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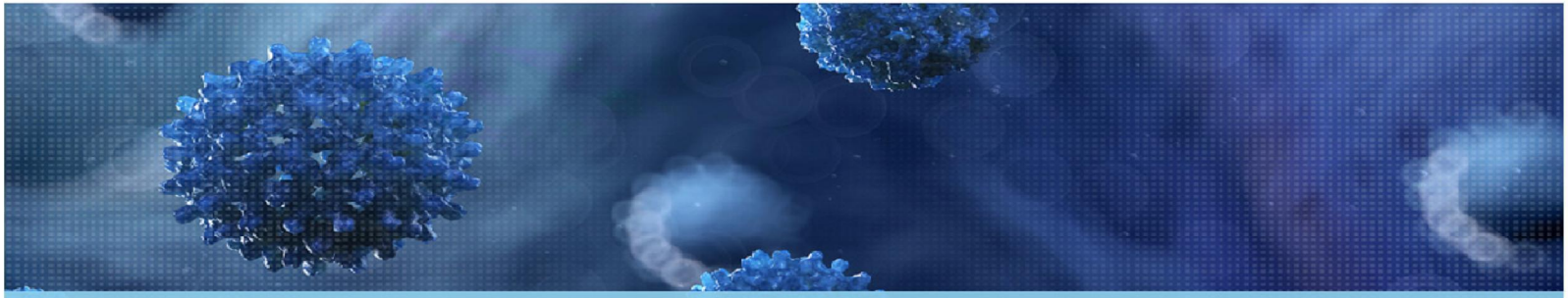
Viroscience lab

WHERE SKILLS MEET TO STUDY & PROTECT



SARS-CoV-2: kinetiek van shedding en immuunrespons

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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

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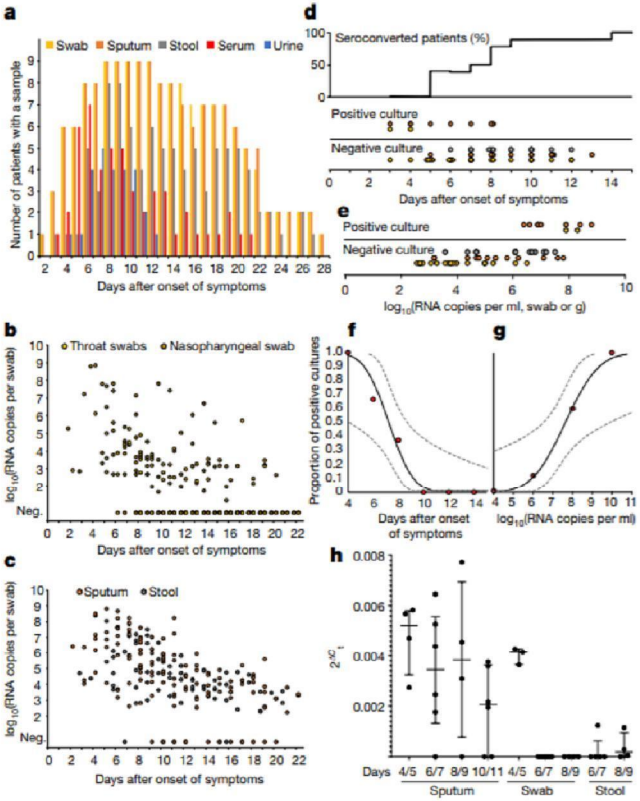
Table 2 | Clinical characteristics of all patients

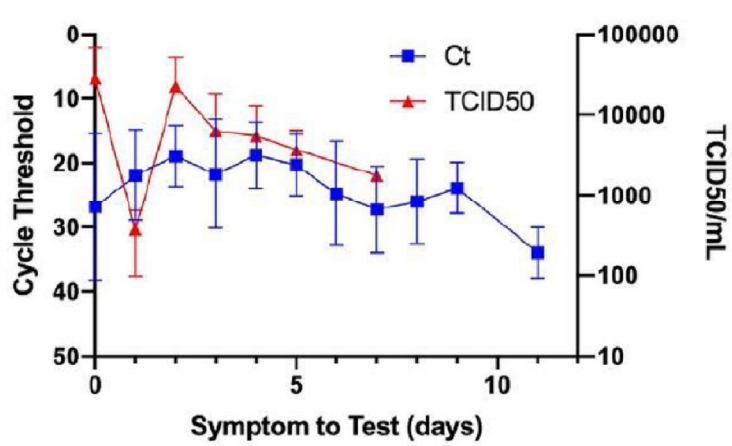
Patient ID no.	Comorbidity	Initial symptoms	Later symptoms	ANC per µl	ALC per µl	CRP (mg l^{-1})	LDH (U l^{-1})
1	Hypothyroidism	Cough, fever, diarrhoea	Diarrhoea	4,870	1,900	46	197
2	None	Sinusitis, cephalgia, cough	Hyposmia, ageusia	3,040	1,200	4.9	182
3	COPD	Arthralgia, sinusitis, cough	Dysosmia, 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; CRC, 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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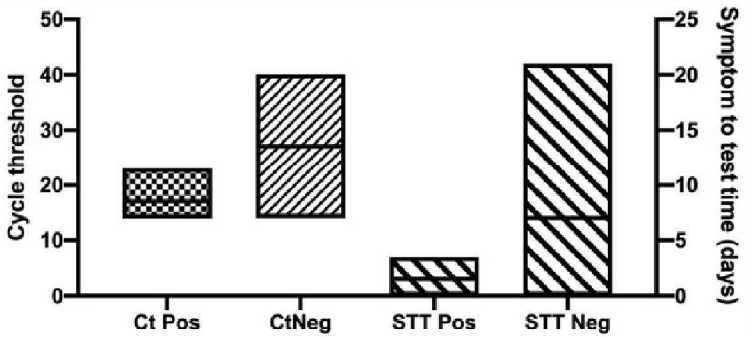
SARS-CoV-2 E gene RT-PCR Ct values and symptom to test time (STT) in samples that were culture positive (Ct +, STT +), or negative (Ct -, STT -)



- 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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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 immunofluorescene read-out

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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)	SCID
	GVHD after allogeneic HSCT	XLA
	HIV-positive with CD4+ T-cell count <200 cells/μl	Interferon receptor deficiency
	Induction chemotherapy for paediatric leukaemia	Hyper IgE syndrome
	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	
Non-severe (mild/moderate)	Maintenance chemotherapy for haematological malignancies	CVID
	Chemotherapy for solid tumours	CGD
	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/μl	
	Methotrexate use for autoimmune disease	
	Daily corticosteroid dosage (based on prednisone) of ≤30 mg (adults) or ≤2 mg/kg (infants) for ≤14 days	
	Other possible immune deficiencies (that is, untreated autoimmune disease, DM, etc.)	

5.1.2e 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)

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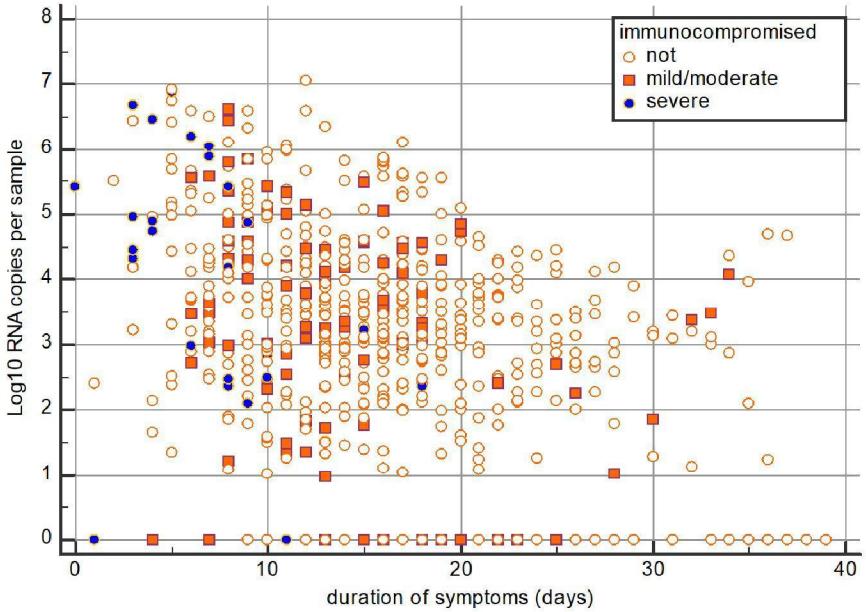
Immune status



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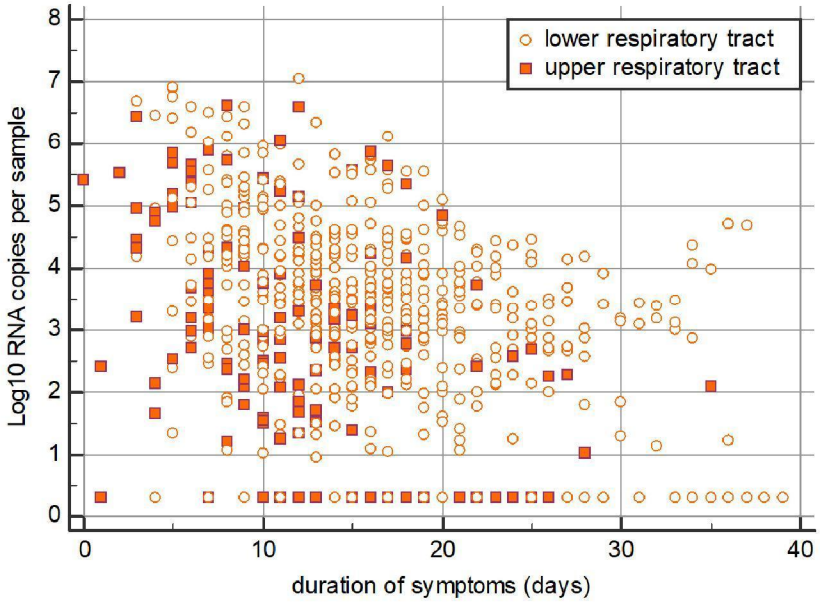
Type of respiratory material



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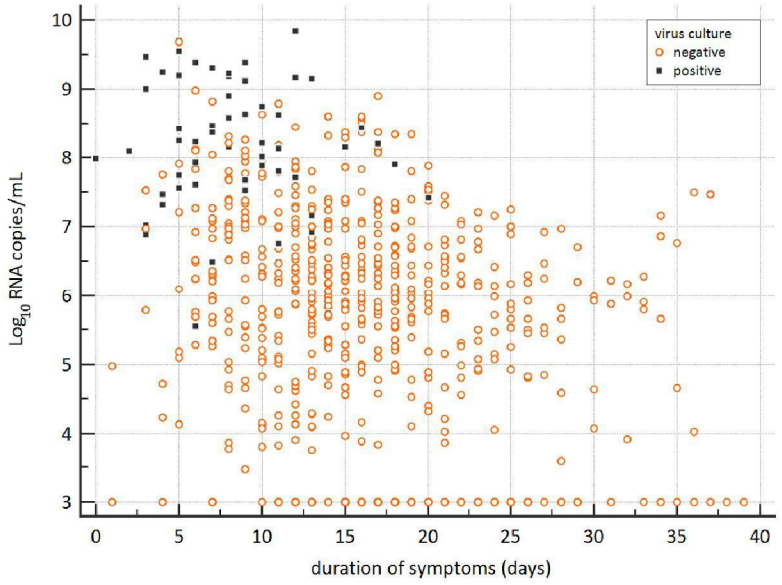
Isolation of infectious virus



- The median viral load was significantly higher in culture positive samples than in culture negative samples:
8,14 versus 5,88 Log_{10} RNA copies/mL, P<0,0001
- 62 samples positive in culture

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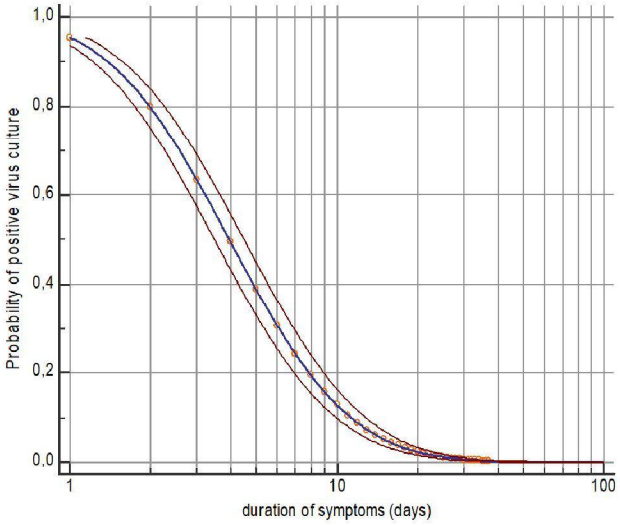
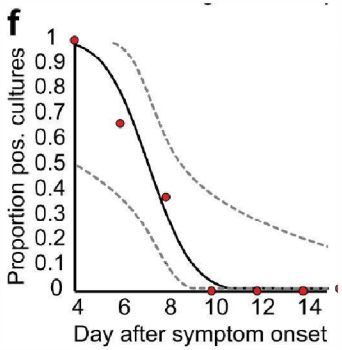
Probability isolation infectious virus versus duration of symptoms

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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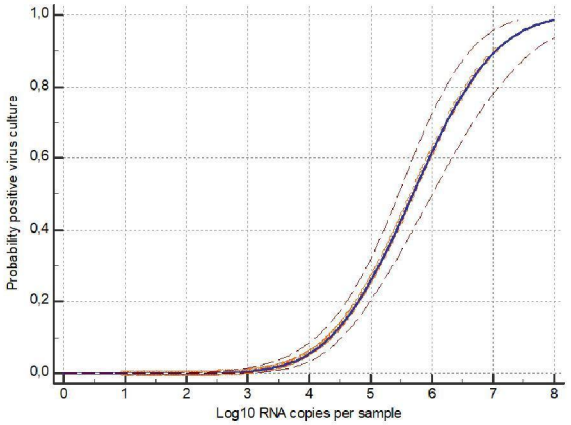
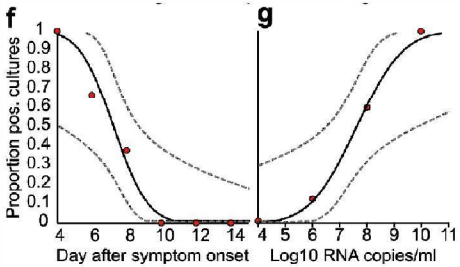
Probability isolation infectious virus versus viral RNA loads



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 Log10

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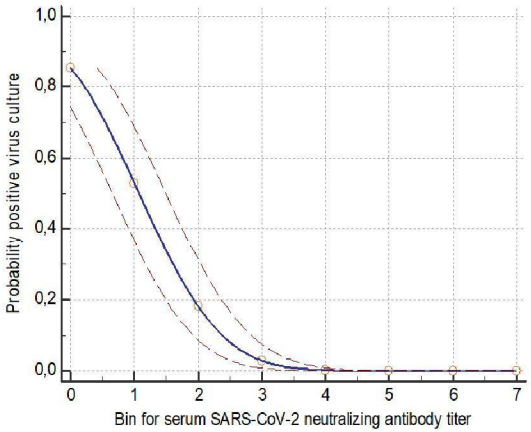
PRNT

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

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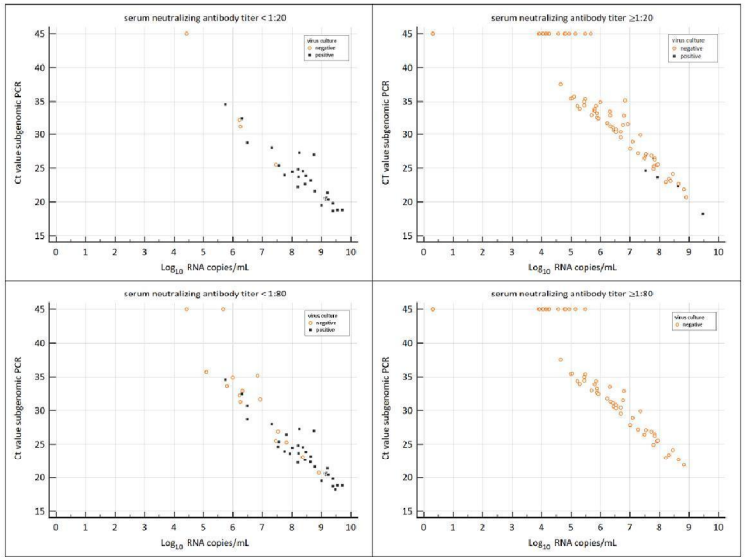
Isolation of infectious SARS-CoV-2 from the respiratory tract.



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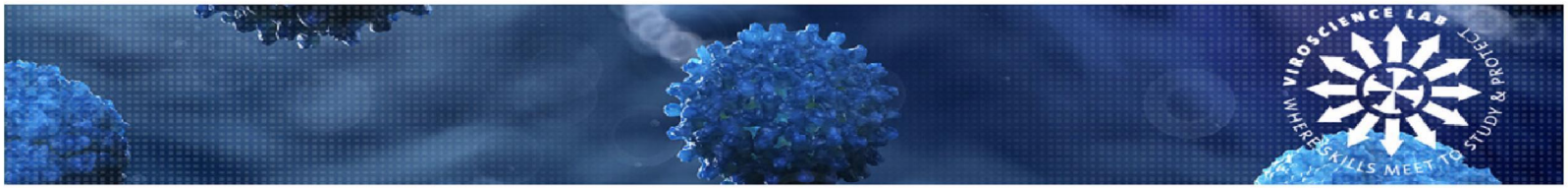

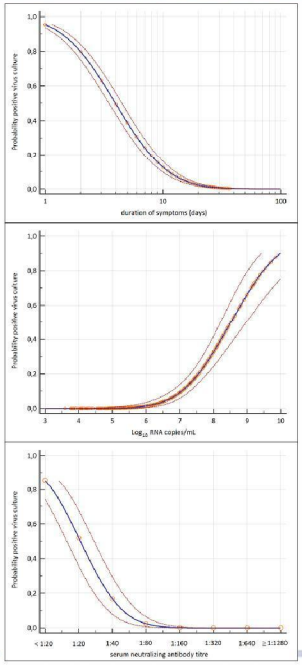

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Multivariate analysis

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 Log_{10} 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.

Acknowledgements

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