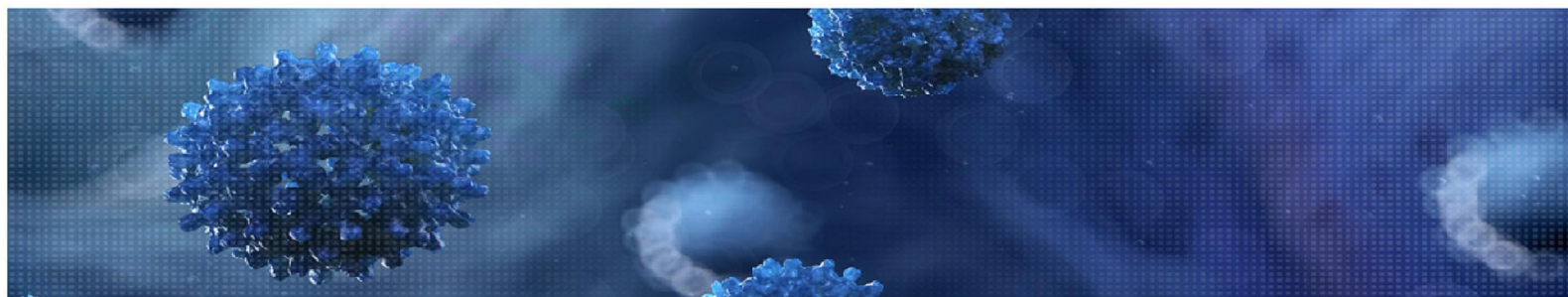




Viroscience lab
WHERE SKILLS MEET TO STUDY & PROTECT



SARS-CoV-2: kinetiek van shedding en immuunrespons

5. 5.1.2e



Key Questions

1. Duration of infectious virus shedding from respiratory tract
2. Determinants of infectious virus shedding:
 - Duration of symptoms?
 - Viral RNA load?
 - Presence of serum neutralizing antibodies?
 - Immune status?

Article

Virological assessment of hospitalized patients with COVID-2019

Nature | Vol 581 | 28 May 2020 | 465

<https://doi.org/10.1038/s41586-020-2196-x>

Received: 1 March 2020

Accepted: 24 March 2020

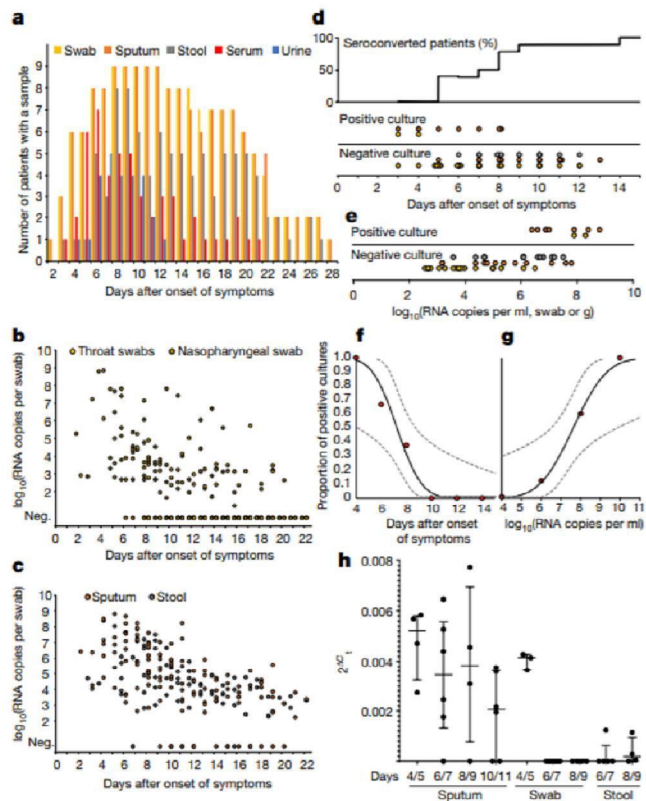
Published online: 1 April 2020

Roman Wölfel^{1,6}, Victor M. Corman^{2,6}, Wolfgang Guggemos^{3,6}, Michael Seilmaier³, Sabine Zange¹, Marcel A. Müller², Daniela Niemeyer², Terry C. Jones^{2,4}, Patrick Vollmar¹, Camilla Rothe⁵, Michael Hoelscher⁵, Tobias Bleicker², Sebastian Brünink², Julia Schneider², Rosina Ehmann¹, Katrin Zwirgmaier¹, Christian Drosten^{2,7} & Clemens Wendtner^{3,7}✉

Table 2 | Clinical characteristics of all patients

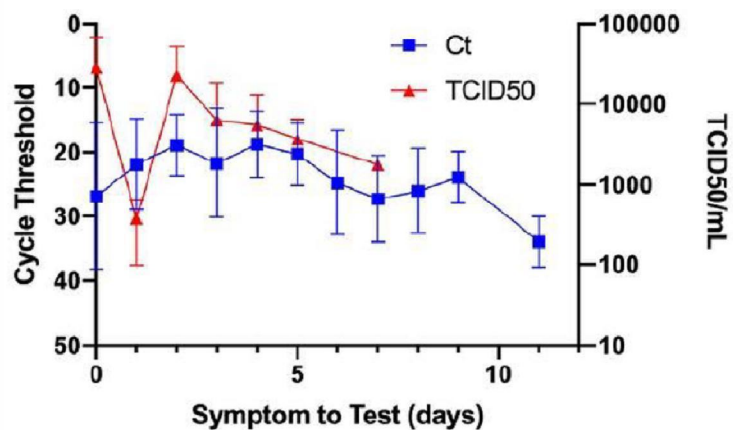
Patient ID no.	Comorbidity	Initial symptoms	Later symptoms	ANC per μl	ALC per μl	CRP (mg l^{-1})	LDH (U l^{-1})
1	Hypothyroidism	Cough, fever, diarrhoea	Diarrhoea	4,870	1,900	46	197
2	None	Sinusitis, cephalgia, cough	Hyposmia, ageusia	3,040	1,200	4.9	182
3	COPD	Arthralgia, sinusitis, cough	Dysosmia, dysgeusia	5,040	2,600	1.3	191
4	None	Otitis, rhinitis	Hyposmia, hypogeusia	2,420	2,220	5.9	149
7	Hypercholesterolaemia	Rhinitis, cough	Fever, dyspnoea, hyposmia, hypogeusia	4,690	900	4.9	209
8	None	Sinusitis, cough		2,500	1,600	1.7	203
10	None	Sinusitis, cough	Fever, cough	2,350	700	7.8	220
14	None	Fever, cough, diarrhoea		5,040	1,500	9.8	220
16	None	None		4,620	900	0.5	201

ALC, absolute lymphocyte count; ANC, absolute neutrophil count; CRP, C-reactive protein; COPD, chronic obstructive pulmonary disease; LDH, lactate dehydrogenase.



- For less than 5% isolation success, the estimated day was 9.78 (95% CI: 8.45-21.78) days post-onset and
- the estimated RNA concentration for less than 5% isolation success was estimate to be 6.51 Log₁₀ RNA/ml (95% CI: -4, 11-5.40).
- 9 samples positive in culture

SARS-CoV-2 viral dynamics as expressed by E gene RT-PCR Cycle threshold (Ct) value and cell culture TCID50/mL, over time (days).
Squares represent Ct values while triangles reflect TCID50.



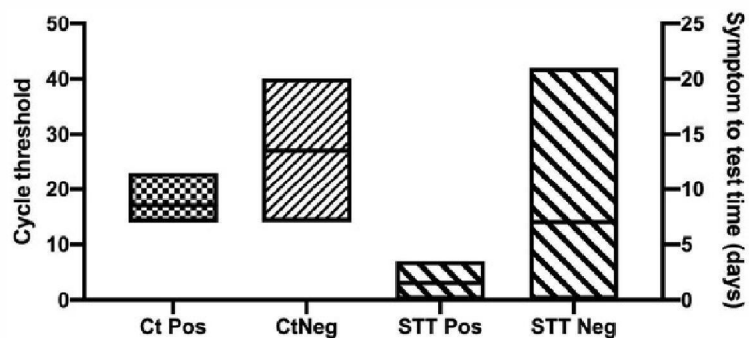
- A total of 90 samples were analyzed.
- Median age of the pts sampled was 45 (30-59).
- 49% of samples were from males.
- SARS-CoV-2 was successfully cultivated from 26 (28.9%) of the samples.
- The samples included in this study included those positive for SARS-CoV-2 by RT-PCR from day of symptom onset (Day 0) up to 21 days post symptom onset.
- 26 samples positive in culture

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SARS-CoV-2 E gene RT-PCR Ct values and symptom to test time (STT) in samples that were culture positive (Ct +, STT +), or negative (Ct -, STT -)



- There was no growth in samples with: a Ct > 24 or STT > 8 days.
- Positive culture samples had a significantly lower Ct when compared to culture negative samples (17 [16-18] vs 27 [22-33], $p < 0.001$)

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et al., accepted CID

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Methods

March and beginning of April 2020:

- Perform virus culture (on surplus) on as many respiratory samples from COVID-19 patients as possible.

For all admitted patients with at least one virus culture result:

- Extract all SARS-CoV-2 PCR data from LabTrain
- Extract all SARS-CoV-2 neutralizing antibody titers from LabTrain
- Extract duration of symptoms, severity of disease and immune status from HiX.

Techniques

- Neutralizing antibody titers: plaque reduction neutralization assay (Okba et al.)
- PCR: in-house (REF) and Cobas6800. Convert ct values to viral load using external calibration curve (quantified E-gene transcript) (Wolfel et al.)
- Virus culture: Vero cells with CPE and immunofluorescence read-out

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

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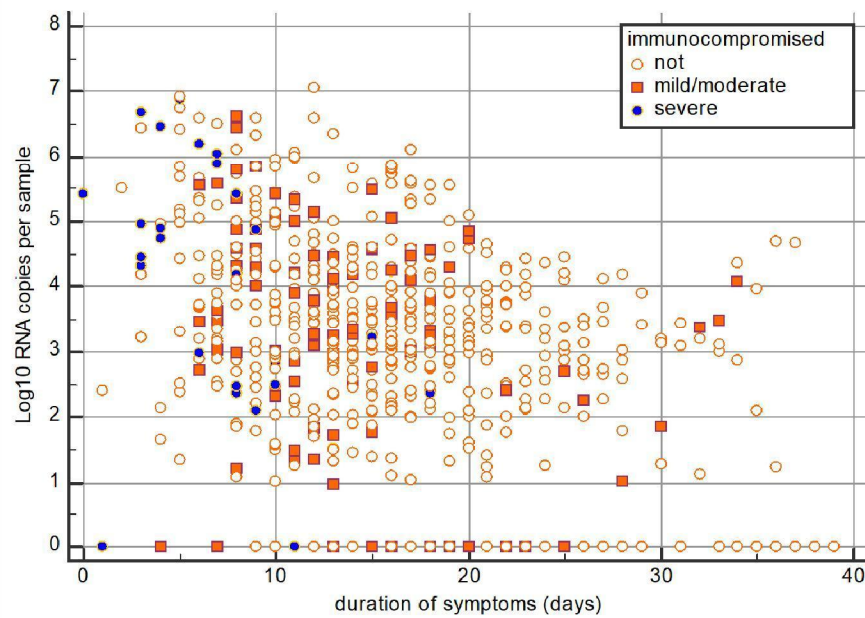
et al., Antiviral Therapy, 2015

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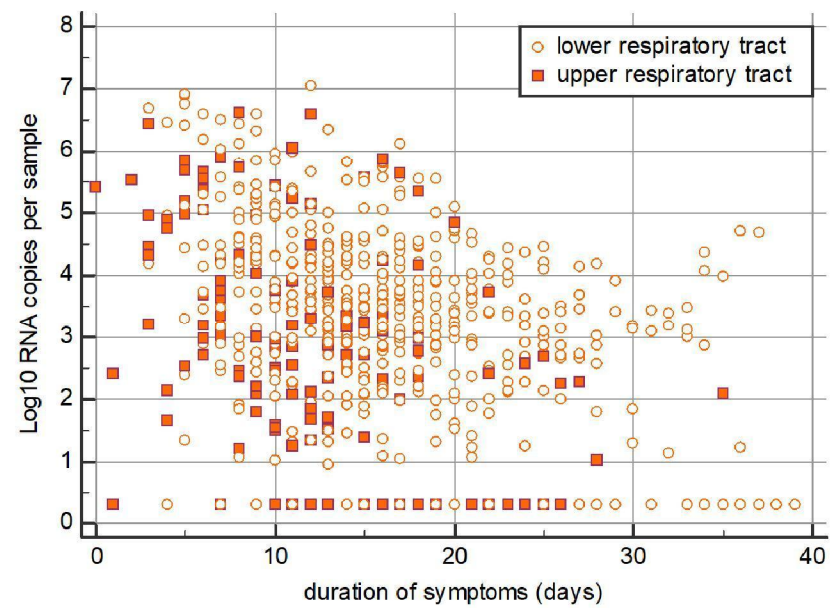
Patient characteristics

Characteristic	All	Intensive care	Ward
Number	129	89 (69.0%)	40 (31.0%)
Male	86 (66.7%)	65 (73.0%)	21 (52.5%)
Age (median – IQR)	65 (57-72)	66 (57 – 72)	63 (57-74)
Immunosuppression			
Moderate	19 (14.7%)	10 (11.2%)	9 (22.5%)
Severe	11 (6.7%)	5 (5.6%)	6 (15.0%)
Disease severity			
Mechanical ventilation	89 (69.0%)	81 (91.0%)	0
Oxygen	43 (33.3%)	8 (9.0%)	35 (87.5%)
Died**	14 (10.9%)	11 (12.3%)	3 (7.5%)
Duration of illness**			
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)

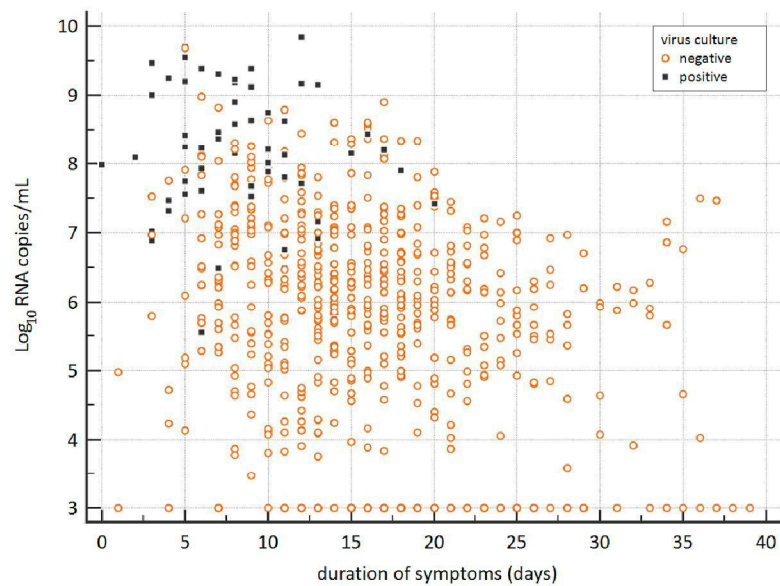
Immune status



Type of respiratory material



Isolation of infectious virus

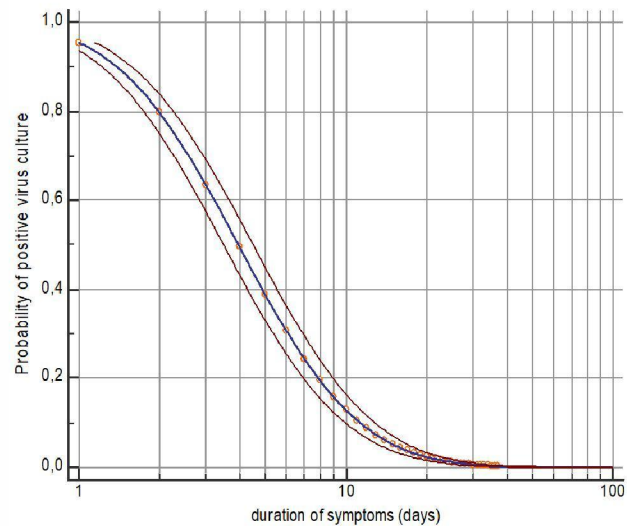


- The median viral load was significantly higher in culture positive samples than in culture negative samples:
8,14 versus 5,88 Log₁₀ RNA copies/mL,
 P<0,0001
- 62 samples positive in culture

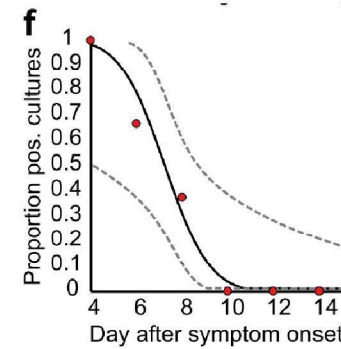
Probability isolation infectious virus versus duration of symptoms



The median time of infectious virus shedding was 8 days (IQR 5 – 11, range 0 – 20)



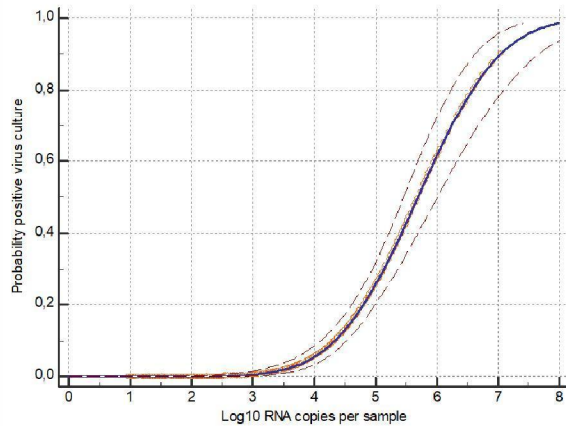
Probability <5% when duration of symptoms is at least 15.2 days (95% CI 13.4 – 17.2)



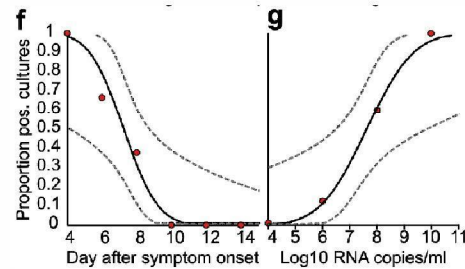
Wolfel et al.
5% = 9,78 days

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Probability isolation infectious virus versus viral RNA loads



The probability of isolation infectious SARS-CoV-2 is <5% when viral load is below 6,63 Log_{10} RNA copies/mL (95% CI 6,24 – 6,91)

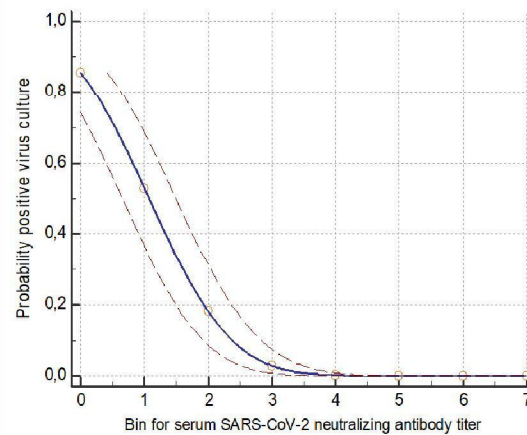


Wolfel et al.
5% = 6,51 Log_{10}

PRNT

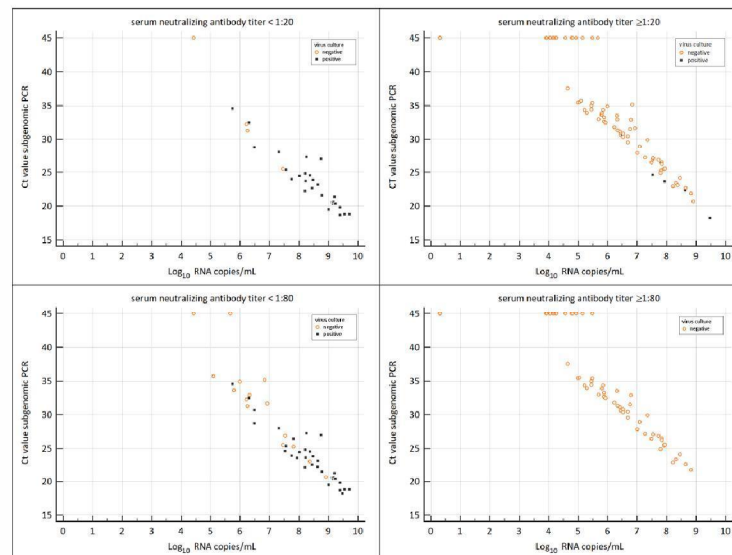


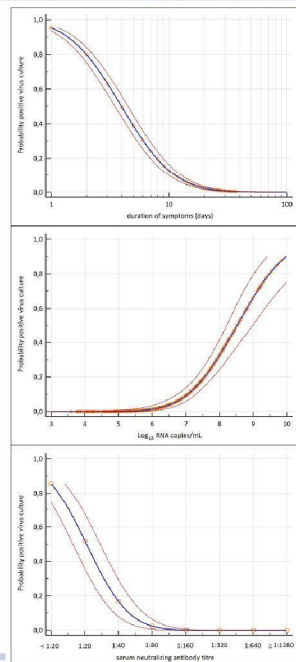
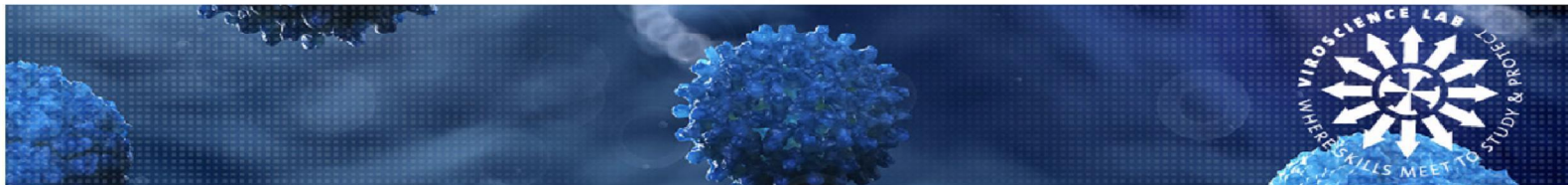
titer	n	POS	NEG
< 1:20	31	27 (87%)	4 (13%)
1:20	10	4 (40%)	6 (60%)
1:40	7	2 (29%)	5 (71%)
1:80	2	0 (0%)	2 (100%)
1:160	4	0 (0%)	4 (100%)
1:320	11	0 (0%)	11 (100%)
1:640	9	0 (0%)	9 (100%)
1:1280	14	0 (0%)	14 (100%)
1:2560	16	0 (0%)	16 (100%)



Probability <5% when PRNT is at least
1:80

Isolation of infectious SARS-CoV-2 from the respiratory tract.





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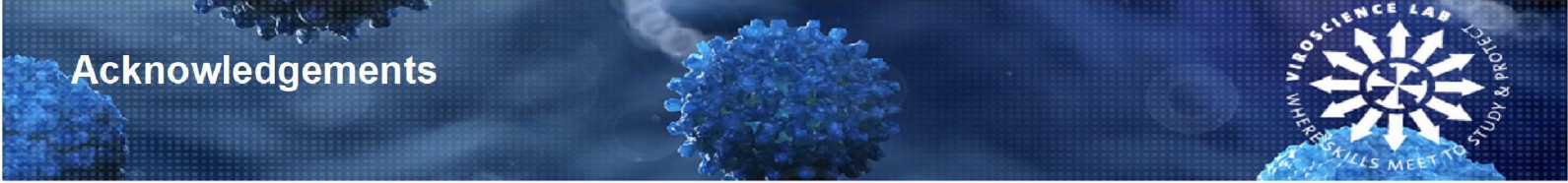
Multivariate analysis

Variabele	Positive (n=33)	Negative (n=79)	Univariate Odds ratio (95% CI)	Multivariate Odds ratio (95% CI)
Viral load >10 ⁵ RNA copies/sample	21 (63.6%)	6 (7.6%)	21.3 (9.1 – 49.8) p<0.001	9.9 (2.0-47.7) P=0.004
Duration of symptoms < 2 weeks	23 (69.7%)	21 (26.6%)	6.4 (2.7 – 23.3) P=0.005	3.2 (0.6 – 18.8) P=0.19
PRNT50% titer 1:20 or higher	6 (18.2%)	75 (94.9%)	0.01 (0.003 – 0.04) P<0.001	0.02 (0.005- 0.11) P<0.001
Immunocompromised yes	10 (30.3%)	10 (12.7%)	3.00 (0.8-11.0) P=0.099	1.7 (0.4 – 7.5) P=0.49



Conclusions

- a viral load above 7 Log₁₀ RNA copies/mL and
- absence of serum neutralizing antibodies were independently associated with isolation of infectious SARS-CoV-2 from the respiratory tract.
- the duration of infectious virus shedding in respiratory tract samples in severe COVID-19 is longer than in mild COVID-19.



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