGTACGGTGTACCGTCGTACCGAGGACTCGGTGCTGC...
```

- ✓ quality (PHRED > Q20)
- ✓ length (> 75 nucleotides)
- ✓ high entropy

- human reads
- bacterial reads
- bovine reads

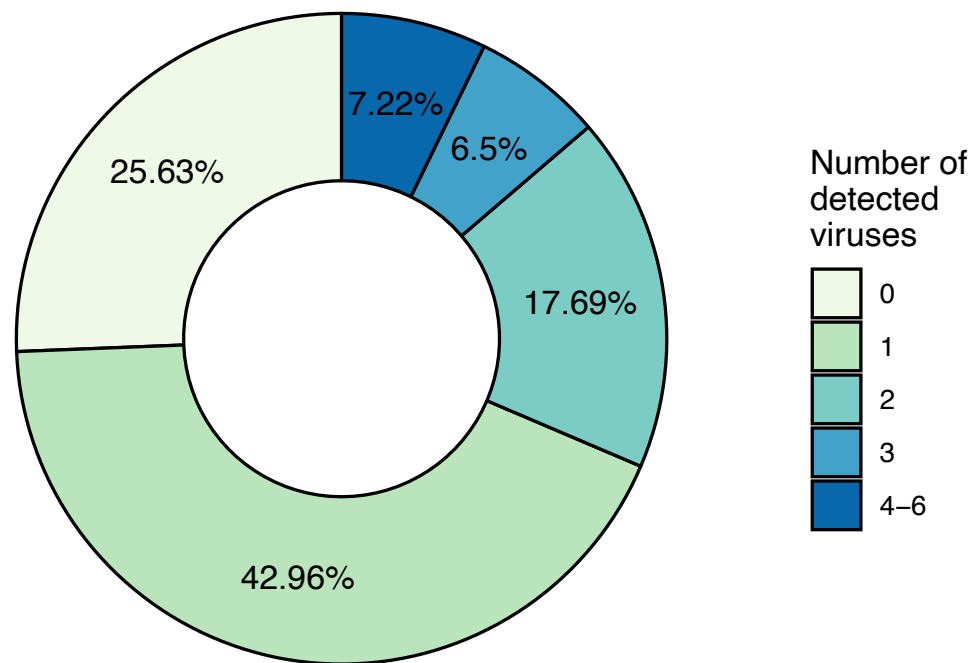
BLAST = Basic Local Alignment Search Tool  
in-house data base with >100'000 virus sequences from GenBank

reads assigned to virus species  
internal control (MS2, T1 phage)  
unaligned reads reported as “unknown”



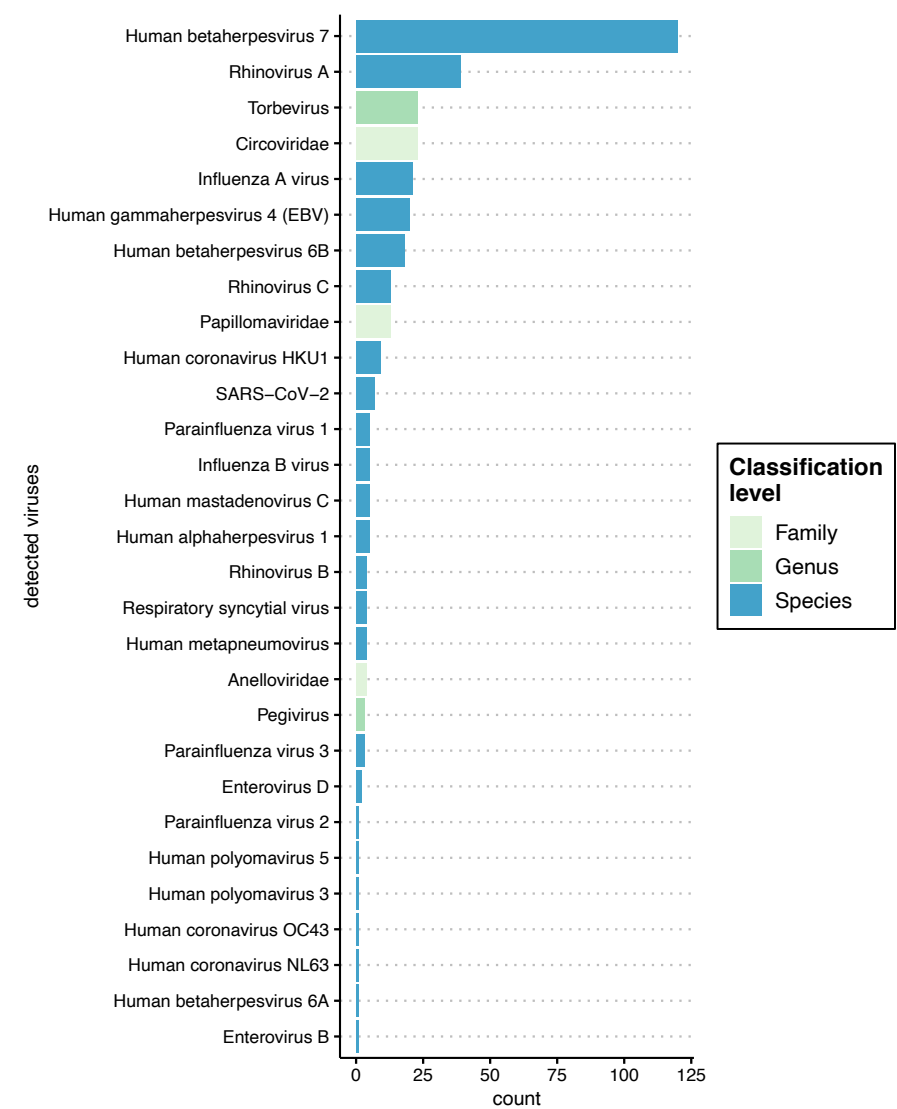
# Multiple and diverse viruses detected in most patients

Viruses per patient



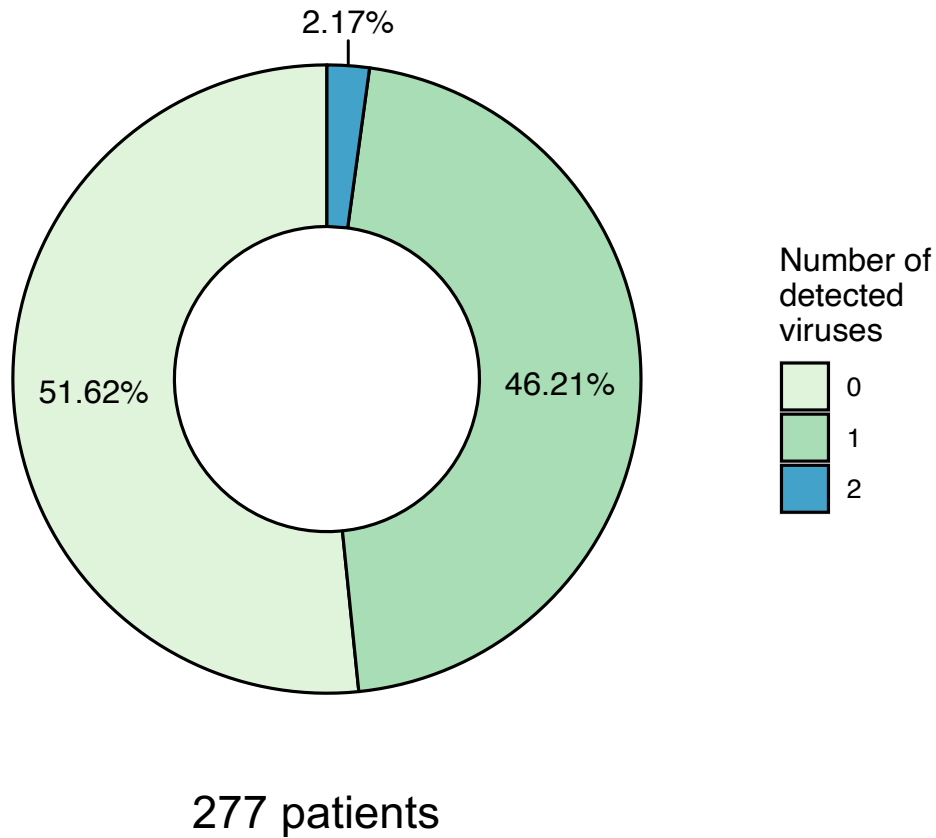
277 patients

Differentiation of detected viruses

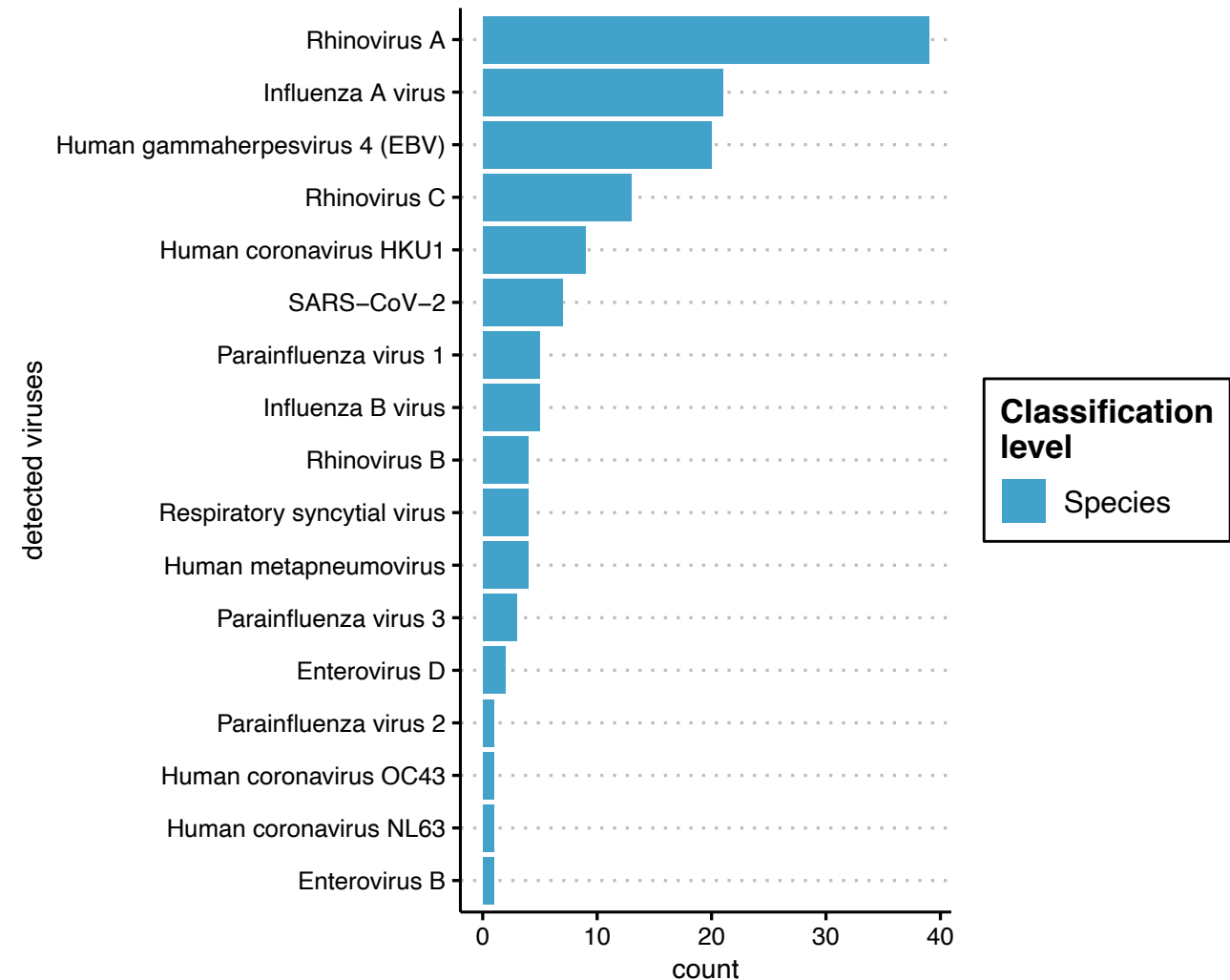


# Respiratory viruses detected in half of the patients

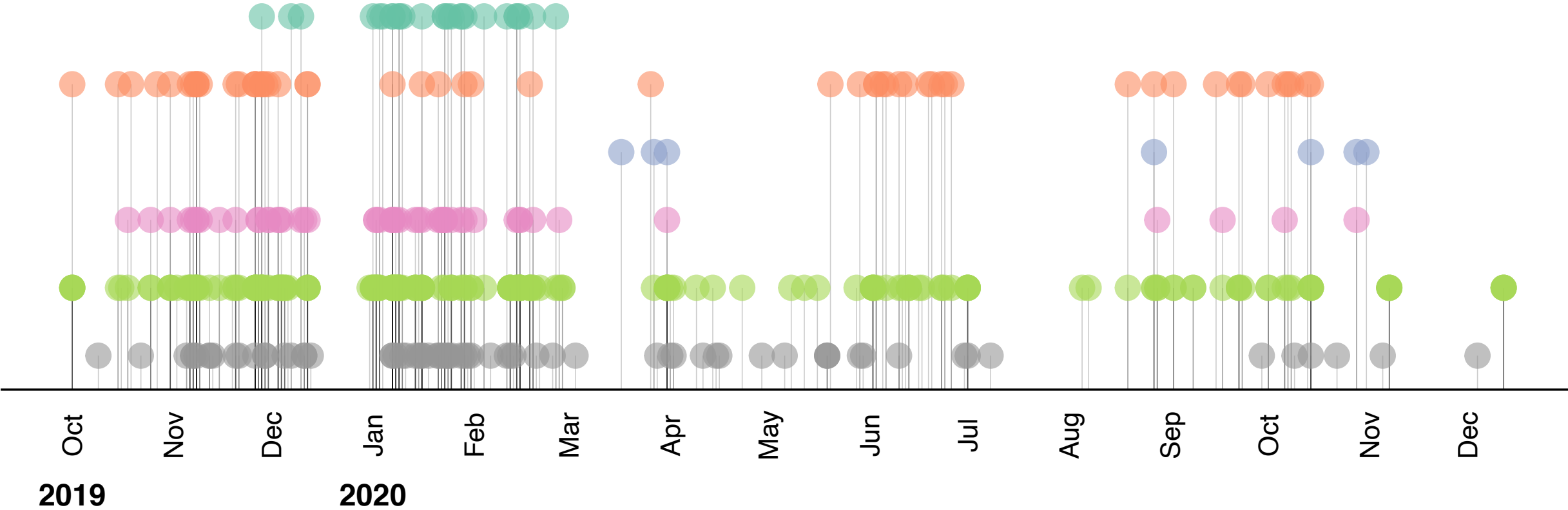
## Detected “respiratory viruses” per patient



## Differentiation of detected “respiratory viruses”

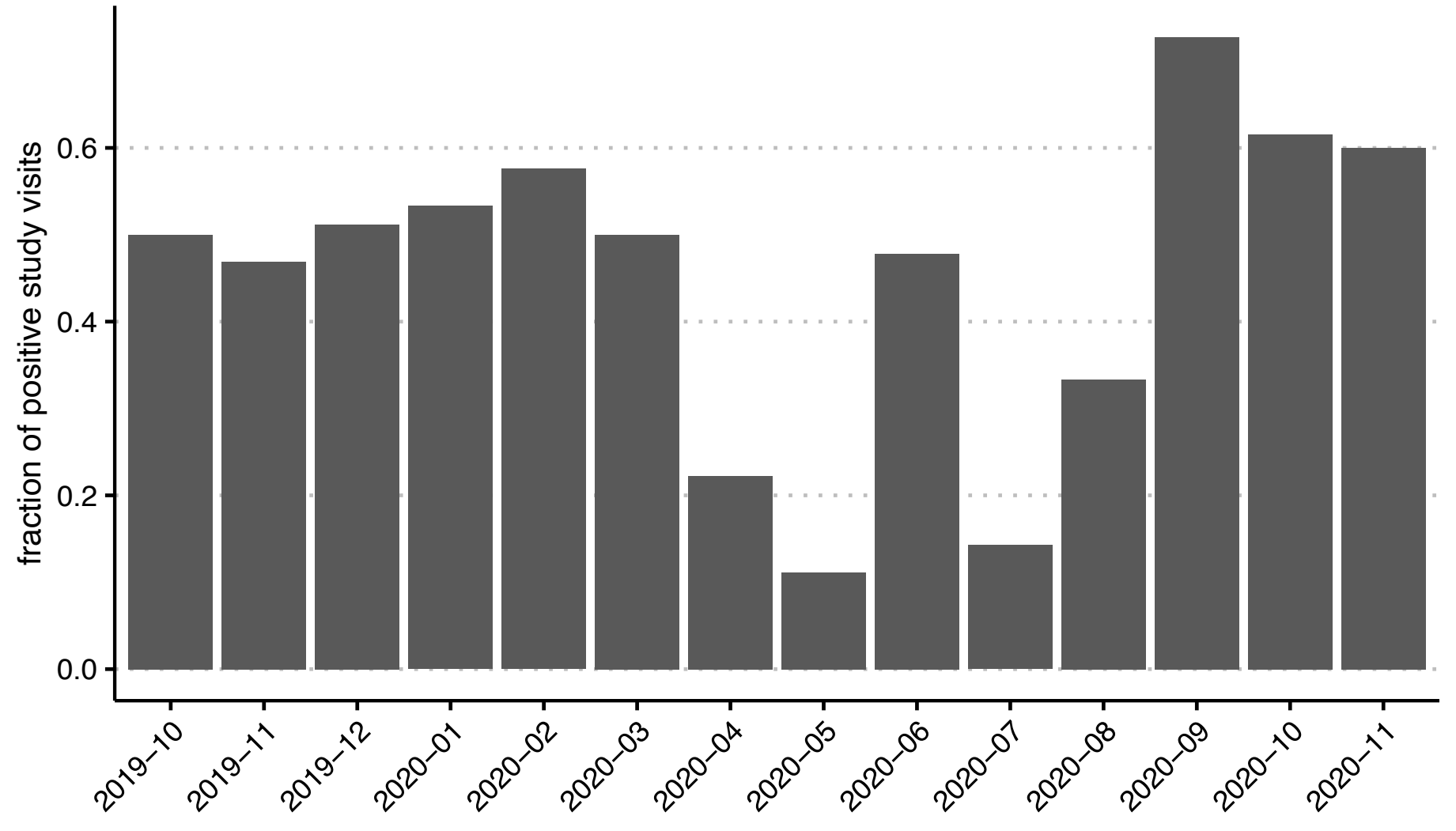


# Timeline shows seasonal and persistent viruses



- Influenzaviruses
- Rhinoviruses
- SARS-CoV-2
- other respiratory viruses
- other viruses
- negative

# Fewer visits with positive virus detection in spring and summer

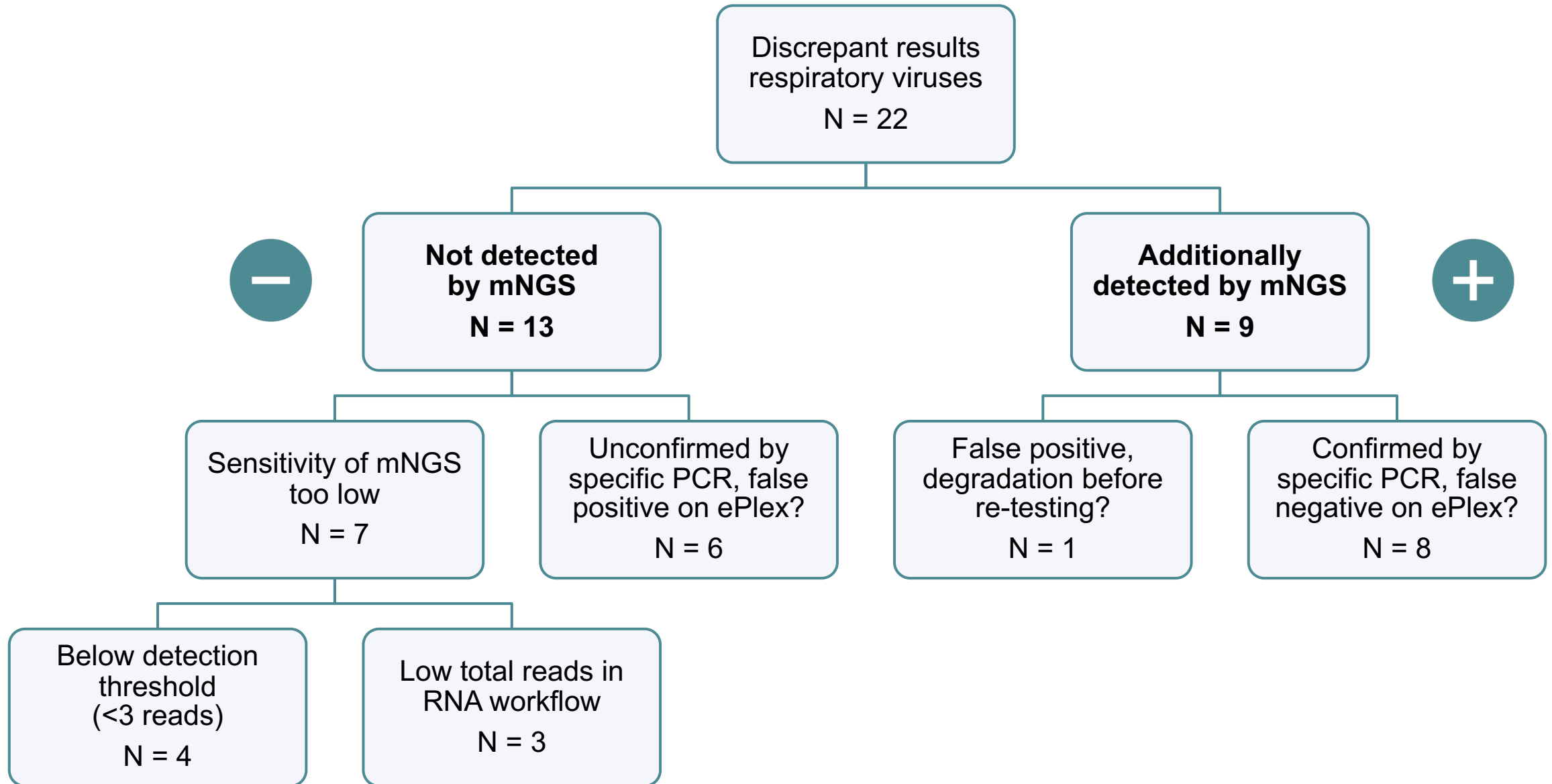


# Metagenomic sequencing shows good performance compared to a conventional test panel for respiratory viruses

			mNGS	ePLEX positive	ePLEX negative
<b>All Targets</b>	OPA = 99.5%	PPA = 89.3%	positive	108	9
		NPA = 99.8%	negative	13	4270
<b>Rhino/Entero</b>	OPA = 97.8%	PPA = 94.8%	positive	55	3
		NPA = 98.6%	negative	3	214
<b>Influenza A</b>	OPA = 98.2%	PPA = 83.3%	positive	20	1
		NPA = 99.6%	negative	4	250
<b>huCoV HKU1</b>	OPA = 99.3%	PPA = 88.9%	positive	8	1
		NPA = 99.6%	negative	1	265

missed by metagenomic sequencing  
detected additionally

# Discrepant results mostly due to low sensitivity in either platform



# Presumed cause and antibiotic treatment

Antibiotic treatment was prescribed to 24 patients (8.7%)

Suspected Cause	Total	mNGS positive	mNGS negative
viral	6	3	3
bacterial	18	7	11

mNGS detected  
a possible viral  
cause in 5 cases

# Conclusions viral metagenomics in URTI

## **Viral metagenomic sequencing**

- Unbiased, detects new viruses (SARS-CoV-2) and genotypes (EV-D68)
- High agreement with respiratory panel

## **In acute URTI**

- Re-evaluation of the GPs presumed diagnosis
- Reduce inappropriate antibiotic use
- Could prospectively influence URTI treatment (reduced turnaround time)

## **Used for surveillance**

- seasonality, anticipate circulating viruses, guiding prevention
- information on implemented hygiene measures during pandemic



# Acknowledgements



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