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## Postmortem Antigen-Detecting Rapid Diagnostic Tests to Predict Infectivity of SARS-CoV-2–Associated Deaths

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We investigated the infectivity of 128 severe acute respiratory disease coronavirus 2–associated deaths and evaluated predictive values of standard diagnostic procedures. Maintained infectivity (20%) did not correlate with viral RNA loads but correlated well with anti-S antibody levels. Sensitivity >90% for antigen-detecting rapid diagnostic tests supports their usefulness for assessment.

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Deaths associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have raised concerns that contact with the corpses of deceased persons might pose a risk for transmitting infection (1). Nasopharyngeal SARS-CoV-2 RNA loads were shown to remain stable up to 20 days postmortem (2), and the maintained infectivity of corpses has sporadically been examined (2–4). In contrast, body surfaces of corpses have been considered non-infectious (5). Systematic studies on the infectivity of corpses and predictive values of standard diagnostic procedures remain scarce.

For this study, we prospectively collected nasopharyngeal swab specimens from 128 SARS-CoV-2 RNA-positive and 72 RNA-negative corpses ≤14 days postmortem to assess infectivity and predictive values of virologic parameters (Table). We excluded corpses exhibiting advanced putrefaction. For initial assessment, we determined RNA loads using quantitative reverse transcription PCR (qRT-PCR) (Appendix, <https://wwwnc.cdc.gov/EID/article/28/1/21-1749-App1.pdf>).

We found SARS-CoV-2 RNA up to 325 hours postmortem, but RNA loads did not correlate with

<sup>1</sup>These senior authors contributed equally to this article.

**Table.** Baseline characteristics of corpses received by the Institute of Legal Medicine, Hamburg, Germany, 2020–2021\*

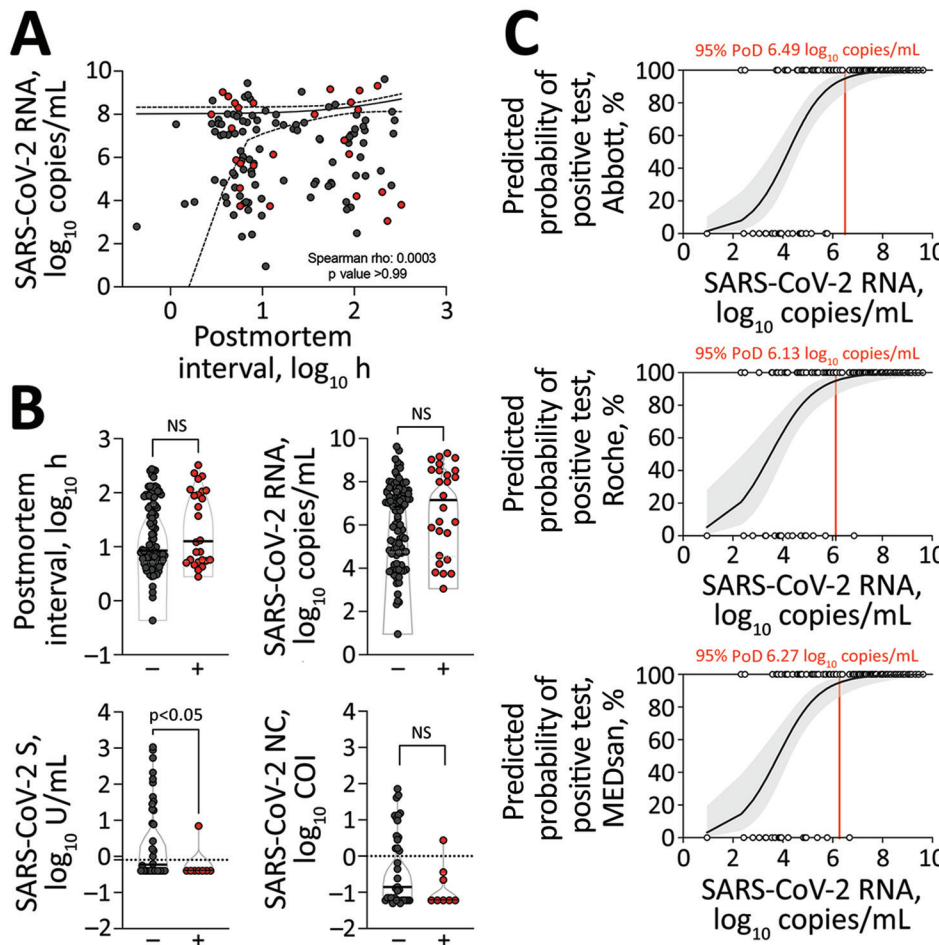
Characteristic	SARS-CoV-2 RNA positive, † n = 128	SARS-CoV-2 RNA negative, † n = 72	Total, n = 200
Age, y, median (IQR)	83.5 (71.5–89.1)	81.0 (73.0–87.0)	82.3 (72.9–88.5)
Sex			
M	71 (55.5)	36 (50.0)	107 (53.5)
F	57 (44.5)	36 (50.0)	93 (46.5)
Place of death			
Home	28 (22.0)	30 (41.7)	58 (29.1)
Nursing home	38 (29.9)	3 (4.2)	41 (20.6)
Hospital	39 (30.7)	25 (34.7)	64 (32.2)
ICU	20 (15.7)	10 (13.9)	30 (15.1)
Other	2 (1.6)	4 (5.6)	6 (3.0)
Postmortem interval, ‡ h, median (IQR)	8.7 (5.3–82.6)	4.9 (3.5–8.8)	7.0 (4.3–49.9)
Putrefactive changes	11 (8.9)	1 (1.4)	12 (6.1)
SARS-CoV-2 RNA load, ¶ copies/mL, median (IQR)	7.0 × 10 <sup>6</sup> (5.5 × 10 <sup>4</sup> –5.2 × 10 <sup>7</sup> )	Below LOD	Not applicable

\*Values are no. (%) except as indicated. In case of missing data points, valid percentages are indicated. ICU, Intensive care unit; LOD, limit of detection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2  
 †B.1.1.7 variants (2/128) identified by multiplex-typing PCR (5). SARS-CoV-2–associated deaths were tested in a multiplex typing PCR for SARS-CoV-2 spike variants.  
 ‡Interval from time of death until initial sampling and cooling at 4°C.

the postmortem interval (PMI;  $r = 0.003$ ,  $p > 0.99$ ) (Figure, panel A). RNA loads were comparatively high (median  $7.0 \times 10^6$  copies/mL, interquartile range [IQR]  $5.5 \times 10^4$ – $5.2 \times 10^7$  copies/mL) (Figure, panel B) and in some cases exceeded loads in the acute phase

of the disease (6), possibly because of postmortem mucosal softening and higher exfoliation of tissue during sample collection.

Virus isolation proved infectivity was maintained in 26/128 (20%) corpses (Appendix). PMI (median 13



**Figure.** Overview of 128 consecutive records of SARS-CoV-2–associated deaths received by the Institute of Legal Medicine, Hamburg, Germany, 2020–2021. A) SARS-CoV-2 RNA loads by postmortem intervals. Spearman correlation was performed; estimates and 95% CI are shown. B) Postmortem intervals, viral RNA loads, quantitative (S), and qualitative (NC) antibody levels compared among culture-positive (+) and culture-negative (-) corpses. Comparisons were performed using Mann-Whitney-U or  $\chi^2$  testing, as appropriate. Median and interquartile ranges are shown. Horizontal dotted lines indicate cutoff value. C) Probability of positive antigen-detecting rapid diagnostic test results depending on viral RNA loads calculated by binomial logistic regression. Robust estimates with 95% CI are shown. Vertical red line indicates 95% PoD with the corresponding viral RNA load. Ag-RDT, antigen-detecting rapid antigen test; COI, cut-off index; NC, nucleocapsid; NS, not significant; PoD, probability of detection; S, spike; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

hours, range 3–325 hours) and SARS-CoV-2 RNA load ( $1.4 \times 10^7$  copies/mL, IQR  $3.7 \times 10^4$ – $3.3 \times 10^8$ ) among culture-positive corpses did not differ significantly from PMI (median 8 hour, range 0–275 hour;  $p = 0.38$ ) and RNA loads ( $7.0 \times 10^6$  copies/mL, IQR  $5.8 \times 10^4$ – $3.9 \times 10^7$  copies/mL;  $p = 0.14$ ) among culture-negative corpses (Figure, panel B). We successfully isolated virus from samples with comparatively low amounts of RNA ( $<1 \times 10^4$  copies/mL), in contrast with previous findings among living patients (6). We observed putrefactive changes in no culture-positive corpses compared with in 11/98 (11%) culture-negative corpses ( $\chi^2 = 3.20$ ;  $p = 0.11$ ), indicative of potentially decreased infectivity.

We confirmed seroconversion in 18/44 (41%) blood samples, 15/43 (35%) anti-nucleocapsid positive and 17/44 (39%) anti-spike positive (range  $<0.4$ – $1066.0$  U/mL; Appendix). Levels of anti-spike antibodies, representing neutralizing antibody levels (7), were not significantly correlated with PMI ( $r = 0.07$ ;  $p = 0.64$ ), but were well correlated with viral RNA levels ( $r = -0.70$ ;  $p < 0.0001$ ). Anti-nucleocapsid antibodies were found in only 1/8 (13%) culture-positive compared with 14/35 (40%) culture-negative corpses ( $\chi^2 = 2.17$ ;  $p = 0.23$ ) (Figure, panel C). Moreover, anti-spike antibody levels differed significantly ( $p = 0.04$ ) between culture-positive (1.22 U/mL, SD 2.32) and culture-negative (86.85 U/mL, SD 240.56) corpses, indicative of inverse association of SARS-CoV-2-specific antibody levels with infectivity (Figure, panel C).

Antigen-detecting rapid diagnostic tests (Ag-RDTs) are considered adequate alternative swift diagnostic tools in living patients (8,9), but knowledge about their postmortem applicability and reliability remains scarce. We tested Ag-RDTs from 3 manufacturers and found excellent performance for postmortem use (Appendix Table 1). Compared with qRT-PCR results, for the Panbio COVID-19 Ag Rapid Test Device (Abbott, <https://www.abott.com>), sensitivity was 80.3% (95% CI 72.3%–86.4%) and specificity 100.0% (95% CI 95.0%–100.0%); for the SARS-CoV-2 Rapid Antigen Test (Roche <https://www.roche.com>), sensitivity was 86.4% (95% CI 79.1%–91.9%) and specificity 98.6% (95% CI 93.0%–100.0%); and for the SARS-CoV-2 Antigen Rapid Test (MEDsan <https://www.medsan.eu>), sensitivity was 84.1% (95% CI 76.6%–90.0%) and specificity 95.8% (95% CI 88.0%–99.0%) (Appendix Figures 1, 2).

We found SARS-CoV-2 RNA load correlated with Ag-RDT positivity in univariate and multivariate analyses ( $p < 0.001$ ), thereby confirming their predictive value (Figure, panel C; Appendix Table 2). Subgroup analyses of corpses with  $>1 \times 10^6$  RNA copies/mL ( $n = 74$ ) revealed 100% (95% CI 95.1%–100.0%)

sensitivity in Abbott ( $n = 74$ ) and Roche and MEDsan ( $n = 73$  each) assays. In contrast, neither PMI ( $p = 0.34$ ) nor putrefactive changes ( $p = 0.90$ ) were predictive for testing positive in Ag-RDTs (exemplarily for the MEDsan assay; Appendix Table 2). Ag-RDT sensitivity in infectious corpses was 92.3% (95% CI 74.9%–99.1%) for Abbott, 96.2% (95% CI 80.4%–99.9%) for Roche, and 96.2% (95% CI 80.4%–99.9%) for MEDsan. We detected 2 SARS-CoV-2 variants of concern despite relatively low viral RNA loads ( $4.83 \log_{10}$ ); the 2 samples tested positive by Abbott and Roche but were missed by MEDsan.

The first limitation of our study is that blood was not available from all corpses, and the serologic assays and Ag-RDTs used are not approved for cadaveric samples. Furthermore, because of a shortage of reagents and supplies, we had to use different tests to quantify RNA, and slight deviations cannot be ruled out.

In summary, we show that cadavers from SARS-CoV-2-associated deaths remain infectious long after death in a considerable proportion of cases. Postmortem infectivity does not correlate with PMI or viral RNA load but correlates with the absence of virus-specific antibodies. Ag-RDTs performed well, enabling rapid on-site detection. Because previous studies among living patients indicate that Ag-RDTs reliably detect all SARS-CoV-2 variants (10), we believe that our results on postmortem Ag-RDTs use can contribute to crisis management in severely affected regions and increase safety in the medical sector worldwide.

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The ethics committee of the Hamburg Chamber of Physicians approved this study (reference no. 2020-10353-BO-ff and PV7311).

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## Monitoring International Travelers Arriving in Hong Kong for Genomic Surveillance of SARS-CoV-2

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We sequenced ≈50% of coronavirus disease cases imported to Hong Kong during March–July 2021 and identified 70 cases caused by Delta variants of severe acute respiratory syndrome coronavirus 2. The genomic diversity detected in Hong Kong was similar to global diversity, suggesting travel hubs can play a substantial role in surveillance.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineage B.1.617 (1) and 3 of its sublineages, B.1.617.1 (Kappa), B.1.617.2 (Delta), and B.1.617.3, were first detected in India. The Delta variant started circulating widely in different continents beginning in late March 2021 (2,3). It was initially classified as a variant of interest in April 2021 and then reclassified as a variant of concern in May 2021.

Hong Kong adopted an elimination strategy to control coronavirus disease (COVID-19). A previous study reported the use of stringent measures (e.g., mandatory COVID-19 testing, travel restrictions) to detect and prevent SARS-CoV-2 importation by COVID-19–positive travelers (4), thereby reducing the risk of new SARS-CoV-2 introductions, and also showed that regional and international airports could be useful sentinel surveillance sites to monitor SARS-CoV-2 circulation. In this study, we tested the feasibility of using surveillance strategies similar to those used in that study to monitor sequence diversity of Delta variant

# Postmortem Antigen-Detecting Rapid Diagnostic Tests to Predict Infectivity of SARS-CoV-2–Associated Deaths

## Appendix

### Methods

#### Patients and Ethics

For the study, we prospectively included a total of 200 corpses received at the Institute of Legal Medicine (University Medical Center Hamburg-Eppendorf, Hamburg, Germany); we excluded corpses exhibiting advanced putrefactive changes (marbling and mummification). All corpses were stored at 4°C upon receipt; we defined postmortem interval as the time from death until cooling. Informed consent was obtained from relatives or legal representatives. We performed data and sample acquisition from November 1, 2020–February 28, 2021. For initial assessment for SARS-CoV-2 RNA, quantitative reverse transcription (qRT-) PCR from nasopharyngeal swab samples was performed as part of routine diagnostics at the Institute of Microbiology, Virology and Hygiene (University Medical Center Hamburg-Eppendorf, Hamburg, Germany). In total, 128/200 corpses were SARS-CoV-2 RNA positive, and 72/200 were SARS-CoV-2 RNA negative. Notably, none of the 72 SARS-CoV-2 RNA–negative deceased patients had had a diagnosis of COVID-19 during their lifetime nor did they have a diagnosed or suspected case of SARS-CoV-2 or COVID-19 at the time of death.

#### Sampling and Molecular Diagnostic

We performed an initial assessment for the presence of SARS-CoV-2 RNA in all corpses received at the Institute of Legal Medicine by qRT-PCR. Following receipt of the initial results (usually <24 h later), we performed, 4 subsequent nasopharyngeal swabs, 1 tested using universal transport medium (MANTACC, <https://www.mantacc.com>) for qRT-PCR and virus isolation, and 3 for antigen-detecting rapid diagnostic tests using the swab supplied with the kit. For quantitative SARS-CoV-2 RNA detection, we used commercially available assays, such as

Cepheid Xpert Xpress SARS-CoV-2 (<https://www.cepheid.com>), Roche cobas SARS-CoV-2 (<https://www.roche.com>), and lab-developed assays (1,2). We used standard RNA reference material (obtained from INSTAND eV, <https://www.instand-ev.de>) for quantification. To calculate  $\log_{10}$  RNA copies/mL ( $y$ ) based on Ct-values ( $x$ ), targets and conversion formulae were used: Cepheid Xpert Xpress SARS-CoV-2:  $y = -0.29x + 12.83$  (target E2); Roche cobas SARS-CoV-2:  $y = -0.308x + 13.81$  (target T2); SARS-CoV-2\_UCT (utility channel test) LDT (lab-developed test):  $y = -0.291x + 12.97$  (target E-gene); NeuMoDx LDT:  $y = -0.425x + 14.8$  (<https://www.neumodx.com>; target E-gene), Roche LightCycler 480 II:  $y = -0.318x + 13.32$  (target E-gene). We did not consider the nonlinearity of RNA quantification within the analysis. We also analyzed all nasopharyngeal swab samples in a multiplex typing PCR (3), detecting del 69/70 and 501Y, enabling us to distinguish SARS-CoV-2 spike variants of concern, such as B.1.1.7 and B.1.351.

#### **Cell Culture and Virus Isolation**

We maintained and cultivated Vero E6 cells under standard conditions (4). For virus isolation, we used 500 $\mu$ L of each swab medium (universal transport medium) taken at the time of antigen-detecting rapid diagnostic (Ag-RDT) testing, and performed infection as described elsewhere (5). We analyzed virus growth after incubation at 37°C for 72h by qRT-PCR as described elsewhere (1).

#### **Serologic Diagnostic**

We obtained cadaveric blood from all corpses evaluated by full autopsy, 44/128 SARS-CoV-2 RNA-positive corpses. We used Roche Elecsys Anti-SARS-CoV-2-NC with the Roche cobas e411 according to manufacturer recommendations, for qualitative detection of SARS-CoV-2 nucleocapsid protein antibodies. We used Roche Elecsys Anti-SARS-CoV-2-S with the Roche cobas e411 according to manufacturer recommendations, for the quantitative detection of SARS-CoV-2 spike antibodies. We set cutoff values according to manufacturer recommendations:  $>1$  COI (Elecsys Anti-SARS-CoV-2-NC) and  $>0.8$  U/mL (Elecsys Anti-SARS-CoV-2-S).

#### **Evaluation of Ag-RDTs**

We performed Ag-RDTs from 3 different manufacturers (Appendix Table 1) according to manufacturer protocols: I) Abbott Panbio COVID-19 Ag Rapid Test Device

(<https://www.abbott.com>), II) Roche SARS-CoV-2 Rapid Antigen Test (<https://www.roche.com>), and III) MEDsan SARS-CoV-2 Antigen Rapid Test (<https://www.medsan.eu>). All 3 Ag-RDTs detect the SARS-CoV-2 nucleoprotein (N). All assays were listed by official authorities to meet the requirements for SARS-CoV-2 testing in Germany (6), but none of them was approved for use in the postmortem setting. Two independent examiners performed Ag-RDT readouts by visual inspection.

### Statistical Analysis

We performed a sample size estimation for the number of cases included, assuming a significance level of  $\alpha = 0.05$  and applying a margin of error of 0.05. We tested data distribution and variance equality by Q-Q plot and homoscedasticity plot. We used a Mann-Whitney-U test to compare differences between 2 independent groups in nonparametric distributed, unpaired datasets. We used  $\chi^2$  testing to compare proportions between groups. We calculated Spearman's rank correlation coefficients to assess the statistical correlation of nonparametric distributed variables. We used binary logistic regression and multivariate logistic regression for multivariate analyses. We included independent variables in the model on a clinical and scientific basis. We calculated Clopper-Pearson 95% confidence intervals for binomial proportions. P values  $<0.05$  were considered statistically significant. We performed statistical analysis using IBM SPSS Statistics, version 27.0.0.0 (<https://www.ibm.com>), and STATA/MP, version 17.0 (<https://www.stata.com>). We used GraphPad Prism software version 9.1.1 (<https://www.graphpad.com>) for data illustration.

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**Appendix Table 1.** Antigen-detecting rapid diagnostic test specifications as provided by the manufacturer for all tests used in the study\*

Test device name	Manufacturer	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Limit of detection, TCID <sub>50</sub> / mL
Panbio COVID-19 Ag Rapid Test Device	Abbott†	93.3 (83.8–98.2)	99.4 (97.0–100.0)	1.50×10 <sup>2</sup>
SARS-CoV-2 Rapid Antigen Test	Roche Diagnostics Deutschland GmbH‡	96.5 (91.3–99.0)	99.7 (98.2–99.9)	4.94×10 <sup>2</sup>
MEDsan SARS-CoV-2 Antigen Rapid Test	MEDsan¶	92.5 (86.2–96.5)	99.8 (98.9–99.9)	1.40×10 <sup>1</sup>

\*TCID<sub>50</sub>, 50% tissue culture infection dose.

†<https://www.abbott.com>

‡<https://www.roche.com>

¶<https://www.medsan.eu>

**Appendix Table 2.** Predictive factors for positive testing by antigen-detecting rapid diagnostic tests investigated in univariate and multivariate logistic regression analyses\*,†

Parameter	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value#	OR (95%CI)	P value#
Abbott assay‡				
Postmortem interval, /h	1.00 (0.99–1.00)	0.70	1.00 (0.99–1.01)	0.70
SARS-CoV-2 RNA load, log <sub>10</sub> , copies/mL	3.65 (2.16–6.17)	<0.0001	3.65 (2.14–6.23)	<0.0001
Putrefactive changes	1.55 (1.03–2.33)	0.04	1.34 (0.78–2.31)	0.29
Roche assay¶				
Postmortem interval, /h	1.01 (1.00–1.02)	0.15	1.01 (1.00–1.03)	0.09
SARS-CoV-2 RNA load, log <sub>10</sub> , copies/mL	3.09 (1.81–5.28)	<0.0001	3.49 (1.95–6.25)	<0.0001
Putrefactive changes	1.22 (0.71–1.79)	0.63	0.66 (0.33–1.31)	0.23
MEDsan assay§				
Postmortem interval, /h	1.00 (0.99–1.01)	0.49	1.00 (1.00–1.01)	0.34
SARS-CoV-2 RNA load, log <sub>10</sub> , copies/mL	3.31 (1.94–5.64)	<0.0001	3.40 (1.97–5.86)	<0.0001
Putrefactive changes	1.32 (0.89–1.95)	0.17	0.96 (0.56–1.65)	0.90

\*OR, odds ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

†True-positive testing served as the dependent variable (compared with qRT-PCR). Independent variables in the model were included on a clinical and scientific basis.

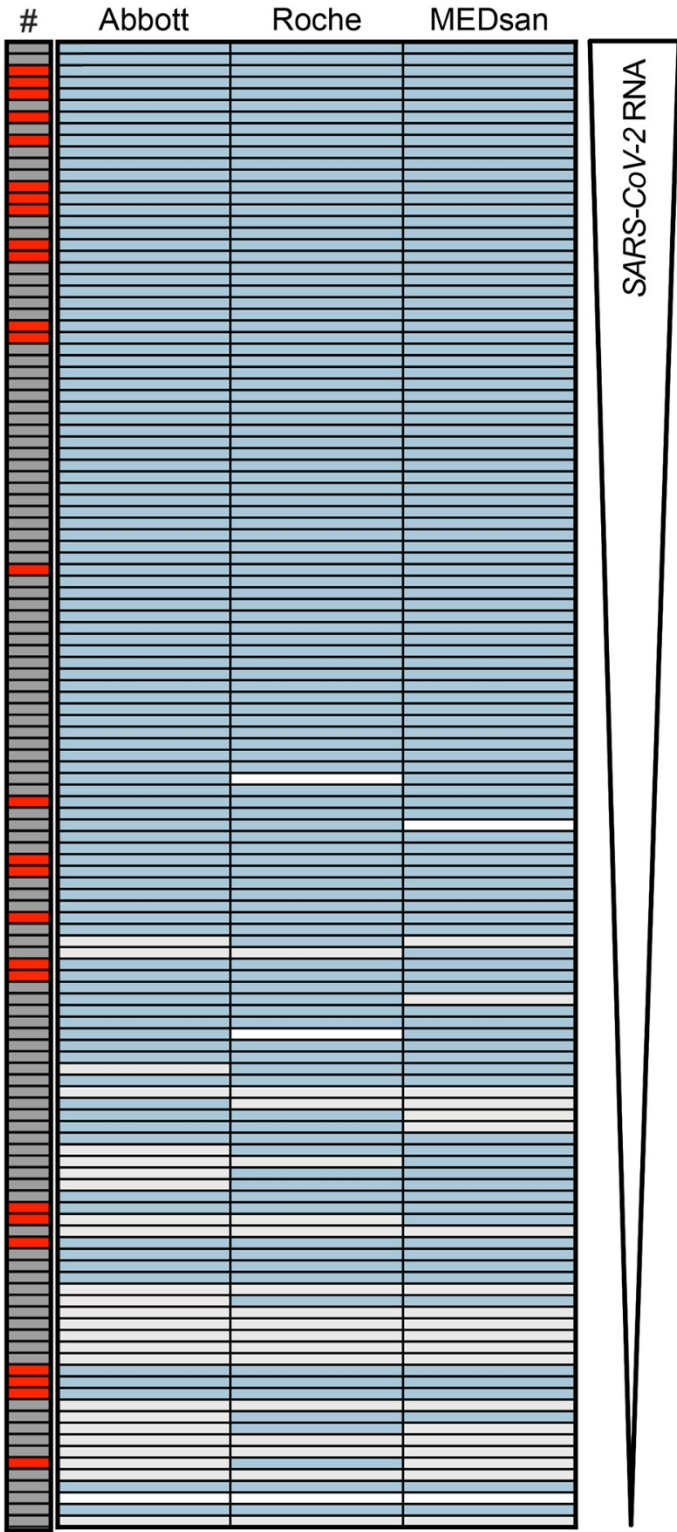
‡<https://www.abbott.com>; model estimator:  $\chi^2 = 56.11$ ,  $p < 0.0001$ .

¶<https://www.roche.com>; model estimator:  $\chi^2 = 41.86$ ,  $p < 0.0001$ .

§<https://www.medsan.eu>; model estimator:  $\chi^2 = 44.22$ ,  $p < 0.0001$ .

#P values <0.05 considered statistically significant.





**Appendix Figure 1.** Antigen-detecting rapid diagnostic test results are illustrated as positive (light blue) and negative (light gray). # indicates virus culture status; culture-positive corpses are marked red.

