Supplementary Appendix of

Diagnostic accuracy of rapid antigen tests in asymptomatic and presymptomatic close contacts of individuals with confirmed SARS-CoV-2 infection: cross sectional study

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Table S1 Baseline characteristics of pre-/asymptomatic close contacts of individuals with a confirmed SARS-CoV-2 infection.

	Stratified by rapid antigen test	
	Veritor	Biosensor
	N = 2,678	N = 1,596
Age [years], mean (SD)	45.9 (17.6)	40.7 (16.4)
Gender, female n (%)	1,370 (51.3)	751 (47.3)
Time interval between last contact and sampling [days], median (IQR), range (min-max)	5 (5 to 5), (0 to 13)	5 (5 to 5), (0 to 11)
Symptoms at time of sampling, n (%)	219 (8.6)	158 (10.1)
Symptom onset, n (%)*	*N = 219*	*N = 158*
At day of sampling	17 (7.8)	14 (8.9)
A day before sampling	64 (29.2)	37 (23.4)
Two days before sampling	51 (23.3)	39 (24.7)
Three or more days before sampling	83 (37.9)	45 (28.5)
Unknown	4 (1.8)	23 (14.6)
Type of symptoms (self-reported), n (%)*[#]	*N = 219*	*N = 158*
Common cold	167 (76.3)	123 (77.8)
Shortness of breath	25 (11.4)	12 (7.6)
Fever	13 (5.9)	9 (5.7)
Coughing	60 (27.4)	24 (15.2)
Loss of taste or smell	6 (2.7)	5 (3.2)
Muscle ache	18 (8.2)	5 (3.2)
Other symptoms	16 (7.3)	15 (9.5)

IQR = inter quartile range; min=minimum; max=maximum; SD=standard deviation.
In the Netherlands, individuals are notified of a close contact by the Dutch public health service test-and-trace program, and/or the Dutch contact tracing mobile phone application (the CoronaMelder app) and/or an individual with a confirmed SARS-CoV-2 infection (index case).
* percentage calculated as proportion of those with symptoms at time of sampling
[#] totals add up to a number higher than the number of individuals with symptoms at the time of sampling because individuals could report more than one symptom.

Table S2 Two-by-two tables used in primary and secondary analysis to determine diagnostic accuracy parameters of the Veritor System (Beckton Dickinson) rapid antigen test. An Excel file was added as a supplement that allows the calculation of 2x2 tables based on the diagnostic accuracy of both Ag-RDTs with differing prevalence or sample size.

Primary analysis			RT-PCR test +	RT-PCR test -	Total
		Veritor test +	149	9	158
		Veritor test -	84	2,436	2,520
		Total	233	2,445	2,678
Secondary (stratified) analysis					
Infectiousness viral load cut-off[$]			RT-PCR test +	RT-PCR test -	Total
		Veritor test +	137	20	157
		Veritor test -	15	2,505	2,520
		Total	152	2,525	2,677
Symptoms at sampling[#]	Yes		RT-PCR test +	RT-PCR test -	Total
		Veritor test +	32	1	33
		Veritor test -	6	180	186
		Total	38	181	219
	No		RT-PCR test +	RT-PCR test -	Total
		Veritor test +	105	8	113
		Veritor test -	74	2,130	2,204
		Total	179	2,138	2,317
Interval between sampling and last contact with index case [days][@]	< 5		RT-PCR test +	RT-PCR test -	Total
		Veritor test +	39	1	40
		Veritor test -	17	322	339
		Total	56	323	379
	5		RT-PCR test +	RT-PCR test -	Total
		Veritor test +	53	1	54
		Veritor test -	32	1,217	1,249
		Total	85	1,218	1,303
	> 5		RT-PCR test +	RT-PCR test -	Total
		Veritor test +	26	4	30
		Veritor test -	20	461	481
		Total	46	465	511

[#] Symptoms were not available from 142 individuals

[$] The viral load cut-off for infectiousness, defined as the viral load above which 95% of RT-PCR test positives had a positive culture, was 5.2 log10 E gene copies/mL. Viral load was unavailable for one Veritor-tested individual with a positive RT-PCR test result.

[@] The interval between the moment of sampling and the last contact with an index case was not available for 488 individuals, mainly because this question was added to the questionnaire later in study.

Table S3 Two-by-two tables used in primary and secondary analysis to determine diagnostic accuracy parameters of the Biosensor (Roche Diagnostics) rapid antigen test. An Excel file was added as a supplement that allows the calculation of 2x2 tables based on the diagnostic accuracy of both Ag-RDTs with differing prevalence or sample size.

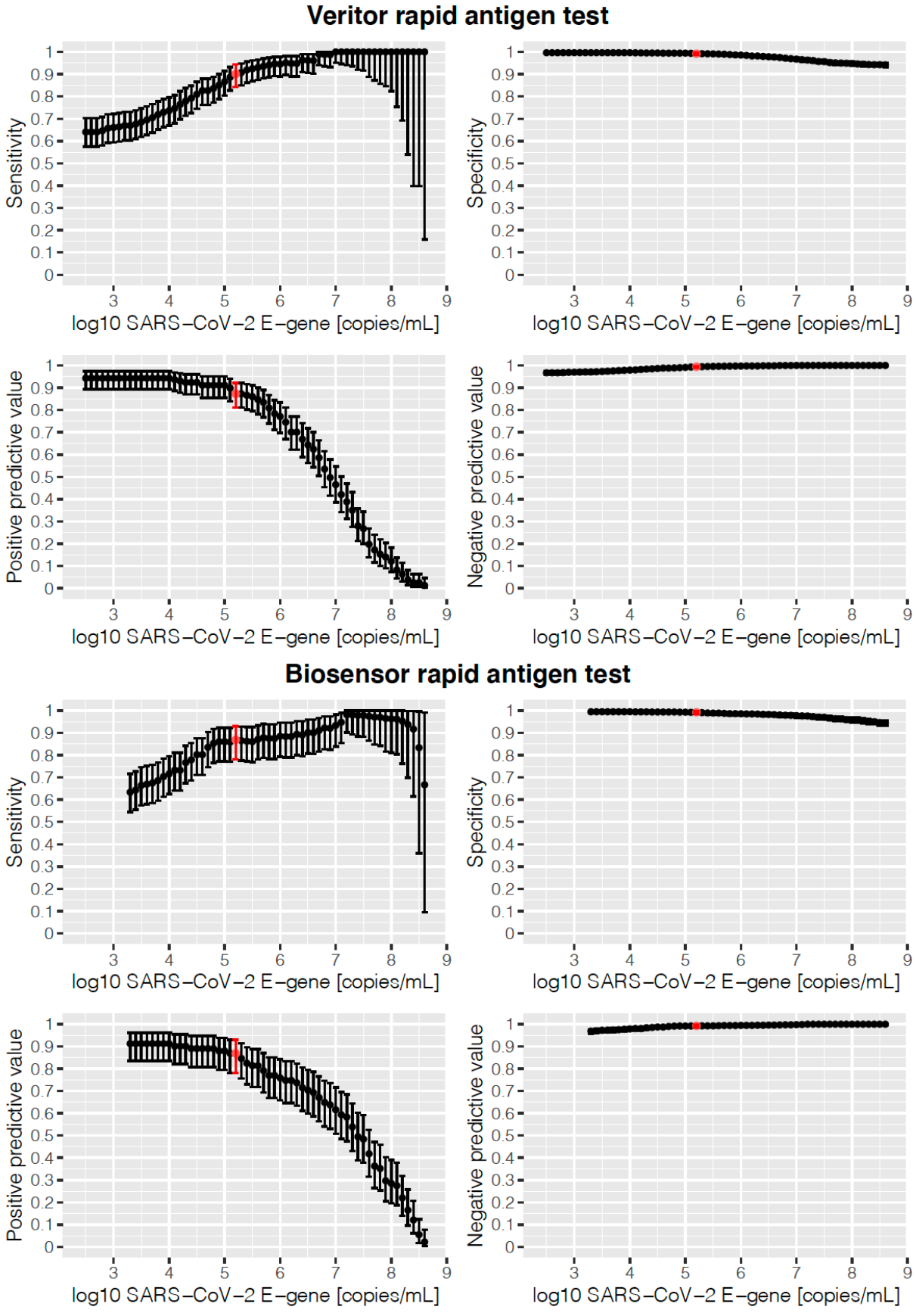
Primary analysis			RT-PCR test +	RT-PCR test -	Total
		Biosensor test +	83	8	91
		Biosensor test -	49	1,456	1,505
		Total	132	1,464	1,596
Secondary (stratified) analysis					
Infectiousness viral load cut-off[$]			RT-PCR test +	RT-PCR test -	Total
		Biosensor test +	79	12	91
		Biosensor test -	12	1,493	1,505
		Total	91	1,505	1,596
Symptoms at sampling[#]	Yes		RT-PCR test +	RT-PCR test -	Total
		Biosensor test +	22	2	24
		Biosensor test -	8	126	134
		Total	30	128	159
	No		RT-PCR test +	RT-PCR test -	Total
		Biosensor test +	60	6	66
		Biosensor test -	41	1,307	1,348
		Total	101	1,313	1,414
Interval between sampling and last contact with index case [days][@]	< 5		RT-PCR test +	RT-PCR test -	Total
		Biosensor test +	15	1	16
		Biosensor test -	5	132	137
		Total	20	133	153
	5		RT-PCR test +	RT-PCR test -	Total
		Biosensor test +	52	5	57
		Biosensor test -	33	1,005	1,038
		Total	85	1,010	1,095
	> 5		RT-PCR test +	RT-PCR test -	Total
		Biosensor test +	9	1	10
		Biosensor test -	4	191	195
		Total	13	192	205

[#] Symptoms were not available from 24 individuals

[$] The infectiousness viral load cut-off, defined as the viral load above which 95% of RT-PCR test positives had a positive culture, was 5.2 log10 E gene copies/mL.

[@] The interval between the moment of sampling and the last contact with an index case was not available for 143 individuals, mainly because this question was added to the questionnaire later in study.

Supplementary Figure 1 Diagnostic accuracy parameters of Ag-RDTs in asymptomatic close contacts, i.e., without symptoms at sampling for different viral load cut-offs. Points highlighted in red indicate a viral load cut-off of 5.2 log10 SARS-CoV-2 E-gene copies/mL, which was considered the infectiousness viral load cut-off determined by viral culture.



Supplementary Material 1: short questionnaire (translated from Dutch)

3-item questionnaire used between 14 December and 19 December 2020 (West-Brabant region) and 15 December and 18 December 2020 (city of Rotterdam).

Short questionnaire on COVID-19 like symptoms and reason for testing

1. At this moment, do you have any COVID-19 like symptoms?
   - ☐ No *END OF QUESTIONNAIRE*
   - ☐ Yes

2. What COVID-19 like symptoms do you currently have?
   Multiple answers possible
   - ☐ Common cold
   - ☐ Shortness of breath
   - ☐ Fever
   - ☐ Coughing
   - ☐ Loss of taste or smell
   - ☐ Muscle ache
   - ☐ I have other symptoms

3. What was the moment you first experienced these symptoms?
   - ☐ Today
   - ☐ Yesterday
   - ☐ Two days ago
   - ☐ Three or more days ago

5-item questionnaire used from December 19 (city of Rotterdam) and 20 (West-Brabant region) onwards

Short questionnaire on COVID-19 like symptoms and reason for testing

1. What is the reason for testing?
   Multiple answers possible
   - ☐ Received notification by public health service (by phone or letter)
   - ☐ Received notification by CoronaMelder app (English: Corona notification app)
   - ☐ Received notification by SARS-CoV-2 infected person
   - ☐ None of the above, requested test because of a SARS-CoV-2 infected person in my immediate surroundings

2. When was your last contact with the infected person?
   Date: ___ - ___ - 20___ *(day – month – year)*

3. At this moment, do you have any COVID-19 like symptoms?
   - ☐ No *END OF QUESTIONNAIRE*
   - ☐ Yes

4. What COVID-19 like symptoms do you currently have?
   Multiple answers possible
   - ☐ Common cold
   - ☐ Shortness of breath
   - ☐ Fever
   - ☐ Coughing
   - ☐ Loss of taste or smell
   - ☐ Muscle ache
   - ☐ I have other symptoms

5. What was the moment you first experienced these symptoms?
   - ☐ Today
   - ☐ Yesterday
   - ☐ Two days ago
   - ☐ Three or more days ago

Supplementary Material 2: Specimen collection, SARS-CoV-2 diagnostic testing, and SARS-CoV-2 virus culture procedures

Specimen collection and SARS-CoV-2 diagnostic testing procedures

Procedures were performed in accordance with standard operating procedures of the public health service and the two laboratories, and the laboratories followed quality management standard ISO15189:2012.

West-Brabant: RT-PCR test and Veritor system Ag-RDT

Two swabs were taken per participant. The first swab was a combined oropharyngeal- and nasal swab (2.5 cm deep from the edge of the nostril) that was placed in universal transport medium (HiViralTM) with MagnaPure LC lysis- and binding buffer (LBB) (Roche Diagnostics Netherlands, Almere, The Netherlands) and transported to Microvida location Roosendaal laboratory for RT-PCR testing. RT-PCR testing was performed using the cobas® SARS-CoV-2 test on the cobas® 8800 platform (Roche Diagnostics International, Rotkreuz, Switzerland). This RT-PCR test has two targets: The E-gene and RdRp-gene. The viral load in genome copies/ml was calculated based on an in-house established standard curve. The second swab was a combined oropharyngeal- and nasal swab (2.5 cm deep) and was placed in a sterile dry tube and frozen at -20°C within 30 minutes after collection before transportation to the Microvida location Amphia laboratory. There, after allowing the specimen to thaw, a trained laboratory technician performed the BD Veritor System (Becton Dickinson, Franklin Lakes, NJ) in accordance with the manufacturer's operating procedure within 6 hours after the specimen was obtained. The Veritor Ag-RDT is a chromatographic immunoassay intended for the direct and qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasal swabs from individuals who are suspected of COVID-19 within the first 5 days of symptom onset. The system is intended to be used with a digital reader although validated for visual reading[1]; we used visual reading.

Interpretation and recording of RT-PCR test results was performed according to the manufacturer's instructions and a trained technician. In case of discrepancies a second in house RT-PCR was performed for confirmation.[2] Results from the Ag-RDT were interpreted and recorded by two persons visually and in case of discrepancies the result from the digital reader was used.

Rotterdam: RT-PCR test and Biosensor Ag-RDT

Two swabs were taken per participant. First, one combined oro- and nasopharyngeal swab (>5 cm deep from the edge of the nostril) was taken for RT-PCR testing, placed directly in universal transport media (HiViralTM) and shipped to the Erasmus MC Viroscience diagnostic laboratory. Routine RT-PCR testing was performed on the combined oro- and nasopharyngeal swab in virus transport medium using the cobas ® SARS-CoV-2 test on the cobas 6800® platform (Roche Diagnostics International, Rotkreuz, Switzerland). Genome copies/ml was calculated based on an in house established standard curve. The virus transport medium from the same oro- and nasopharyngeal swab was also directly inoculated onto Vero cells clone 118, without prior freezing.[3]

A second nasopharyngeal swab (>5 cm deep from the edge of the nostril) was taken subsequently from the same nostril using the swab included in the kits for the Biosensor test (Roche Diagnostics, Basel, Switzerland). This test was carried out immediately on-site following manufacturer's instructions.

Interpretation and recording of RT-PCR test and Ag-RDT results was performed independently by two persons according to the manufacturer's instructions. In case of discrepancies, the results were additionally interpreted by a laboratory specialist.

Output amplification

During the study period, the Veritor and Biosensor test were applied according to manufacturer instructions, as such no output amplification methods were used.

SARS-CoV-2 virus culture

At the Erasmus MC Viroscience diagnostic laboratory, samples of Rotterdam participants with a positive RT-PCR test result were inoculated onto Vero cells, and incubated for seven days. Once cytopathic effects were visible, the presence of SARS-CoV-2 virus was confirmed with immunofluorescent detection of its nucleocapsid protein (Rabbit polyclonal antibody Sino Biological inc., Eschborn, Germany). Samples from participants from West-Brabant were not cultured.

Viral load calculation

SARS-CoV-2 RT-PCR tests were conducted in two laboratories (Erasmus Medical Center Viroscience diagnostic laboratory and Microvida) that use similar RT-PCR platforms (cobas 6800 and 8800 at Erasmus Medical Center Viroscience diagnostic laboratory and Microvida respectively), but SARS-CoV-2 viral culture in only one of those two laboratories (Erasmus Medical Center Viroscience diagnostic laboratory). Since Ct-values often differ between laboratories, several steps were indeed undertaken to enable conversion of laboratory-specific SARS-

CoV-2 RT-PCR Ct values into standardised SARS-CoV-2 viral loads and viral load cut-off for virus culturability. At the Erasmus Medical Center Viroscience diagnostic laboratory a first standard curve was created by testing dilutions of a publicly available quantified SARS-CoV-2 E-gene transcript (European Virus Archive EVAg[4]) using the RT-PCR protocol described by Corman et al.[5] The relationship between this E-gene RT-PCR Ct-value and E-gene copies/ml was determined by linear regression analysis. Subsequently, this E-gene standard curve was used to create a second standard derived from cell-cultured SARS-CoV-2 virus. Dilutions of this second standard were used to prepare a secondary standard curve by linear regression this standard curve was used to convert Ct values obtained from participant samples to SARS-CoV-2 viral loads (copies/ml). To determine whether the two cobas PCR platforms provided comparable data, both laboratories tested the same SARS-CoV-2 viral load panel obtained from the National Public Health Institute (RIVM). The Ct values generated in the two laboratories corresponded well. A linear regression model with Ct value as the outcome, and laboratory (Erasmus Medical Center Viroscience diagnostic laboratory or Microvida) and viral load as covariates indicated that the laboratory was not associated with the Ct value (p = 0.29). In a separate linear regression model, no evidence of an interaction between the laboratory and viral load was found (p-value for interaction = 0.86). A conversion factor was taken into account to correct for differences in initial sample volume and RT-PCR dilutions steps. The specific mathematical formulas to calculate the viral load (copies/mL) from Ct-values were $62.5 * e^{\frac{43.1-Ct}{1.607}}$ for Rotterdam, and $62.5 * e^{\frac{43.1-Ct}{1.607}} / 3\frac{1}{3}$ for West-Brabant, where in West-Brabant the viral loads were divided by a factor $3\frac{1}{3}$ to account for the lower volume of medium used per swab by Microvida (1.8 mL) as compared to Erasmus MC (6 mL). The infectiousness viral load cut-off was defined as the viral load above which 95% of RT-PCR test positives showed *in vitro* infectivity in cell culture.

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