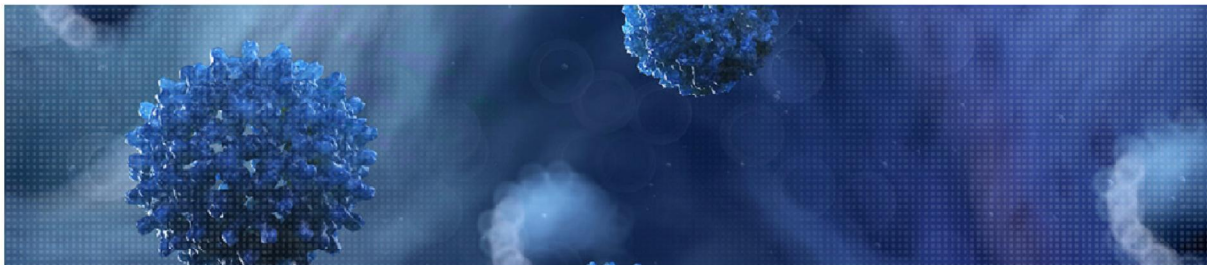




Viroscience lab
WHERE SKILLS MEET TO STUDY & PROTECT



Shedding of infectious virus in COVID-19

5.1.2e

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Key Questions



1. What is the **duration** of infectious virus shedding from respiratory tract?
2. **Which factors determine** shedding of infectious virus:
 - Duration of symptoms?
 - Viral RNA load?
 - Presence of serum neutralizing antibodies?
 - Immune status?
 - sgRNA?



Evidence from literature



n	disease severity	Main findings	reference
9	mild	<ul style="list-style-type: none"> No recovery infectious virus after 8 days post onset disease (p.o.d.) Recovery infectious virus <5% after 9,78 days p.o.d. Recovery infectious virus <5% if viral load < 6,51 Log₁₀ RNA copies/mL 	Wolfel, nature, 2020
90	unknown	<ul style="list-style-type: none"> No recovery infectious virus after 8 days p.o.d. No recovery infectious virus if ct-value > 24 	Bullard, clin infect dis, 2020
253	asymptomatic - severe	<ul style="list-style-type: none"> Recovery infectious virus in samples with Ct >35 was 8,3% Recovery infectious virus 10 days p.o.d. was 6% 	Singanayagam, Eurosurveillance, 2020



Methods




March and beginning of April 2020:

- Virus cultures on respiratory samples from hospitalized COVID-19 patients

For patients with at least one virus culture result:

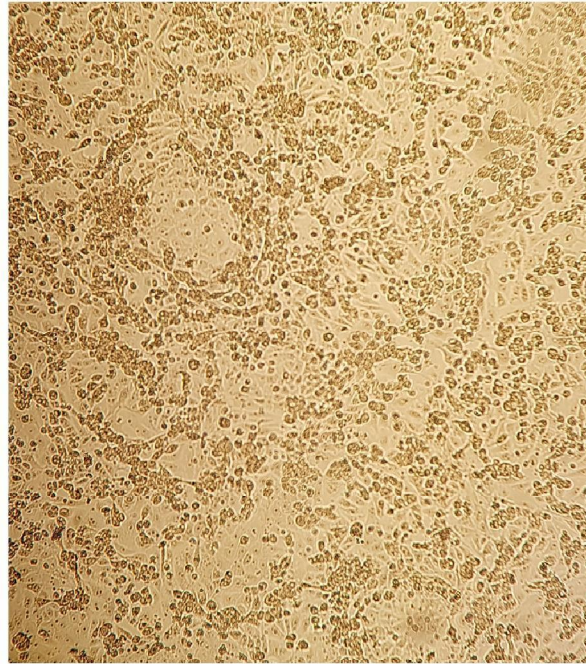
- SARS-CoV-2 RNA loads (Wolfel et al.)
- Serum neutralizing antibody titers (Okba et al.)
- sgRNA PCR (Wolfel et al.)
- Duration of symptoms, severity of disease and immunocompromised status.

Multivariate analysis:

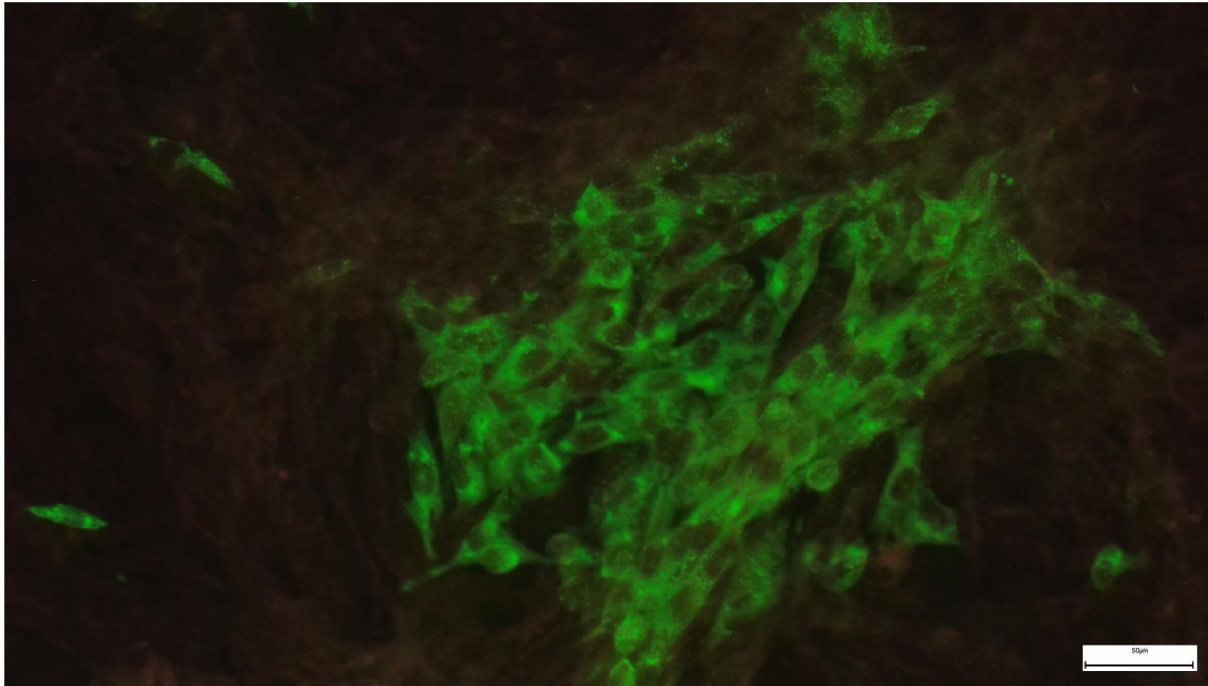
- Assess factors associated with shedding infectious virus: duration of symptoms, immunocompromised status, viral RNA loads, presence serum neutralizing antibodies
 - Generalized estimating equations to account for repeated measurements obtained from the same patient during hospitalization.
- 



Vero E6
Uninfected
Day 2



Vero E6
SARS-CoV-2 infected
Day 2



Vero E6
SARS-CoV-2 infected
Day 2

Immunocompromised score

Table 1. Criteria used to categorize the level of immune compromise

Level of immune suppression	Reason for immune compromise (acquired condition/iatrogenic/drug-induced)	Inborn immunodeficiency
Severe	Allogeneic HSCT (<12 months) GVHD after allogeneic HSCT HIV-positive with CD4 ⁺ T-cell count <200 cells/ μ l Induction chemotherapy for paediatric leukaemia Chemotherapy with >7 days neutropenia SOT patients Lung transplant (always) <6 months and induction Rx >1 year SOT and rejection (<3 months) Use of immunomodulating biologicals Daily corticosteroid dosage (based on prednisone) of >30 mg (adults) or >2 mg/kg (infants) for longer than 14 days	SCID XLA Interferon receptor deficiency Hyper IgE syndrome
Non-severe (mild/moderate)	Maintenance chemotherapy for haematological malignancies Chemotherapy for solid tumours Autologous HSCT 1 year after SOT and no rejection HIV-positive with or without HAART, with undetectable viral load and CD4 ⁺ T-cell count >200 cells/ μ l Methotrexate use for autoimmune disease Daily corticosteroid dosage (based on prednisone) of \leq 30 mg (adults) or \leq 2 mg/kg (infants) for \leq 14 days Other possible immune deficiencies (that is, untreated autoimmune disease, DM, etc.)	CVID CGD



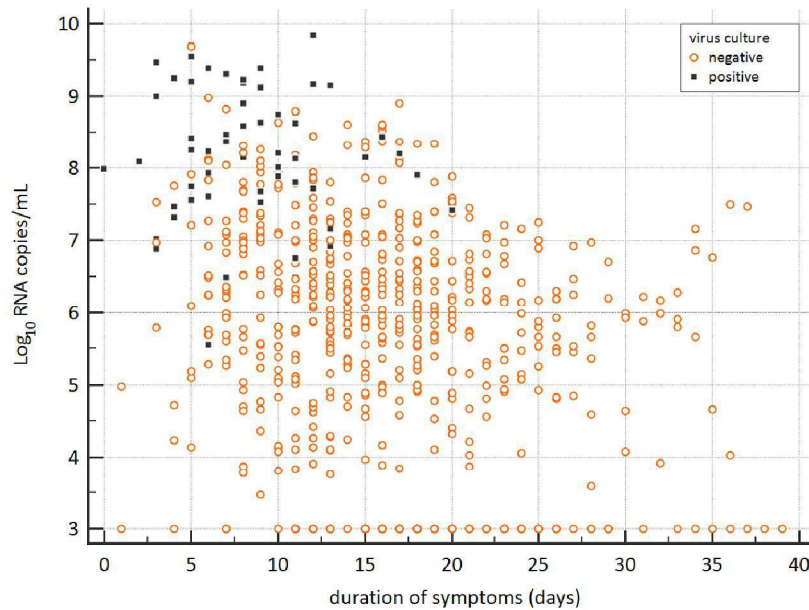
Patient characteristics



Characteristic	All	Intensive care	Medium care	p-value (ICU vs ward)
Number	129	89 (69.0%)	40 (31.0%)	
Male	86 (66.7%)	65 (73.0%)	21 (52.5%)	0.04
Age (median – IQR)	65 (57-72)	66 (57 – 72)	63 (57-74)	0.90
Immunocompromised				0.04
Moderate	19 (14.7%)	10 (11.2%)	9 (22.5%)	
Severe	11 (8.5%)	5 (5.6%)	6 (15.0%)	
Clinical parameters				
Mechanical ventilation	81 (62.8%)	81 (91.0%)	0	
Supplemental oxygen	43 (33.3%)	8 (9.0%)	35 (87.5%)	
Died	14 (10.9%)	11 (12.3%)	3 (7.5%)	
Duration of illness*				0.009
Median (IQR)	18 (13-21)	18 (13-22)	15 (12-18)	
Tests per patient, Total (mean per person)				
Culture	690 (5.3)	601 (6.8)	89 (2.2)	
PRNT	112 (0.9)	82 (0.9)	30 (0.8)	
PCR	688 (5.3)	599 (6.7)	89 (2.2)	

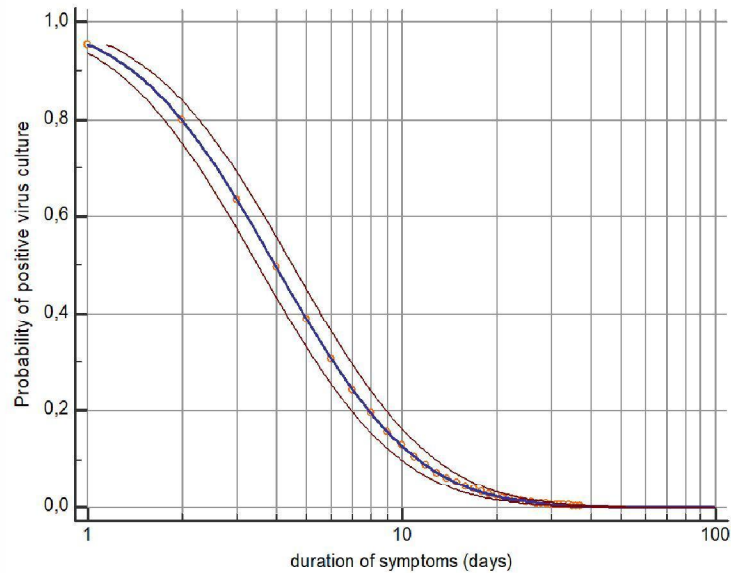
* As of April 17th 2020. PRNT = plaque reduction neutralization titer.

Duration of infectious virus shedding



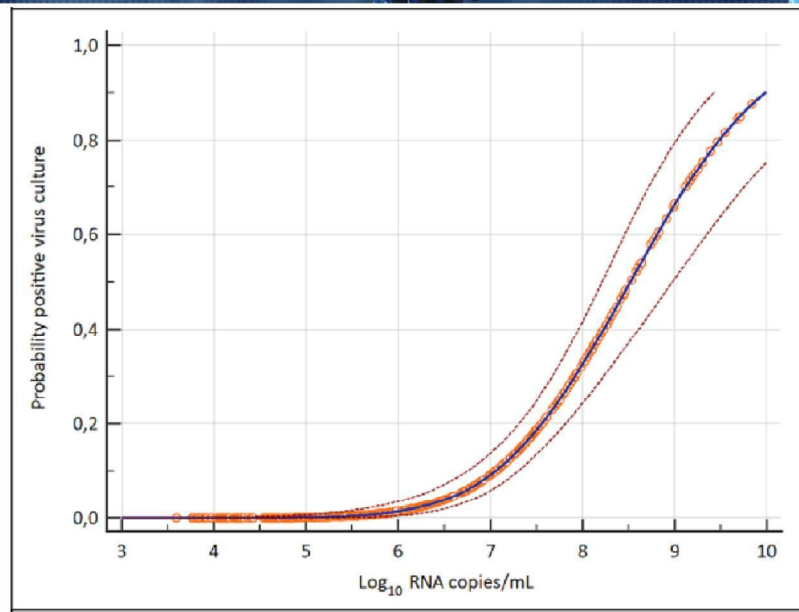
- 23/129 patients positive
- 62/690 samples positive
- Median duration shedding: 8 days
- IQR: 5 – 11 days
- Range: 0 -20 days

Isolation of infectious virus versus duration of symptoms



Probability $\leq 5\%$ when duration of symptoms is ≥ 15.2 days (95% CI 13.4 – 17.2)

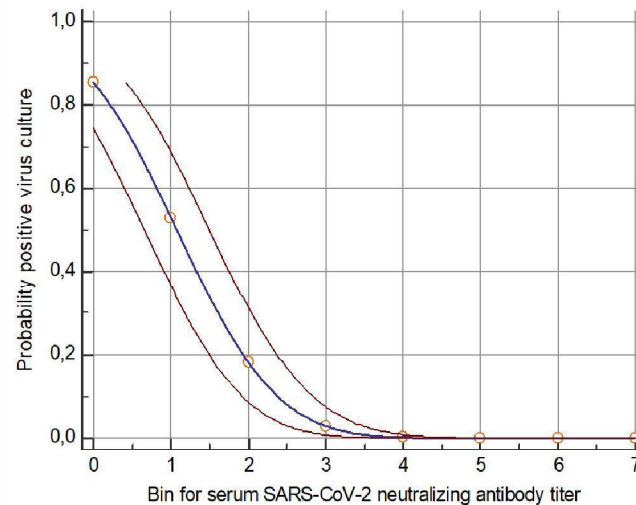
Isolation of infectious virus versus viral RNA loads



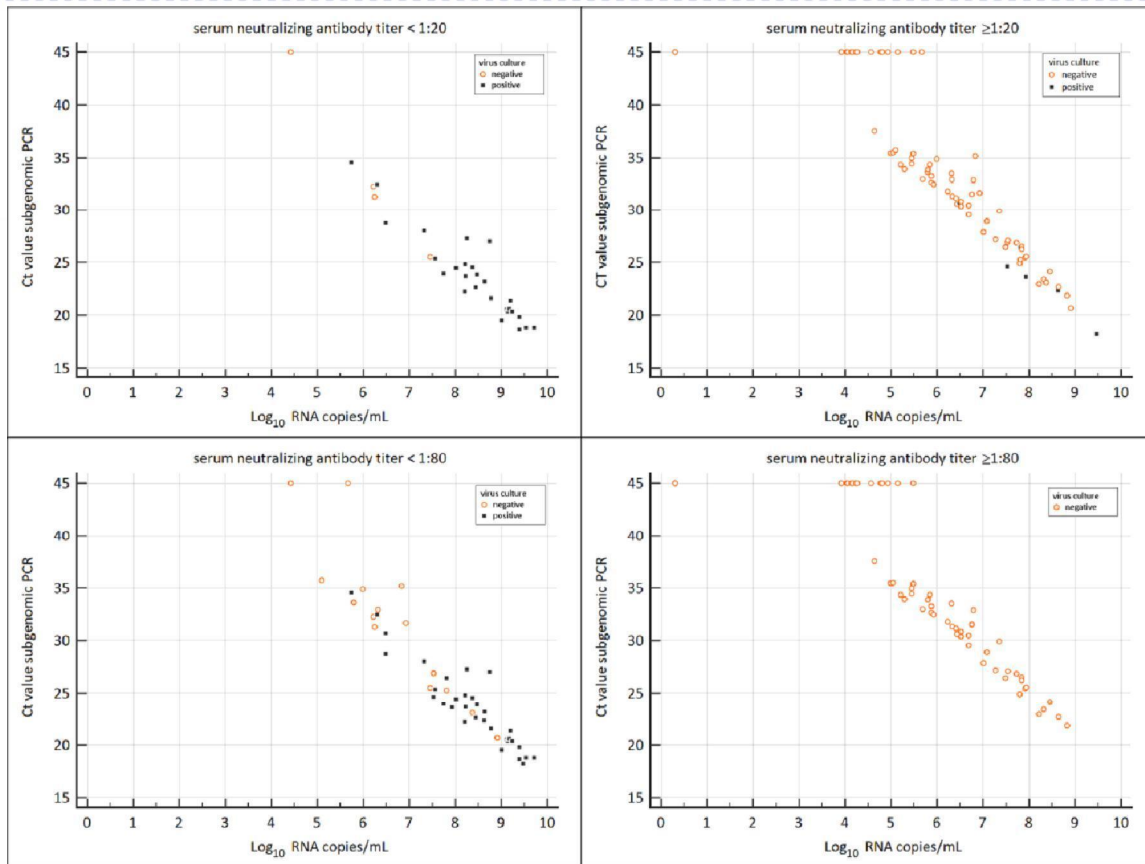
Probability < 5% when viral load < **6,63 Log₁₀ RNA copies/mL** (95% CI 6,24 – 6,91)

Isolation of infectious virus versus neutralizing antibody titer

titer	n	POS	NEG
< 1:20	31	27 (87%)	4 (13%)
1:20	10	4 (40%)	6 (60%)
1:40	7	2 (29%)	5 (71%)
1:80	2	0 (0%)	2 (100%)
1:160	4	0 (0%)	4 (100%)
1:320	11	0 (0%)	11 (100%)
1:640	9	0 (0%)	9 (100%)
1:1280	14	0 (0%)	14 (100%)
1:2560	16	0 (0%)	16 (100%)



Probability < 5% when serum neutralizing antibody titer is $\geq 1:80$

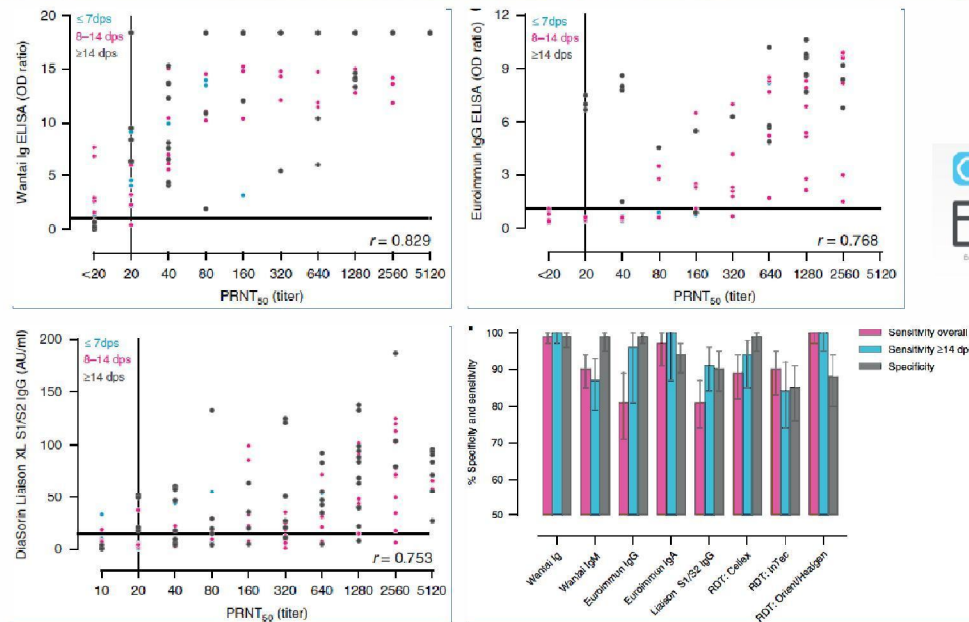




Key determinants infectious virus shedding

Variable	Positive virus culture (n=33)	Negative virus culture (n=79)	Univariate Odds ratio (95% CI)	Multivariate Odds ratio (95% CI)
Viral RNA load >10 ⁷ RNA copies/mL	29 (87.9%)	22 (27.8%)	18.8 (5.5 – 64.2), p<0.001	14.7 (3.7-58.1), p<0.001
Duration of symptoms < 7 days	20 (60.6%)	17 (21.5%)	5.6 (1.7 – 18.1), p=0.004	2.1 (0.4-11.7), p=0.31
Serum neutralizing antibody titer 1:20 or higher	6 (18.2%)	75 (94.9%)	0.01 (0.003 – 0.05), p<0.001	0.01 (0.002-0.08), p<0.001
Immunocompromised yes	10 (30.3%)	10 (12.7%)	3.00 (0.8-11.0), p=0.098	2.0 (0.7 – 5.3), p=0.22

Serology and quantitative PCR for IPC?



Corine geurtsvankessel et al., Nature Communications, 2020



Conclusions

Duration of infectious virus shedding in hospitalized patients with **severe** COVID-19 is longer than what has been reported for in **mild** COVID-19

Factors independently associated with detection of infectious virus:

- High viral RNA load in respiratory samples
 - Absence of serum neutralizing antibodies
- 