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Bioclinical Study of Coronavirus Disease 2019 in the Laboratory of Virological Hemorrhagic Fever in Guinea

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Abstract

Introduction: This study aimed to describe the clinical and microbiological characteristics of individuals confirmed to have coronavirus disease 2019 (COVID-19) in the laboratory of virological hemorrhagic fever in Guinea. **Method:** This was a cross-sectional and descriptive study conducted from March 19 to June 19, 2020, in the virological hemorrhagic fever laboratory in Guinea. Samples collected from individuals living in the Conakry region, which were positive for the reverse-transcriptase polymerase chain reaction test for COVID-19, were included in this study. **Results:** Among the confirmed cases (N = 743), the males (n = 440, 59%) were more than the females (n = 303, 41%). The mean age of the confirmed cases was 33.31 ± 16.08 years. Almost all the confirmed cases (n = 515, 69.3%) had contact with a confirmed case of COVID-19. At the time of collection, 78.7% (481) of the cases were asymptomatic, while 35.3% (262) were symptomatic. At the time of the sample collection, the main symptoms were fever (51.53%), cough (49.24%), physical asthenia (46.18%), sore throat (33.2%), headache (28.62%), anosmia (9.92%), nausea, vomiting, and abdominal pain (5.34%), and diarrhea (1.53%). Only 2% of the cases had comorbidities, including diabetes (n = 7) and hypertension (n = 5). The viral load of the asymptomatic and symptomatic patients was similar with the cycle threshold value, all of which varied from 10 - 19, 20 - 29, and 30 - 36, respectively. In the first test, 27 samples were declared undetermined; two days later, samples were re-taken and re-tested and 18 of them were positive for COVID-19. The average length of hospitalization was 14 days for patients admitted to the hospital. On the other hand, the non-

hospitalized patients isolated themselves at home for 22 days. We found an association between age and the presence of symptomatology ($p < 0.001$). **Conclusion:** The main clinical manifestations of COVID19 patients in our study were fever, cough, generalized myalgia, asthenia, headache, diarrhea, and dyspnea. The risk of being symptomatic increases with age. However, younger patients are at a lower risk of experiencing severe symptoms of COVID-19.

Keywords

COVID-19, Coronavirus Disease, Viral Load, RT-PCR, Conakry

1. Introduction

The coronavirus disease 2019 (COVID-19) is an emerging infectious disease caused by the coronavirus strain severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1] [2]. On December 31, 2019, the Chinese health authority alerted the World Health Organization (WHO) on several cases of pneumonia of unknown etiology in the city of Wuhan in central China's Hubei province [3].

On January 7, 2020, the Chinese authorities officially announced that the disease was caused by a novel strain of coronavirus. Deep sequencing analysis of lower respiratory tract samples indicated a novel coronavirus, which was named 2019 coronavirus (2019-nCoV) [4].

The first cases in China in the city of Wuhan presented common symptoms, including fever, fatigue, and dry cough according to the report by Wang *et al.* [5].

In France, Gousseff *et al.* [6] declared that the first cases diagnosed presented symptoms of fever, cough, dyspnea, myalgia, odynophagia, and diarrhea. The first case detected in Finland presented symptoms of runny nose, nausea, fever, cough, and asthenia from nasopharyngeal samples analyzed in a Finnish laboratory on January 29, 2020 [7]. On February 17, 2020, the Egyptian Ministry of Health informed the WHO of the first case detected in their country. The patient, who showed no "symptoms", was transferred to the hospital and quarantined for treatment [8]. The laboratories performed real-time reverse-transcriptase polymerase chain reaction (RT-PCR) tests for three regions of the viral genome: the envelope (E), the RNA-dependent RNA polymerase (RdRp), and the nucleocapsid (N) [7].

In summary, the most common symptoms for COVID-19 were fever, cough, fatigue, and difficulty breathing. In the most severe situations, there is an appearance of an acute respiratory distress syndrome that can lead to death, especially in people who are more fragile as a result of aging or comorbidities. However, it is worth-noting that there is a high incidence of asymptomatic COVID-19 cases [9].

On March 12, 2020, the Guinean state announced the first known imported

case of COVID-19. Community transmission of SARS-CoV-2 has occurred at least since March 21 in the capital Conakry, which resulted in the first COVID-19-related death on April 15, 2020 [10]. According to the National Institute of Statistics in 2018, the capital Conakry has 2,095,705 inhabitants with a density of 5150 inhabitants per km^2. It covers an area of 45,000 ha or 450 km^2 [11]. From the foregoing, the role of the laboratory was to assist in the national diagnostic response. Considering the clinical and biological variations in COVID-19 cases in different countries or continents, serious and fatal complications can occur in the elderly or people with comorbidities who are infected by SARS-CoV-2.

This underlines the importance of advancing our knowledge of this virus and understanding its biological variations in the clinics in Guinea. Therefore, this study aimed to determine the clinical and microbiological characteristics of patients with COVID-19 in the region of Conakry.

2. Material and Methods

2.1. Study Setting

This study was conducted in the laboratory of Virological hemorrhagic fevers of Guinea (LFHVG), which was located in the commune of Ratoma in Nongo Contéya. The information collected from the notification forms enabled our data collection. The data collection sheet was appended in this paper.

2.2. Method

We conducted a descriptive cross-sectional study lasting three months from March 19, 2020 to June 19, 2020. We conducted exhaustive recruitment of individuals for three-months sampling of all samples that tested by LFHG and met our inclusion criteria from 19 March 2020 to 19 June 2020.

We included all samples collected from the region of Conakry that were declared positive by the RT-PCR test at LFHVG and whose notification forms were fully completed. We excluded the samples collected outside the zone of Conakry even they were declared positive by the RT-PCR test in LFHVG. Samples that were collected by LFHVG but were tested by other reference laboratories were also excluded.

The data were collected on a standardised and validated form that included a socio-demographic section, then a clinical section and finally a biological section. An example of this form has been added in the appendix.

The nasopharyngeal samples were collected after obtaining the informed consent of the patient and analyzed in LFHVG. During the data collection, a form was completed and the results obtained were used solely for scientific purposes. Medical confidentiality was ensured throughout our study and the data collected were used anonymously.

2.3. Ethics Statement

All study procedures complied with the Declaration of Helsinki and were ap-

proved by the National Ethics Committee for Health Research of Guinea (CNERS) (No. 068/CNERS/21). Samples were collected after obtaining the patients' informed consent and laboratory research permit.

2.4. Data Collection

For all suspected cases with symptoms and/or travel history, including the contacts of a confirmed case of COVID-19, suspected cases of COVID-19-related death, and those that had undergone massive screening, nasopharyngeal swabs were collected in viral transport medium. Samples were sent to the laboratory at 4˚C on ice packs for tests within 24 hours of collection, Swabs that were not processed within 24 hours of collection were stored at −20˚C or lower and tested within 30 days of collection. The commercial Daan Gene kit (DAAN Gene Co, Ltd., of Sun Yat-SEN University, China) was used according to the manufacturer's instructions. RNA was extracted from 200 µl of the samples. RNA was eluted in 50 µl of elution buffer and used as a template for RT-PCR.

The ORF1ab gene of 2019-nCoV was qualitatively detected by the FAM channel, the N gene of 2019-nCoV was qualitatively detected by the JOE channel, the E gene of 2019-nCoV was qualitatively detected by the ROX channel and the internal reference was detected by the CY5 channel. RT-PCR amplification was carried out in 4 steps: 50˚C for 15 minutes (one cycle); 95˚C for 3 minutes (one cycle); 95˚C for some seconds then 60˚C for 40 seconds (five cycles); 95˚C for some seconds then 60˚C for 40 seconds (40 cycles).

All COVID-19 cases were confirmed by the above RT-PCR protocol by using the triple target detection kit. We used Ct cutoffs of 36 and 40, with Ct < 36 indicating positive, Ct > 40 indicating negative, and 36 - 40 indicating an undetermined status that need to be repeated (test 1 and test 2). Two groups of samples were studied: those who were followed up in an outpatient setting (at home) and those who were hospitalized in a hospital. The viral load monitoring, which was conducted every 7th day, enabled the determination of the length of hospitalization.

2.5. Variables Investigated

The prevalence of COVID-19 during our study period in the LFHV was determined by the proportion of positives among all participants. The frequency of all data was determined on the positives collected during our period. The duration of hospitalization or isolation was determined by the average duration of the positives who participated in the study.

To determine the factors associated with symptomatology, we dichotomized the symptomatology variable into (YES/NO) and then looked for an association by performing the Khui2 and Fisher exact test at the 5% significance level.

Data were collected from the survey forms and entered into the Epidata software (version 3.1). These data were exported and analyzed with IBM SPSS Statistics for Windows (version 21, IBM Corp., Armonk, N.Y., USA).

The Microsoft Office 2013 software package (Word, Excel, and Powerpoint) was used for entering the data, designing Tables 1-4 and Figure 1 and Figure 2, and producing slides.

2.6. Role of Funding Source

The study was supported by funds from the Guinea Viral Hemorrhagic Fevers Laboratory. The funding agencies had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Table 1. Basic characteristic of patients.

Sex n, (%)		
Male	440	59
Female	303	41
Age range n, (%)		
0 - 18	113	15.20
19 - 26	128	17.20
27 - 59	447	60.20
≥60	55	7.40
Average age (±SD): 33.31 ± 16.08		
Residence n, (%)		
Ratoma	390	52.50
Matoto	173	23.30
Dixinn	49	6.60
Kaloum	80	10.80
Matam	51	6.90
Occupation n, (%)		
Unemployed	129	17.36
Civil servant	261	35.13
Liberal	249	33.51
Pupil/Student	104	14.00
Time to obtain in Hour n, (%)		
72	662	89.10
>72	81	10.9
Status at time of Sample n, (%)		
Alive	736	99.10
Death	7	0.99
The average length of hospitalization according to Location n, Average		
House	18	22
Hospital environment	84	14

Table 2. Clinical characteristic.

Reason for Screening n, (%)		
Contact	515	69.30
Suspect	132	17.80
Mass Screening	96	12.90
Clinical Status n, (%)		
Asymptomatic	481	78.70
Symptomatic	262	35.30
Signs and Symptoms n, (%)		
Fever	135	51.53
Cough	129	49.20
Asthenia	121	46.18
Sore Throat	87	33.20
Headache	75	28.62
Runny nose	56	21.37
Myalgia	54	20.61
Anosmia	26	9.92
Dyspnea	20	7.63
Chest pain	16	6.10
Joint pain	13	4.96
Anorexia	12	4.58
Nausea and vomiting	8	3.05
Abdominal pain	6	2.29
Diarrhea	4	1.53
Comorbidity n, (%)		
Diabetes	7	46.70
HTA	5	33.30
Pregnancy	1	6.70
Asthma	1	6.70
Sickle cell disease	1	6.70

Table 3. Biological characteristics of patients.

CT viral load Symptomatic asymptomatic		
[10 - 19]	75	67

Continued

[20 - 29]	100	289
[30 - 36]	87	125
	Test 1 n, (%)	
Positive	725	96
Not known	27	4
	Test 2 n, (%)	
Positive	9	33
Negative	18	67

Table 4. Distribution of symptomatology by age group and viral load.

Variable		Symptomatic		p
		Yes n (%)	No n (%)	
Age range (years old)	0 - 18	27 (3.63)	86 (11.57)	0.001
	19 - 26	50 (6.73)	78 (10.50)	
	27 - 59	153 (20.59)	294 (39.57)	
	≥60	32 (4.30)	23 (3.01)	
Viral load	[10 - 19]	75 (10.09)	67 (9.02)	0.745
	[20 - 29]	100 (13.46)	289 (38.90)	
	[30 - 36]	87 (11.71)	125 (16.82)	

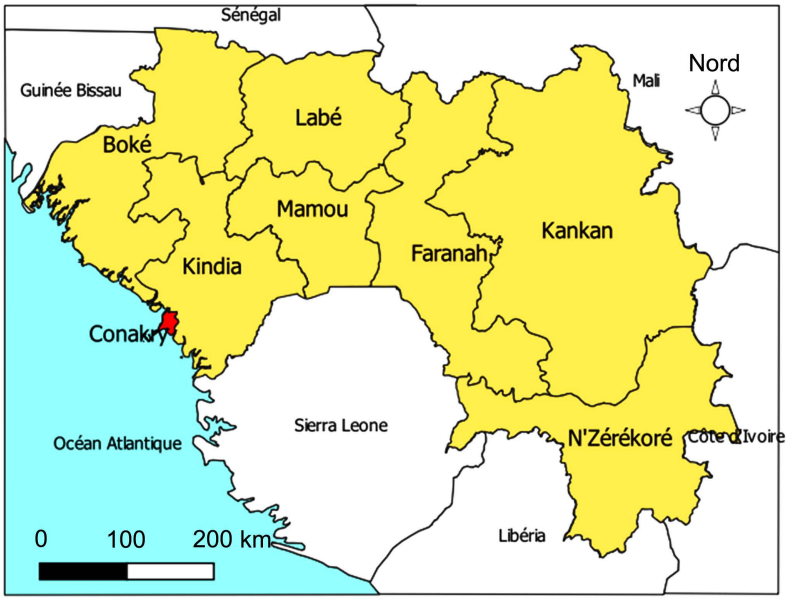

Figure 1. Map of the Republic of Guinea divided into administrative regions. The capital Conakry, which is the study area, is shown in red.

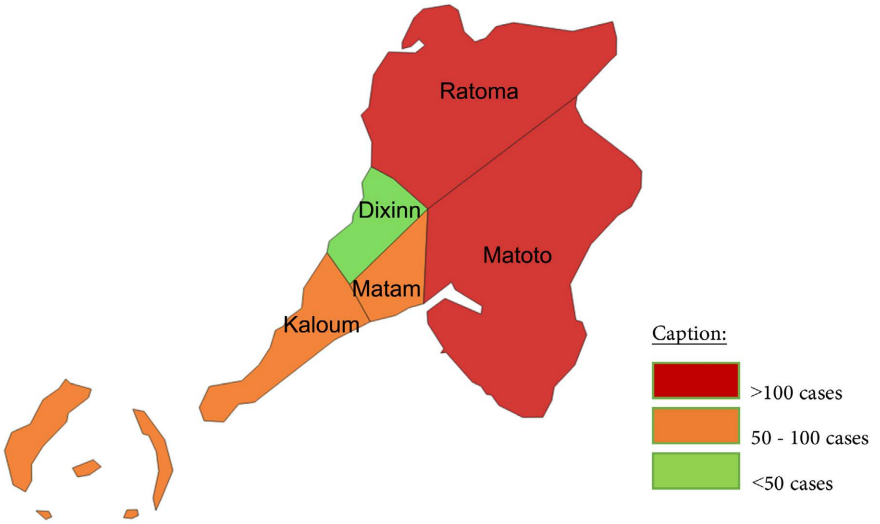

Figure 2. Map of the capital Conakry divided into its 5 main communes coloured according to the number of cases of COVID-19.

3. Results

Out of 4319 samples collected, 743 were positive for SARS-Cov 2 during the study period, which amounted to a COVID-19 positivity rate of 17% (95% CI: 0.57 - 0.91).

The cases were predominantly male as evidenced by a male-female sex ratio of 1.45. The most represented age group was 19 - 26 years, with a frequency of 54.90% and an average age of 33.31 years. Almost all (99%) samples were taken at the time of diagnosis from living people. The time to obtain the results was more than 72 hours for 10.9% of the samples. The basic characteristics of the samples are described in detail in **Table 1**.

Among the COVID-19 cases, 69.3% had contact with a confirmed case of COVID-19. At the time of sampling, 78.7% of the cases were asymptomatic. The main symptoms at the time of sampling were fever (51.53%), cough (49.24%), physical asthenia (46.18%), sore throat (33.2%), headache (28.62%), anosmia (9.92%), nausea, vomiting and abdominal pain (5.34%), and diarrhea (1.53%). 2% of the cases presented comorbidities, including diabetics (n = 7) and hypertension (n = 5). The frequency of asymptomatic cases was high in the 0 - 19 years age group (74.5%) and low in the ≥60 years age group (44%). The clinical characteristics of these cases are described in detail in **Table 2**.

During the study period, 27 samples were declared indeterminate (inconclusive). At the resumption of the test (test 2), 18 samples were declared positive after two days. The viral load of the asymptomatic and symptomatic patients was similar. The average length of stay in the hospital was 14 days for the hospitalized cases but 22 days for those confined to their homes. The biological characteristics of these cases are described in detail in **Table 3**.

During the study period we made an association between age and symptomatology which showed a strong relationship with a p less than 0.001. We noted the

presence of more symptomatology from the third age. The association between gender and having COVID-19 was found with a p Value 0.020 with a strong trend for male gender.

4. Discussion

The cases were predominantly male as evidenced by a male/female sex ratio of 1.45, which indicates that men are more prone to coronavirus infections than women.

This finding is similar to that of several authors who reported male predominance in COVID19 infection. For instance, Meng *et al.* [12] reported a slight male predominance of 51% in China, while Zhi *et al.* [13] reported a male predominance of 71%, suggesting men may be more prone to coronavirus infections than women.

The association between gender and having COVID-19 was found with a p Value 0.020 with a strong trend for male gender, which according to a meta-analysis conducted in 2016 by Kelly Moran and Sara Del Valle [14], the higher rate of male mortality or infection could also be explained by behaviors. In many cultures, men are more likely to smoke, which is linked to a worse prognosis. On the contrary, women are inclined to adopt more protective conducts. Furthermore, compared with men, women are more likely to wear face masks, wash their hands, and avoid public transportation during previous outbreaks of respiratory viruses, such as bird flu and SARS. Such differences in attitudes and behaviors between men and women have continued in the current pandemic, according to a survey conducted in March and April of 2020 by the National Bureau of Economic Research [15]. Responses from 21,649 people in eight developed countries indicated that women are more likely to take COVID-19 seriously and agree to comply with public safety measures than men.

The most represented age group in our study was 19 - 26 years, representing 54.90% of the whole cohort. The mean age of all the cases was 33.31 ± 16.078 years, ranging from 3 months to 98 years. However, Chang *et al.* [16] reported a median age of 34 years in China. This result is different from that of Richardson *et al.* [17] who reported an average age of 63 years in America, and that of Docherty *et al.* [18] who reported an average age of 73 years in England.

Our study revealed that the municipality of Ratoma was the most affected area, accounting for 52.3% of cases. This could be explained by the geographical location of the LFHVG, which is located in the municipality.

The civil servant and liberal professions were the 2 most affected professions, accounting for 68.82% of all the cases. We have not found why these various professions were more affected; therefore, an in-depth study is required to investigate this finding.

Almost all the samples were collected at the time of notification from living persons. This was due to the fact that the late implementation of massive screening of community deaths was carried out by the Guinean health authority.

The time taken to obtain the results was over 72 hours for 10.9% of the sample, which could be explained by the fact that the LFHVG conducted approximately 200 tests per day. The testing capacity was limited as the LFHVG received samples from all regions of the country. There was an extended treatment period of several hours before results were obtained.

Among the cases, 69.3% had contact with a confirmed case of COVID-19, which is the main means of transmission. This finding is consistent with a previous study showing that the transmission is mainly via respiratory droplets and close contacts with infected cases [19]. Overall, our and others' evidence demonstrated that human transmission for SARS-CoV-2 is primarily via respiratory droplets and close contacts [5].

At the time of the sample collection, 78.7% of the cases were asymptomatic. This finding is approximately similar to that of DO Kpamy *et al.* [20] who reported that 69.79% of the cases in Guinea were asymptomatic. This difference could be explained by the fact that, at the time of collection, a sample can be positive according to RT-PCR one week before the symptoms begin to appear, which is known as the incubation period.

Our results are different from those of Mizumoto *et al.* [19] who investigated 3711 passengers or crew members of the cruise ship Diamond Princess that were quarantined at the port of Yokohama (Japan), which represents a quasi-experimental model of infection with SARS-CoV-2. In their study, among 634 confirmed cases of SARS-CoV-2 infection, 17.9% were asymptomatic [21]. The main symptoms were respiratory, cerebral, and digestive symptoms. This could be explained by the tropism of coronavirus in the lung, brain, and digestive system [22]. Our results are consistent with the findings of several studies conducted around the world, including studies from Gousseff *et al.* [6] in France and Plaçais *et al.* [23] and Du Wenjun *et al.* [24] in China.

The frequency of asymptomatic and mild symptoms was high in the 0 - 18 years (74.5%) age group, which agrees with the results of Du, Wenjun *et al.* [24] who concluded that, indeed, a weaker inflammatory response to lung damage causes milder clinical symptoms in children than in adults.

The viral load detected in the asymptomatic patients during our study was similar to that of the symptomatic patients. This result is comparable to that of Rothe *et al.* [25] in Germany, who stated that asymptomatic people are potential sources of 2019-nCoV infection may warrant a reassessment of the transmission dynamics of the current outbreak.

All samples collected during our study period were nasopharyngeal samples, as nasopharyngeal samples contain larger viral load compared to oropharyngeal samples, as stated by Zou, Lirong *et al.* [26] who analyzed the viral load of nasal and throat swabs obtained from 17 symptomatic patients against the day of symptom onset. Higher viral loads (inversely related to cycle threshold values) were detected soon after symptom onset, with higher viral loads detected in the nose than in the throat.

In our study, 27 samples were declared indeterminate (inconclusive) according to the results of the kit; probably these samples were at the window period of elimination of the virus when the samples were collected. 27 samples were taken again after two days for retesting, 18 samples were declared positive; therefore, those declared negative were considered to have eliminated the virus.

In our study, 18% of the cases were confined to their homes because they were asymptomatic and/or showed mild symptoms. The average length of hospitalization was 14 days; however, those confined to their homes stayed up to 22 days for quarantine. In this regard, in-depth studies are required to determine the reasons for the difference in length of hospitalization or isolation between the two groups.

Our study showed an association between age groups and being symptomatic, especially in the third age group. Our results corroborate with the findings of the study of the ISARIC [27]. However, we found no association between viral load and symptomatology.

Our study has several limitations. First, for all the samples, the diagnosis was confirmed with swabs from the upper respiratory tract and no oropharyngeal swabs were used. Second, we did not perform blood tests and were unable to report physical signs by the time patients are hospitalized. Third, the widespread use of RT-PCR tests resulted in a shortage of test kits and thus an extended waiting period (*i.e.*, several hours) was needed before obtaining results.

5. Conclusions

The main clinical manifestations of the novel 2019-nCoV are fever, cough, generalized myalgia, asthenia, headache, diarrhea, and dyspnea. In particular, asymptomatic patients have the same viral load as the symptomatic patients and the asymptomatic patients are capable of spreading the virus.

The risk of being symptomatic increases with age. However, younger patients are at a lower risk of experiencing severe symptoms of COVID-19.

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Ethics Approval and Consent to Participate

The studies were approved by the National Ethics Committee for Health Research of Guinea (CNERS) (No. 068/CNERS/21).

Human and Animal Rights

All study procedures complied with the Declaration of Helsinki.

Consent for Publication

Samples were collected after obtaining the patients' informed consent and labor-

atory research permit.

Availability of Data and Materials

Not applicable.

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None.

Conflicts of Interest

Declared none.

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Appendix

Bioclinical study of coronavirus disease in Conakry:

SURVEY FORM

N°……

1) Sociodemographic characteristics:

Sex: M ☐ F ☐

Age: ….

Profession:……

Municipality:……..

2) Clinical characteristics:

Delay in obtaining results:…… (Collection date ……/ ……/ ……/ et Date of issue of result: ……../……../…….)

Status at the moment of notification:

Alive ☐ Deceased ☐ Date of Death: ……/……. /……../

Category:

Contact ☐ Suspect ☐ Mass Screening ☐ Control ☐

Asymptomatic: Yes ☐ No ☐

Symptomatic: Yes ☐ No ☐

Fever: Yes ☐ No ☐

Cough: Yes ☐ No ☐

Breathing difficulties: Yes ☐ No ☐

Physical asthenia: Yes ☐ No ☐

Myalgia: Yes ☐ No ☐

Headaches: Yes ☐ No ☐

Others ……

If control:

At the time of notification, is the case in hospital? Yes ☐ No ☐

If yes: Date of hospitalisation: ……../……../……../

Name of health facility: ……

If not hospitalised, has the case been kept in containment? Yes ☐ No ☐

If yes, where……

Comorbidity:

Pregnancy (Trimester: ……) ☐ Postpartum (<6 weeks) ☐

HTA ☐ HIV ☐

Diabetes ☐ Chronic Kidney Disease ☐

Hepatitis ☐ Lung disease ☐

Cancer ☐

3) Characteristic Biological:

Diagnostic de Laboratoire: PCR en temps réel:

a) Nature of the sample:

Nasopharyngeal swab ☐ Oropharyngeal swabSang ☐

b) Rank of the Test:

Test 1: ☐ Test 2: ☐ Control: ☐

c) Laboratory conclusion:

Test 1:

Positive: ☐ Viral load:…….

Undetermined: ☐ Re-testing: Yes ☐ No ☐

Negative: ☐

Test 2 (Re-testing declared undetermined in test 1):

Positive: ☐ Viral load:…….

Negative: ☐

Control:

Control Rank: 1 ☐ 2 ☐ 3 ☐ 4 ☐.: ……..

Control time (time taken between admission and control)

Jour: ……

Control results:

Positive: ☐

Negative: ☐