



SARS-CoV-2 antigen detection in deceased bodies: implications for infection prevention

Meenakshi Sharma¹, Vandana Vijayeta Kiro⁴, Sharad Srivastav⁴, Nasim Mansoori¹, Parin Lalwani³, Amit Lathwal⁵, Richa Agrawal⁶, Kapil Dev Soni⁶, Nirupam Madaan⁵, Rajesh Malhotra², Anjan Trikha³, Sanjeev Lalwani¹ & Purva Mathur⁴

¹Division of Forensic Pathology & Molecular DNA Laboratory, Departments of ²Orthopaedics, ³Anaesthesia & Critical Care, ⁴Microbiology (Laboratory Medicine), Jai Prakash Narayan Apex Trauma Centre, Departments of ⁵Hospital Administration & ⁶Anaesthesia & Critical Care, All India Institute of Medical Sciences, New Delhi, India

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Background & objectives: High transmissibility of the SARS-CoV-2 has significant implications on healthcare workers' safety, preservation, handling, transportation and disposal of the deceased bodies. The objective of this study was to detect SARS-CoV-2 antigen in nasopharyngeal samples and its implications in handling and care of COVID-19 deceased bodies.

Methods: A study was conducted at a dedicated COVID-19 centre on deceased individuals from April to December 2020. Rapid antigen test (RAT) and reverse transcription (RT)-PCR was compared on all the SARS-CoV-2 positive cadavers recruited in the study.

Results: A total of 115 deceased individuals were included in the study. Of these, 79 (68.7%) were male and 36 (31.3%) were female and majority were in the age group of 51-60 yr [31 (27%)]. SARS-CoV-2 antigen test was positive in 32 (27.8%) and negative in 83 (72.1%) individuals. The mean time interval between deaths to the sample collection was 13.2 h with interquartile range of eight to 20 h. Reverse transcription (RT)-PCR was used as the reference test and 24 (20.9%) cases were true positive; 93.6 per cent [95% confidence interval (CI) 88.8-98.4%] sensitivity, 45.2 per cent (95% CI 35.5-55%) specificity, 60.2 per cent (95% CI 50.6-69.8%) positive predictive value and 88.8 per cent (95% CI 82.7-95%) negative predictive value of antigen test was computed.

Interpretation & conclusions: SARS-CoV-2 antigen test was positive beyond 19 h in COVID-19 deceased individuals. Antigen test was found to be highly sensitive in the deceased. Patients, suspected of having died due to COVID-19, can be screened by this method. As infectiousness of the virus in the deceased bodies cannot be directly concluded from either the antigen or RT-PCR test, yet possible transmission cannot be completely ruled out. Strict infection control measures need to be followed during the handling and clearance of COVID-19 cadavers.

Key words Antemortem - antigen - CBNAAT - COVID-19 - infection control - nasopharyngeal swab - rapid test - RAT - RT-PCR - SARS-CoV-2

As of June 11, 2022, a total of 6,268,956 COVID-19 deaths were reported by the World Health Organization (WHO), making it a global health issue of great concern of this century^{1,2}. Worldwide, COVID-19 presented an unusual challenge to the governments due to the high transmissibility of the virus^{3,4}. Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has been categorized by the Advisory Committee on Dangerous Pathogens as hazard group 3 organisms⁵ which implies (a) the hazard may cause a serious human disease and may pose a significant risk to laboratory staff, (b) the virus may be contagious to other human being, and (c) prophylaxis and/or treatment are usually available⁶. At present, the usual mode of transmission of COVID-19 is human-to-human contact, mainly through respiratory aerosols and droplets generated from an affected person without proper usage of face mask⁷. The transmissibility of the virus relies upon various factors such as the time of exposure, optimum use of preventive measures and personal factors such as the quantity of virus in respiratory discharges⁸. Other potential ways of transmission could be the transference of virus from virus-tainted surfaces to the mucous membranes of the person, particularly to the nose, eyes and mouth^{8,9}. Infections in hospitals and other healthcare facilities are of utmost concern^{10,11}. Especially in the context of SARS-CoV-2, other than the safety of the healthcare workers, the potential risk of transmission of this virus to the morgue staff, funeral agencies, transportation services, families of the deceased person, religious representatives and organizations undertaking burials and cremations is also crucial. Information about the risk of viral transmission to these individuals from deceased bodies is sparse. Since the commencement of the COVID-19 pandemic, viability of SARS-CoV-2 viruses in different conditions and dead human tissue has been a matter of concern^{12,13}, knowledge of which has a wide range of implications, starting from the cautious handling of laboratory samples to disease mitigation measures and also the final disposition of the corpse⁶.

SARS-CoV-2 RNA has been exhibited in several samples, relating to the graveness of symptoms and organ involvement^{14,15}. Our group conducted the first systematic analysis of rapid antigen test (RAT) in India; based on which, RAT was rolled out for screening in certain clinical indications by the Indian Council of Medical Research (ICMR)¹⁶. Based on a study, RATs, conducted on suspected COVID-19 patients, showed

sensitivity of 61.7 per cent and specificity 98.26 per cent¹⁷.

Rapid antigen test, due to its easy availability, affordability, execution and interpretation, has been in use for swift detection of SARS-CoV-2 in areas where conducting molecular tests are not feasible. We are aware of studies where RAT against quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and persistence of viral RNA in various tissues have been conducted on decedents in limited numbers¹⁸⁻²¹. In the current study, we evaluated RAT positivity in a large cohort of COVID-19 decedents and also studied their clinico-epidemiological profile; antigen testing in deceased was evaluated against RT-PCR test.

Material & Methods

Case selection: This study was carried out at Jai Prakash Narayan Apex Trauma Centre, All India Institute of Medical Sciences (AIIMS), New Delhi. AIIMS is a 2500-bedded tertiary care hospital, with about 1000 beds dedicated to COVID-19 care at the peak of the COVID-19 surges. The study was approved by the Institution Ethics Committee (Ref. No.: RP-22/2020).

From April to December 2020, 1,342 individuals died due to COVID-19 at our hospital. Among these, 115 deceased individuals were randomly selected and tests were conducted as per availability of time and workforce. Their antemortem tests for SARS-CoV-2 RNA were conducted with reverse transcription (RT)-PCR, cartridge-based nucleic acid test, rapid antigen test (RAT) or a combination of these. Evaluation of all documents available at the centre concerning the deaths was performed descriptively. There were no exclusion criteria for the study. Postmortem RAT and RT-PCR tests were conducted for comparison of the SARS-CoV-2 positive deceased in the mortuary. Rapid antigen test was performed as soon as the sample was collected, according to the instructions provided by the Standard Q COVID-19 Antigen Kit (SD Biosensor, South Korea). For RT-PCR, sample collection and sample processing were performed in accordance with the protocol developed in a previous study by the same authors²².

Corresponding to the test results, descriptive analysis was conducted to present the distribution of individual characteristics. The duration between death and the sample collection was defined as the time interval. The length of stay was ascertained from the admission time to the time of death. Median and interquartile range (IQR) was measured for both the

time interval and the length of stay. Chi-square test and Mann-Whitney U test were performed for categorical variables and continuous variables, respectively, to examine if there was any correlation between the characteristics and test outcomes. Adjusted *P* values of 0.05 or less were considered significant. Box plot was used to check for any outliers in examining time interval and duration of stay. Validity of RAT test was measured by calculating sensitivity, specificity, positive predictive value, negative predictive value and percentage positivity against RT-PCR as the reference test. For statistical analyses, STATA version 11.1 software (Stata Corp., College Station, TX, USA) was used.

Results

Demographic details: A total of 115 deceased individuals were included in the study. Among them, 79 (68.7%) were male and 36 (31.3%) were female; majority were in the age group of 51 to 60 yr (31, 27%). SARS-CoV-2 antigen test was positive in 32 (27.8%) and negative in 83 (72.1%) cases. The time interval between deaths to the sample collection was at a mean of 13.2 h with IQR of eight to 20 h. Figure depicts the box and whisker plot graph related to time interval (h) in relation to RAT results where, there are total six extreme values: four for negative test results.

Clinical-epidemiological analyses: With regard to the antemortem clinical features, dyspnoea (74; 64.9%) was the most common symptom, followed by fever (53; 46.5%), cough (36; 31.3%), gastrointestinal disorders (32; 28.1%), neurological symptoms (21; 18.4%) and others (11; 9.6%). Most of the deceased subjects included in the study had significant comorbidities. The most frequently documented comorbidities were diabetes (41; 35.6%) followed by hypertension (39; 33.9%), chronic kidney disease (16; 13.9%), coronary artery disease (16; 13.9%), malignancy (15; 13%) and chronic kidney disease (14; 12.2%).

The major contributing factors that led to death in these patients were septic shock in 77 (66.9%), followed by acute respiratory distress syndrome in 41 (35.6%), pneumonia in 31 (26.9%), acute kidney injury in 13 (11.3%) and metabolic acidosis in 11 (9.6%) patients. Table I provides descriptive details of the aforementioned findings. None of the factors exhibited any correlation with the positivity of the test.

Rapid antigen test (RAT) analysis: Table II depicts the antigen positivity and negativity in different age

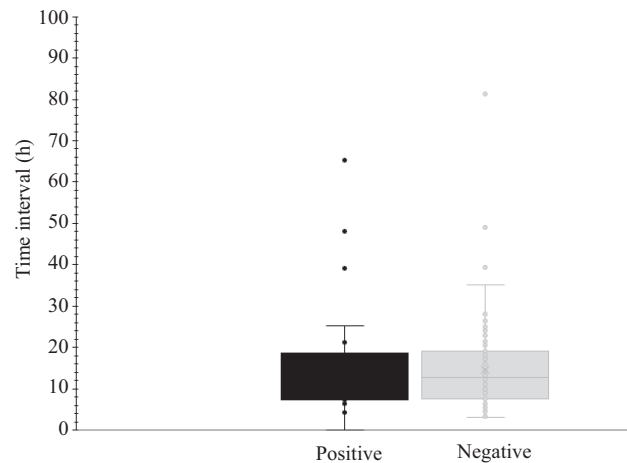


Figure. Box and Whisker plot displaying the time interval (h) in relation to RAT results with outliers in the negative and positive categories. The outlines of the boxes indicate the 25 and 75 per cent percentile, the solid central lines depict the median. End of line show the minima and maxima. Outliers shown as black and grey bold points for positive and negative results, respectively. RAT, rapid antigen test.

groups. Antigen positivity was observed more in males [20 (62.5%)] than females [12 (37.5%)]. Among the deceased who tested positive, it was observed that sample was collected at an average time interval of 11.3 h with IQR range of 8.1 to 18.7 h. The time duration between the last molecular tests to the death of the individuals was seven days with IQR of two to 12 days.

An RT-PCR test was done simultaneously with antigen test. Fifty three per cent cases were RT-PCR positive. Out of these, 66 per cent cases had cycle threshold (Ct) value ≤ 25 (strongly positive); 30.2 per cent cases had Ct value 25-30 (moderately positive); and 3.8 per cent had Ct value >30 (weakly positive). The sensitivity, specificity, positive predictive value and negative predictive value of antigen test were computed as 93.6 per cent (95% CI; 88.8-98.4%), 45.2 per cent (95% CI; 35.5-55%), 60.2 per cent (95% CI: 50.6-69.8%) and 88.8 per cent (95% CI: 82.7-95%), respectively (Table III).

Discussion

The World health Organization (WHO) reiterates that SARS-CoV-2 transmission occurs mainly by inhaling contaminated respiratory droplets, through fomites and in being close proximity to infected individuals²³. Though WHO released in its report in March 2020, that there was no evidence of people getting infected by COVID-19-related dead bodies,

Table I. Overview of associated signs/symptoms, comorbidities and cause of death with respect to rapid antigen test results

Signs/Symptoms	Positive (n=32)	Negative (n=83)	Total (n=115)	<i>P</i>
Fever	17 (53.1)	36 (43.9)	53 (46.5)	0.375
Cough	11 (34.4)	25 (30.5)	36 (31.3)	0.688
Dyspnoea/respiratory problem	18 (56.2)	56 (68.3)	74 (64.9)	0.226
Gastrointestinal/abdominal pain/diarrhoea/anorexia	11 (34.4)	21 (25.6)	32 (28.1)	0.349
Neurological symptoms	6 (18.8)	15 (18.3)	21 (18.4)	0.955
Decrease urine output	2 (6.2)	5 (6.1)	7 (6.1)	-
Others	3 (9.4)	8 (9.8)	11 (9.6)	-
Comorbidities				
Diabetes	7 (21.9)	34 (41)	41 (35.6)	0.055
CKD	7 (21.9)	9 (10.8)	16 (13.9)	0.126
CLDs	5 (15.6)	9 (10.8)	14 (12.2)	0.482
CVDs	2 (6.2)	14 (16.9)	16 (13.9)	0.14
Tuberculosis/bronchial asthma	4 (12.5)	3 (3.6)	7 (6.1)	0.074
Cancer	5 (15.6)	10 (12)	15 (13)	0.61
Hypothyroidism	2 (6.2)	4 (4.8)	6 (5.2)	0.757
Hypertension	11 (34.4)	28 (33.7)	39 (33.9)	0.948
Asthma	1 (100)	0	1 (0.9)	-
Alcoholism	1 (50)	1 (50)	2 (1.7)	-
Gastrointestinal	1 (50)	1 (50)	2 (1.7)	-
Head injury	0	6 (100)	6 (5.2)	-
Heart diseases	0	9 (100)	9 (7.8)	-
Others*	3 (27.3)	8 (72.3)	11 (9.6)	-
Cause of death				
Septic shock	21 (65.6)	56 (67.5)	77 (66.9)	0.85
Cardiogenic shock	2 (6.2)	4 (4.8)	6 (5.2)	0.757
Pneumonia/LRTI	9 (28.1)	22 (26.5)	31 (26.9)	0.861
ARDS	9 (28.1)	32 (38.5)	41 (35.6)	0.295
VAP	1 (3.1)	3 (3.6)	4 (3.5)	0.898
MOF/MODS	2 (6.3)	4 (4.8)	6 (5.2)	0.757
Metabolic acidosis	2 (6.3)	9 (10.8)	11 (9.6)	0.453
AKI	5 (15.6)	8 (9.6)	13 (11.3)	0.364
Pulmonary embolism	0	1 (1.2)	1 (0.9)	-
Refractory hyperkalaemia	3 (9.4)	8 (9.6)	11 (9.6)	0.966
Hypoxia	4 (12.5)	3 (3.6)	7 (6.1)	0.074
COPD	0	1 (1.2)	1 (0.9)	-
Respiratory failure	1 (3.1)	3 (3.6)	4 (3.5)	0.898

*Others category for comorbidities included diseases such as AIDS, downs syndrome, obesity and ovarian cyst. *P*<0.05 was considered significant. CKD, chronic kidney diseases; CLDs, chronic liver diseases; CVDs, cardiovascular diseases; LRTI, lower respiratory tract infection; ARDS, acute respiratory distress syndrome; VAP, ventilator-associated pneumonia; MOF, multiple organ failure; MODS, multiple organ dysfunction syndromes; AKI, acute kidney injury; COPD, chronic obstructive pulmonary disease

but the probability of infection to health professionals from COVID-19 corpses cannot be ruled out. On account of this, the Ministry of Health, Italy,

issued recommendations discouraging autopsies on COVID-19 cadavers²⁴. However, autopsy holds a prominent place in examining the pathological changes,

Table II. Overview of demographic details according to rapid antigen test (RAT) results

Characteristics	RAT results			P
	Positive (n=32)	Negative (n=83)	Total (n=115)	
Age (yr), n (%)				
<20	3 (9.4)	2 (2.4)	5 (4.3)	0.42
20-29	0	5 (6)	5 (4.3)	
30-39	4 (12.5)	14 (16.9)	14 (15.7)	
40-49	6 (18.8)	13 (15.7)	19 (16.5)	
50-59	9 (28)	22 (26.5)	31 (27.0)	
60-69	6 (18.8)	11 (13.2)	17 (14.8)	
>70	4 (12.5)	16 (19.3)	20 (17.4)	
Gender, n (%)				
Male	20 (62.5)	59 (71.1)	79 (68.7)	0.374
Female	12 (37.5)	24 (28.9)	36 (31.3)	
Time interval from death to RAT, median (IQR)	11.38 (8.17-18.7)	14.55 (7.4-20.5)	13.2 (8-20)	0.903
Time interval between RAT and last molecular test, median (IQR)	6 (3-11.5)	7 (3-12)	7 (3-12)	0.848
Length of stay (days)				
<2	6 (18.8)	16 (19.3)	22 (19.1)	0.949
>2	26 (81.2)	67 (80.7)	93 (80.9)	

P<0.05 was considered significant. IQR represented as median (minimum-maximum). IQR, interquartile range

Table III. Depicting sensitivity and specificity results of rapid antigen test (RAT)

RAT	RT-PCR		Total	Sensitivity per cent (95% CI)	Specificity per cent (95% CI)	PPV per cent (95% CI)	NPV per cent (95% CI)	Prevalence per cent (95% CI)
	Positive	Negative						
Positive	24	3	27	93.62 (88.83-98.41)	45.28 (35.53-55.04)	60.27 (50.68-69.86)	88.89 (82.73-95.05)	47 (37.22-56.78)
Negative	29	44	73					
Total	53	47	100					

NPV, negative predictive value; PPV, positive predictive value; CI, confidence interval; RT-PCR, reverse transcription-PCR

pathogenesis and cause of death in COVID-19 and can generate important scientific information.

There are studies where SARS-CoV-2 viral RNA have been detected in swabs of dead subjects who expired due to COVID-19²⁵⁻²⁷. Plenzig *et al*^{25,28} detected the SARS-CoV-2 viral genome after postmortem interval of 17 days and also on exhumed bodies after four months of their demise. In another study, the RNA was detectable yet in 70.3 and 66.6 per cent of the cases within two and 24 h, respectively, after death²⁰. Syamsun *et al*²¹ conducted a study on nasopharyngeal swab of 33 dead bodies using the GeneXpert system, in which SARS-CoV-2 viral RNA was found to be positive in 13 (39.39%). In their study, sample was taken at a duration of 1-4 and 8-12 h after somatic death. Recently, in a comparative study carried out by Zacharias *et al*¹⁸ on 30 deceased subjects, 80 per

cent were positive for SARS-CoV-2 using qRT-PCR, and 56.7 per cent (17/30) were antigen positive. Thus, in their study, RAT exhibited an overall sensitivity of 70.8 per cent (95% CI; 50.8-85.1%) and a specificity of 100 per cent (95% CI: 61-100%)¹⁸. In our study, sensitivity and specificity of the RAT test were estimated as 93.6 (95% CI: 88.8-98.4%) and 45.2 per cent (95% CI: 35.5-55%), respectively.

Scimeca *et al*¹⁹ reported the presence of SARS-CoV-2 viral RNA 24 h after death in 60 per cent cases. Out of these, more than 50 per cent cases showed high viral load though 20 per cent of the deceased were positive for *E*, *N* and *RdRp* gene using RT-PCR. Another study observed the presence of SARS-CoV-2 viral RNA in 66.6 per cent patients who died due to COVID-19 within 24 h of death and 70.3 per cent within 2 h. Out of the 27 cases, 12 tested in the

affirmative for *E* gene (8 cases with Ct values <25 and 4 between 25 and 35)²⁰. In the present study, RT-PCR investigation revealed that 53 per cent cases were positive for SARS-CoV-2 viral RNA (*E* gene). Among these, 66 per cent cases had high viral load with Ct value ≤ 25 ; 30.2 per cent had moderate viral load with Ct value 25-30 (moderately positive) and 3.8 per cent had Ct value >30, indicating low viral load (weakly positive). It is noteworthy that 24 (20.9%) cases analyzed were true positive in the present study.

Our study findings reiterate and reflect the fact that postmortem examination for at least within 24 h after death possess a great risk without appropriate personal protective equipment (PPE) and other infection control measures in the mortuary. Therefore, viral contamination may carry on or increase, in the early hours post death¹⁹. Due to this, healthcare workers and close contacts might be at risk to acquire infections. This is especially true when a patient dies and the body is transported for last rites. Multiple people handle the body, including clinical teams, morgue attendants, ambulance drivers and family members. This could also involve funeral agencies, religious representatives and organizations undertaking burials and cremations. In view of prevention of infection from the deceased, measures such as use of PPE, adoption of safer practices of handling and preservation of corpse, infection limiting infrastructural arrangements and regular disinfection practices of mortuary by fumigation *etc.* are prescribed^{23,29,30}. It is also advised that institutes handling COVID-19 patients conduct regular trainings of all health workers on proper handling of the dead. This must include ambulance drivers, sanitation and morgue staff.

One of the limitations of our study was that we could include dead bodies of only 115 individuals within the constraints of quick disposal and ensuing workload. Furthermore, tissue culture could not be carried out to examine the viability and replication potential of the viral genome detected from the cadavers. Further research is therefore needed including tissue culture of samples collected from deceased persons in the context of SARS-CoV-2 infection.

To conclude, RAT gives result within 15 to 30 min and thus has a great advantage of fast diagnosis of SARS-CoV-2 infection. In this study, the antigen was detected as long as 19 h post death; therefore, in

the absence of sophisticated molecular tests, simple RATs can be used for screening suspected COVID-19 deaths at home which will help in handling of the decedents according to the COVID-19 norms to prevent further potential transmission of infection. Although, infectiousness of the virus in the deceased cannot be directly concluded from either the antigen or RT-PCR test, yet possible transmission from them cannot be ruled out. This study thus reiterates on strict infection and prevention control measures while handling, transporting and disposing off COVID-19 deceased bodies.

Declaration: The datasets used and/or analyzed during this study can be made available by the corresponding author on request.

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Conflicts of Interest: None.

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For correspondence: Dr Purva Mathur, Department of Laboratory Medicine & Hospital Infection Control, Jai Prakash Narayan Apex Trauma Centre, All India Institute of Medical Sciences, New Delhi 110 029, India
e-mail: purvamathur@yahoo.co.in